PREFACE

ANNUAL REPORT

MARIO NEGRI INSTITUTE, MILAN www.marionegri.it

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PREFACE

In 2013 the Mario Negri Institute for Pharmacological Research celebrated 52 years since its foundation. The date marked another step in the Institute’s history too: the Ministerial Decree published in the Italian Gazzetta Ufficiale No, 34, p.17, on 9 February 2013 recognized the Institute as an ‘IRCCS’ – an institute for research and healthcare. That means it is considered part of the National Health Service, and comes under the heading ‘Pharmacology and clinical research for neurological, rare and environmental diseases’. The Institute has been doing preclinical and clinical research in these areas for many years.

This good news, however, unfortunately accompanies a generally worrying financial situation, not only in Italy but many other countries too, especially in southern Europe. This economic crisis has certainly affected scientific research in Italy, and – contrary to what should normally be done in times of difficulty – the Government has completely abandoned the whole sector to itself. Despite these problems, the Mario Negri Institute has been hard at work, producing worthwhile experimental and clinical results. Many of the findings have already been published, and others are ‘in press’. More than 463 articles were accepted in international scientific journals in 2013. As usual, research is described here department by department, though sometimes for single laboratories. Details of the findings are given in the body of the report, and here we shall just make some general observations.

In 2013 we had regrettably to watch the Italian Parliament display its ‘disdain’ for science with laws in which that researchers’ opinions had been replaced by pseudo-arguments put forward by charlatans. Debate on the European Directive on animal experimentation is a typical example of this attitude. Huge efforts will be needed to ensure the public understands that no progress can ever be achieved toward curing human beings – and animals too – without using animals in research.

Another example is the ‘Stamina’ episode, which involved public health structures prescribing and administering products whose content was kept secret, with no regard whatsoever for all the barriers normally in place to safeguard patients who volunteer for clinical trials. Here again, experts’ opinions were completely ignored, and great confusion was caused among the public.

This year, as in the past, the Institute has focused on its main basic areas of research: oncology, neurosciences, cardiovascular and renal diseases, organ transplantation, rare diseases, cell biology, molecular biochemistry, and epidemiology. The approach is always the same – to develop around the overall area a complex series of strategies ranging from basic research to pharmacokinetics, pharmacology, controlled clinical trials, epidemiological analysis and, where possible, the epidemiology of healthcare services.

Several important trials have been completed. One, on lung tumors, showed that docetaxel, an old drug, was more active than erlotinib, a new one that is much more expensive. Another trial found that L-acetylcarnitine together with riluzol prolonged survival for patients with amyotrophic lateral sclerosis – ALS – a rare disease. We hope to be able to confirm this finding in a trial with a larger number of patients. Another rare disease, uremic hemolytic syndrome, can benefit substantially from treatment with eculizumab.

Studies have started on about 200 people who have reached the age of 100, completing the study on about 2000 people aged over 80, investigated in the Monzino Study with the aim of identifying factors that protect against dementia. A clinical trial now in progress is designed to establish the efficacy and toxicity of several opioids – oxicodone, fentanyl and buprenorphine – compared with morphine in cancer patients.

Research is continuing on environmental pollutants in water, soil and foodstuffs, particularly PCBs and dioxin; the same laboratories have developed a method for quantifying drugs of abuse and medicinal drugs in waste waters.

Under the heading of diseases of old age, the REPOSI trial is giving important results on the prevalence of multiple simultaneous pathologies and the consequent polypharmacology – multiple drugs. In the cardiovascular area a series of studies have examined drugs that provide neuroprotection after prolonged cardiac arrest. An important European study has been started, to describe the epidemiology of head trauma and examine the molecular and phenotypic markers involved in brain damage.

New methods have been introduced for studying cerebral and peripheral amyloidosis, using the worm C. elegans. Promising results have already been achieved with the use of nanoparticles to improve drug entry into tumors, and to pass the blood/brain barrier.

Under the heading of transplants, a new method has been developed that achieves complete maturation in vivo of functioning renal organs, generated from suspensions of single embryonal renal
cells. This is an important step towards building a functioning kidney. Gene therapy is currently being developed to prevent chronic rejection of a transplanted solid organ. In a controlled clinical trial octreotide had beneficial effects on the progression of renal polycystic disease. Another study identified some predictors of the response to rituximab in patients with idiopathic membranous nephropathy.

An on-line register has been set up of the controlled clinical trials conducted at the Mario Negri Institute, where anyone can look to see how each trial is progressing. There is no space here to give even brief details of the large number of trials but information can be found in the text (www.marionegri.it).

An essential part of research is training young scientists so that in the laboratory they not only have a chance to express their ideas, but can also earn a qualification. The Institute offers a professional course recognized by the Lombardy Region (950 students have earned diplomas to date) and, at a more advanced level, runs a Ph.D. degree course in collaboration with the UK Open University (more than 80 students have passed); there is also a research doctorate recognized by the Ministry of University and Research in Italy (ten diplomas have been awarded so far).

Finally, an essential part of the Institute’s work involves providing information at all levels. There is the Rare Diseases Information Center (www.marionegri.it click on Centro Malattie Rare), and the Drug Information Center – also easily reached on the Institute website (www.marionegri.it). The Institute works constantly to provide information for physicians, nurses and patients’ associations, and to the public, using all the media available. From 2000 to 013, a total of 1863 articles for the lay public were published. The site www.partecipasalute.it has developed fast. A European project is in progress to inform the public on the details and advantages of controlled clinical trials.

Research is moving through increasingly difficult times and researchers are struggling to make ever-greater efforts. All possible assistance is needed from all possible sources - the government, public bodies, charities and private persons.

Silvio Garattini
Mario Negri
INSTITUTE FOR PHARMACOLOGICAL RESEARCH

Milan

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departments and laboratories
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Scientific Documentalist
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Head: Giulia TARABOLETTI, Biol.Sci.D.
Unit located in Bergamo

Molecular Cancer Therapeutics Unit
Head: Maria Rosa BANI, Biol.Sci.D., Ph.D.

Laboratory of Cancer Cachexia AIRC Start-Up
Head: Rosanna PICCIRILLO, Biotec. Med. D., Ph.D.

Laboratory of Methodology of Biomedical Research
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Laboratory of Clinical Research
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Head: Oscar CORLI, M.D.
CURRICULA VITAE

Maurizio D’Incalci obtained his Medical Degree *cum Laude* from the University of Milan in 1977. After specializing in Pharmacology at the Mario Negri Institute of Milan in 1979 and in Oncology at the University of Genoa in 1981, he worked in the Laboratory of Molecular Pharmacology of the National Cancer Institute in Bethesda, MD, USA. Since 1986 he has been chief of the Laboratory of Cancer Chemotherapy at the Mario Negri Institute and since 1996 he has become chief of the Department of Oncology at the Mario Negri Institute.

He has been President of the Pharmacology and Molecular Mechanisms Group of the European Organization for Research and Treatment of Cancer (EORTC). From 1994 to 1997 he was Chairman of the New Drug Development Coordinating Committee and from 1997 to 2000 he was chairman of the Research Division of the EORTC. He has been member of the Board of the EORTC from April 2000 to 2003.

Since 1995 he is member of the Board of Directors of the Nerina and Mario Mattioli Onlus Foundation. From 1997 to 2012 he has been the Preclinical Coordinator of the Southern Europe New Drug Organization (SENDO) and from 2005 to 2012 he has been the Chairman of the New Agents Committee (NAC) of SENDO.

From 2003 to 2013 he has been member of the Ethic Committee of the Centro di Riferimento Oncologico (CRO) of Aviano.

From 2006 he is president of the Scientific Committee of the Mario Negri Gynecologic Oncology group (MaNGO).

From 2007 he is member of the Scientific Committee of the Italian Association for Cancer Research (AIRC).

From 2009 to 2013 he has been member of the Board of Directors of the Italian Cancer Society (SIC).

From 2010 he is member of the Scientific Committee of the Buzzi Unicem Onlus Foundation for the research, diagnosis and cure of malignant mesothelioma.

From November 2013 he is member of the Ethic Committee of the Fondazione del Piemonte per l’Oncologia – IRCCS of Candiolo.

He is on the editorial board of many international cancer-related scientific journals and from September 2000 to December 2010 he has been Editor for Experimental Oncology of the European Journal of Cancer. Dr D’Incalci is author of more than 470 papers on cancer chemotherapy published in peer reviewed international journals, and of several chapters in books on cancer chemotherapy.

Selected publications


Massimo Broggini followed the faculty of Science of the University of Milan, got the specialization in Biochemistry at Mario Negri Institute, and the PhD degree at the Open University, London, UK.
He worked in the laboratory of Molecular Pharmacology of the National Cancer Institute of Bethesda, Md, in 1986. From 1991 he is the head of the Molecular Pharmacology Unit of the Mario Negri Institute and from 1999 he is the head of the Laboratory of Molecular Pharmacology of the same Institute. His main fields of interest are the study of the mechanism of action of new anticancer agents, the search of altered proteins and genes in human cancer and the study of oncosuppressor genes. He is member of the "Pharmacology and Molecular Mechanisms Group" of the European Organisation for the Research and Treatment of Cancer (EORTC) and of the American Association for Cancer Research. He is in the Editorial board of the European Journal of Cancer, Frontiers in Cancer Genetics and American Journal for Cancer Research.

He is author of more than 150 articles published in international journals.

Selected publications


Irene Floriani got her degree in Biological Sciences at the University of Milan in 1988, her degree in Biostatistics and Experimental Statistics at the University of Milan-Bicocca in 2003 and her PhD at Open University Research School of London in 2005. After ten-year experience in pharmaceutical industries, in 2002 she became Head of the Biometry and Data Management Unit of Laboratory of Clinical Research in Oncology and since 2006 she is Head of Laboratory of Clinical Research (until 2012 Laboratory of Clinical Trials).

From 1999 to 2006 she was member of Ethics Committee of Istituto Scientifico Eugenio Meda. From 2002 to 2013 she was member of Ethics Committee of the San Paolo Hospital of Milan. From 2004 to 2013 she was member of Ethics Committee of Hospital Sant'Anna of Como (from 2010 to 2013 as chairman). From 2010 to 2013 she was member of Ethics Committee of Hospital of Valtellina and Valchiavenna. Since 1998 he has been member of Ethics Committee of Fondazione IRCCS Carlo Besta Neurological Institute (from 2002 to 2013 as vice-president). Since 2013 he is chairman of Ethics Committee of Manzoni Hospital of Lecco. Since 2011 he is in the editorial board of the European Journal of Cancer, Frontiers in Cancer Genetics and American Journal for Cancer Research.

Selected publications

Raffaella Giavazzi obtained her Biological Sciences degree (1979) at the University of Milan and her PhD in Pharmacology at the Mario Negri Institute of Milan (1984), followed by a specialization in pharmacology (1994) at the University of Milan. From 1981 to 1983 she was a post-doc fellow in the Cancer Metastasis and Treatment Laboratory, NCI-FCRDC, Frederick, MD, and from 1983 to 1985 Assistant Professor at the Department of Cell Biology of M.D. Anderson Hospital and Tumor Institute, University of Texas System Cancer Centre in Houston, TX.

From 1986 to 1993 she was Head of the Cancer Metastasis Treatment Unit and since 1993 she has been the Head of the Laboratory of Biology and Treatment of Metastasis at Mario Negri Institute for Pharmacological Research. She was adjuvant Professor of Oncology at the Medical School of the University of Brescia (2007-2010) and of the University of Pisa (1999-2010) and in the Teaching Committee for the PhD course in Physiology-Pharmacology-Molecular and Cellular Toxicology at the University of Siena. Since 2012 she is a member of the Board of Directors (CdA) at the University of Trento. She was consulting scientist for the NCI-Drug Therapeutics Program, USA (1996-2006), and member of the Executive Committee at the Southern Europe New Drug Development Organization (1988-2012). She was in the Board (1994-2012) and President (2005-2007) of the Italian Cancer Society, member of the Executive Committee of the European Association for Cancer Research (2008-2012) and in the Board of the International Metastasis Research Society (2000-2004). From 2008 she is a member of the Pezcoller Foundation Scientific Committee.

In 1996 she was Honorary Research Fellow and Visiting Professor, Division of Oncology, Richard Dimble Department of Cancer/ICRF, London, UK. In 2003 she received the Researcher Career Award “Italian League Against Tumor” and in 2012 she gave the “Giorgio Prodi Lecture” at the Italian Cancer Society.

She is on the Editorial Board of a number of international scientific journals. She has published approximately 200 articles on “peer reviewed” scientific journals and is co-author of several chapters in books on cancer biology and therapy. She has been invited as speaker at numerous national and international congresses on cancer research.

Selected publications


Rosanna Piccirillo graduated summa cum laude in Medical Biotechnologies in 2001 with a thesis in Experimental Oncology at the Istituto Nazionale dei Tumori in Milan. In 2006, she obtained the international PhD in Molecular and Cellular Biology at the San Raffaele Scientific Institute in Milan, studying the intracellular sorting and transport of a protein implied in a human genetic disease (Ocular Albinism Type 1). In 2006, this original research work was awarded with the prestigious Premio Sapio...
Junior per la Ricerca Italiana (http://www.premiosapio.it/2011/pagine/dynamic_art.php?id=6&table_name=2012_edizioni). In 2007, she worked as Visiting Assistant Researcher in the Department of Human Genetics at the University of California, Los Angeles (UCLA), where she acquired useful biochemical skills. From 2007 to 2012, she worked as Postdoctoral Research Fellow in the lab headed by Prof. Alfred L. Goldberg in the Cell Biology Department at Harvard Medical School in Boston, MA, where she expanded her knowledge about protein ubiquitination and degradation in neurodegenerative diseases as well as in muscle atrophy.

Since March 2012, she is head of the laboratory Cancer Cachexia AIRC Start-up in the Oncology Department at Mario Negri Research Institute, where she is leading a research group aimed at dissecting the molecular mechanisms causing muscle wasting during cancer growth in the attempt to block this devastating condition.

Selected Publications


*+: These authors contributed equally to this paper.


Valter Torri got his Medical degree in 1985 and the specialization in medical Oncology in 1989 at the University of Milano.

Education: 1985: MD Degree with full honors cum Laude, University of Milano; 1988 Post-Doctoral Degree in Pharmacological Research, Mario Negri Institute, Milan; 1989 Post-Doctoral Degree in Medical Oncology, University of Milano; 1989-1991 Research Fellow at the Biometric Research Branch of Cancer Treatment Evaluation Program, NCI, Bethesda, MD (USA).

Areas of Interest: Statistical aspects of clinical research methodology with focus on Controlled Clinical Trials in Oncology; Systematic Overview of the medical literature; Methodological aspects of diagnostic test evaluation.

Present Position: Head of Laboratory of Methodology of Biomedical Research, Oncology Department, Mario Negri Institute, Milano.

Chronology of Professional Appointments: 1983-1985: Clinical research Fellow in Internal Medicine at the University Hospital, University of Milan; 1985-1989: Research assistant at the Clinical Trial Unit of the Laboratory of Clinical Epidemiology, Mario Negri Institute for Pharmacological Research, Milano; 1989-1991: Research fellow at the Biometric Research Branch of Cancer Treatment Evaluation Program, NCI, Bethesda, MD (USA); 1994: Head of Biometric Unit of the Laboratory of Cancer Clinical Epidemiology, Oncology Department, Mario Negri Institute for Pharmacological Research, Milano, Italy; 1995 Vice Director of the Italian “Cochrane” Center; 2001: Head of Laboratory of Clinical Research In Oncology, Oncology Department, Mario Negri Institute, Milano. 2006: Head of Laboratory for the development of new pharmacological strategies , Oncology Department, Mario Negri Institute, Milano; 2011: Head of Laboratory of Methodology of Biomedical Research.

Member of Consiglio Direttivo Nazionale dell’Associazione Italiana di Oncologia Medica.

Member of Independent data monitoring committee of International Randomised Clinical trials in NSCLC and ovarian carcinoma.

Co-author of more than 200 papers published on peer reviewed journals and of 5 chapters of scientific books relative to clinical research methodology for therapeutic and diagnostic studies.

Selected publications


**Maria Rosa Bani** got her Biological Sciences degree at the University of Milan in 1998 attaining the Italian Government Qualification to practice as Biologist in 1990. She obtained the specialization in Pharmacological Research from the Department of Education of the Regional Government of Lombardia in 1991 and the specialization in Biomedical Research from the Department of Education of the Regional Government of Abruzzo in 1993. In 2005 she was awarded the degree of Doctor of Philosophy (PhD), Discipline of Life Sciences of the Open University Research School (UK).

From 1991 to 1995 she was a Post Doctoral Fellow at the Cancer Research Division, Sunnybrook Health Science Centre, University of Toronto (Canada); from 2000 to 2001 she was Guest Scientist at the Advance Technology Centre, National Cancer Institute, National Institute of Health (USA). From 1996, she was a Fellow Research Scientist at the Mario Negri Institute for Pharmacological Research, Laboratory of Biology and Treatment of Metastasis and she became a staff research scientist in 2003. Since 2004 she was appointed Head of the Molecular Cancer Therapeutics Unit in the same laboratory.

She has been the Scientific Manager of STROMA and ADAMANT, two Integrated Projects funded in the 6th and 7th Framework Programs of the European Commission.

She is a member of the American Association for Cancer Research (AACR), the European Association for Cancer Research (EACR) and the Italian Cancer Society (SIC).

Maria Rosa Bani research interests are in the field of cancer biology and preclinical therapeutics, with a focus on studying endothelial cell biology and understanding the role of endothelium in cancer progression for a translational opportunity. She is author of 37 peer reviewed publications, 2 book chapters and 70 abstracts of which 17 selected for oral presentations at international meetings.

**Selected publications**


Michela Cinquini

Michela Cinquini got her degree in Statistical Science in 2005 at the University of Milano-Bicocca and her specialization in “Specialist in Pharmacological Research” at the Mario Negri Institute in 2008. She has been working at Mario Negri Institute since 2004. She is now head of the “Systematic reviews methodology and guidelines production” unit by the Laboratory of Methodology of Biomedical Research. In 2009-2010 she worked as a Fellow at the Centre for Statistics in Medicine - Oxford, UK (Supervisor Doctor Altman DG).

Since 2006 she has been teaching in several post-doctoral Masters in Clinical Research Methodology at Ferrara and Parma University and since 2010 in Systematic reviews at Milano University. Since 2008 she has been member of the Italian Cochrane Centre.

Research interest: Statistical and methodological aspects of Systematic reviews and Meta-analysis of intervention; Quality evaluation of evidence-based medicine and production of oncological guidelines using the GRADE approach.

Selected publications


Oscar Corli

Oscar Corli got his Medical degree in 1974 at the University of Milan and the specialization in Anesthesiology and Intensive Care in 1977. From June 1975 to January 1994 he was an Assistant Director of Anesthesiology Department at “Vittore Buzzi” Hospital (Milan). From February 1994 to January 2008 he was a Director of Palliative Care Unit at “Istituti Clinici di Perfezionamento” Hospital (Milan) and from 2008 up to now he was a director of C.E.R.P. and presently he be the head of Palliative care Unit.

Founding member of the S.I.C.P. (Italian Society of Palliative Care ), then national secretary from 1986 to 1994 , then national president of SICP from 1994 to 1997.

Founding member of the E.A.P.C. (European Association for Palliative Care) and a founding member of SIMPA (Italian School of Medicine and Palliative Care ).


Member of the Commission for Palliative Care (Regione Lomabardia) in 1997-98 and member of the "Pain Management" - Department of Medicines and Pharmacovigilance (Ministerial Decree of 24.03.2003) and national coordinator of the same committee at the AIFA since January 2004, confirmed the coordination on 6 July 2005 until 2006. Member of the Commission "Pain therapy, palliative care and end of life" - Ministry of Health - in December 2006 -2007. Editor of Italian Observatory Palliative Care (www.oicp.org).

Selected publications

- Greco MT, Corli O, Montanari M, Deandrea S, Zagone V, Apolone G; Writing Protocol Committee; Cancer Pain Outcome Research Study Group (CPOR SG) Investigators. Epidemiology and pattern of care of breakthrough cancer
Giovanna Damia obtained her Medical Degree cum Laude from the University of Milan in 1985. After specializing in Pharmacology at the Mario Negri Institute of Milan and in Oncology at the University of Milan, she worked as a post-doctoral fellow in the Laboratory of Experimental Immunology of the National Cancer Institute, Frederick, USA. She worked as a research fellow in the Laboratory of Cancer Chemotherapy at the Mario Negri Institute and since April 2003 she has become chief of the DNA Repair Unit at the Mario Negri Institute. From 1992 to 1995 she has been consultant of the General Secretariat of the Progetto Finalizzato CNR “Applicazioni Cliniche della Ricerca Oncologica”. Since September 2005 she is Deputy Editor for Experimental Oncology of the European Journal of Cancer. Her main fields of interest are: mechanism of action of anticancer drugs, cell cycle checkpoints and natural compounds.

Selected publications


Eugenio Erba has obtained his Biological and Biochemistry Analysis Degree at the University of Urbino. He worked as a research fellow in the Laboratory of Cancer Chemotherapy at the Mario Negri Institute and since 1984 he is head of the Flow Cytometry Unit in the Department of Oncology at the Mario Negri Institute of Milan. He has worked as a visiting fellow in the Department of Istochemistry and Cytochemistry of the University of Leiden, The Netherlands in 1983. Since 1997 he is Teacher of Post-Graduate Studies in Cytometry at the University of Milan and Co-ordinator and Teacher of Post-Graduate Studies in Cytometry for the Italian Cytometry Group. He has been President of the Italian Cytometry Group from 1999 to 2001. Since 2001 he is member of the Executive Board of the Italian Cytometry Group.

Scientific areas of interest: studies on the mechanism of action of different compounds with provided antitumoral activity evaluating the mechanism of cell death and cell cycle phase perturbations induced on different human cancer cell lines by using flow cytometry. Co-ordinator of working-group in a quality control study on flow cytometric DNA content analysis in human tumors.

Selected publications


Roldano Fossati got his Medical Degree cum Laude from the University of Milan in 1980, his Post-Doctoral Degree in Endocrinology cum Laude from the University of Verona in 1983 and his Post-Doctoral Degree in Medical Statistics from the University of Milan in 1992. He has been consultant at the Mario Negri Institute since 1983 and, at present, he is head of the Gynecology and Oncology Unit of the Laboratory of Clinical Research.

Areas of Interest: Statistical and methodologic aspects of clinical research with focus on Controlled Clinical Trials in Oncology; Systematic Overview of the medical literature.

Selected publications

Roberta Frapolli, got her degree in Pharmacological Chemistry (110/110 summa cum laude) at the University of Milan in 2000. In 2003, she specialised in Pharmacology at "Mario Negri" Institute for Pharmacological Research of Milan. From 2004 to 2005, she was researcher at Prassisi, Institute for Research sigma-tau, and from 2005 to 2013 research scientist at the "Mario Negri" Institute for Pharmacological Research, Department of Oncology, Laboratory of Cancer Pharmacology. Since January 2013 she is head of the Preclinical Experimental Therapeutics Unit.

Main research activities: preclinical antitumor activity studies; development of experimental models of soft tissue sarcomas and mesothelioma to evaluate new compounds and combinations. Pharmacokinetics studies in animal models.

Selected publications
Lital Hollander holds a Bachelor’s Degree in Medical Sciences from the School of the Medicine of the Hebrew University in Jerusalem, a post-graduate certificate in Clinical Research from the UniversitàStatale di Milano, and a Master’s Degree in Public Health from the University of Liverpool. Since 1994 she has conducted research programs on HIV infection and reproductive health in people living with HIV in collaboration with Italian, European and US (CDC) Institutions. In parallel she has been involved in Health Promotion, Advocacy and Health Policy initiatives aiming to improve the involvement of people living with HIV in all areas concerning their health, rights and well being. She has collaborated with the Department of Oncology since 2007. As of 2013 she has become Head of Unit for Research Design and Planning. Her fields of interest include:

1. Research methodology and its application to the design of clinical research and experimental instruments
2. Exposure and outcome measures in oncology and their application in vulnerable populations
3. Use of evidence base in regulatory and health policy areas
4. Management and reform of complex adaptive systems with particular attention to academic and health organizations.

Selected publications


Marlen Victoria Llerena Mesa got her degree in Pharmaceutic Science at the University of Havana (Cuba) in 1993. In 2003 she got the Lead Auditor Certificate according to ISO 9000-2000 standard at the Institute for Standardization Research, Havana, Cuba. In 2005 and 2006 she got the title of Master in Pharmacologic Science and in Clinical Trials, respectively. Since April 2012 she has been head of the Quality Assurance Unit. Main areas of interest are the control and improvement of the quality assurance system, the approval of standard operative procedures (SOPs) and development of a documentation system meant to guarantee the traceability of all the activities in accord to the Norme of Good Clinical Practices (GCP) and legal directives.

Selected publications

Mirko Marabese got his Biological Sciences degree at the University of Milan in 2001 attaining the Italian Government Qualification to practice as Biologist in 2002. He obtained the specialization in Pharmacological Research from the Mario Negri Institute for Pharmacological Research in 2005. In the same year he was awarded the degree of Doctor of Philosophy (PhD), Discipline of Life Sciences of the Open University Research School (UK). From 2001, he was a Fellow Research Scientist at the Mario Negri Institute for Pharmacological Research, Laboratory of Molecular Pharmacology and he became a staff research scientist in 2008. From 2003 to 2004 he was a Visiting Fellow at Apoptosis & Cancer Laboratory at Medical Research Council (MRC) Toxicology Unit of Leicester (UK). Since 2011 he was Head of the Molecular Genetics Unit in the Oncology Department at Mario Negri Institute for Pharmacological Research.

The research activities of the Molecular Genetics Unit are focused on the characterization from a molecular point of view the tumors and to understand the role of the gene alterations in relationship with the therapy response. Thanks to the strong collaboration with clinician in hospitals, the recent activities are focused on non small cell lung cancer. In particular the aim of the research is to define a strategy to bypass the chemotherapy resistance of KRAS mutated tumors that account for 25% of all lung tumors. At the same time, the research team is also focused on the acquired resistance after chemotherapy of the small cell lung cancer.

Selected publications


Sergio Marchini was graduated summa cum laude, in Biological Science, University of Milan in 1993, attaining the Italian Government Qualification to practice as Biologist in 1996. He obtained the specialization in Pharmacological Research from the Department of Education of the Regional Government of Lombardia in 1997 and the in 2000 he was awarded in advanced studies in Pharmacology, University of Pavia, Italy. In 2003 he got the Ph. D. degree at the Open University, London UK. Professional Positions: 2001-up to now: permanent position as a researcher at the "Mario Negri" Institute for Pharmacological research. Since 2011 he was appointed Head of of Translational Genomic Unit, Laboratory of Cancer Chemotherapy. In 2001, he was visiting scientist at MGH, Boston, MA, US and 1998 he was visiting scientist at the Birmingham University (U.K.), Department of Medical Genetic. Honour and Awards: 2001: First rank in the prize "ONLUS-AICC 2001" for young Italian scientists. 1995: First rank in the prize "MIGLIORI POSTER S.I.C." XIII Riunione Nazionale di Oncologia Sperimentale e Clinica (Verona, 15-18 ottobre 1995). Research activities: translational research activities are mainly focused on ovarian cancer tumors as well as on mixoid liposarcomas. By exploiting "-omic" approaches on different cohort of tumor biopsies, the research activities of the Translational Genomic Unit are focused on defying the strong resistance of KRAS mutated tumors and then integrate the transcriptional (miRNA, and gene expression) and mutational landscape (target resequencing) of ovarian cancer and mixoid liposarcomas tumors to identify molecular determinant with prognostic and diagnostic value.
Selected publications


Davide Poli got his master’s degree in Physics at the University of Milan in 2007 and his specialization in “Biochemical Research Technician” at the Mario Negri Institute for Pharmacological Research in 2004. Since November 2012 is a Head of Coordination, Management and Monitoring in the Laboratory of Clinical Research.

His areas of interest are: design of eCRF in Clinical Trials, new electronic aspects of Clinical Research especially towards technologies of Web-based Electronic Data Capture, methodology and data management aspects in Clinical Research.

Selected publications

- Ocular Hypertension Treatment Study Group, European Glaucoma Prevention Study Group (EGPS), Poli D. The accuracy and clinical application of predictive models for primary open-angle glaucoma in ocular hypertensive individuals. Ophthalmology, Volume 115, Number 11 pp. 2030-2036, November 2008


- The European Glaucoma Prevention Study (EGPS) Group, Poli D. Results of the European Glaucoma Prevention Study. Ophthalmology, Volume 112, Number 3, pp. 366-375, March 2005

Luca Porcu obtained his degree as “Biochemical Research Technician” from the Mario Negri Institute for Pharmacological Research in 2005. From 2001 to 2007 he has been employed as Coordinator and Data Manager of Clinical Trials in the Clinical Epidemiology Laboratory; from 2007 to 2009 he has been employed as Contract Research Associate in charge for the auditing of Clinical Trials; from 2007 up to now he is employed for data analysis, meta-analysis, statistical computing in Biomedical Research in the Laboratory of Methodology for Biomedical Research.

His scientific focus is the methodology of Biomedical Research, in particular the probabilistic models implemented in the oncological setting (e.g.: probabilistic models fitting Survival-Post-Progression endpoint), the statistical methodology for rare tumors, pros and cons of Progression-Free-Survival as a primary efficacy endpoint.
Selected Publications

- Rossi A, Torri V, Garassino MC, Porcu L, Galetta D. The impact of personalized medicine on survival: Comparisons of results in metastatic breast, colorectal and non-small-cell lung cancers Cancer Treat Rev. 2013 Sep 25
- Romano M, Frapolli R, Zangarini M, Bello E, Porcu L, Galmarini CM, Garcia-Fernández LF, Cuevas C, Allavena P, Erba E, D’Incalci M. Comparison of in vitro and in vivo biological effects of trabectedin, lurbinectedin (PM01183) and Zalypsis® (PM00104) Int J Cancer. 2013 Apr 16

Eliana Rulli got her master’s degree in Biostatistics and Experimental Statistic in 2007, her degree in Statistical Science in 2004 at the University of Milano-Bicocca and her specialization in "Specialist in Pharmacological Research " at Mario Negri Institute in 2007. She has been working at institute Mario Negri since 2003, at this time she is in charge of Statistic unit at the Laboratory of Clinical Trials. She is member of the Ethics Committee of the association "La Nostra Famiglia" - IRCCS "E. Medea". Areas of interest: methodology and statistical aspects of clinical research, systematic reviews and quality assessment of medical literature

Selected publications


Giulia Taraboletti got her degree cum laude in Biological Sciences at the University of Pavia (Pavia, Italy) in 1983, and the specialization in Pharmacological Research at the Mario Negri Institute, Milano, Italy in 1986. From 1986 to 1988 she was a post-doctoral fellow at the Laboratory of Pathology, NCI, NIH, Bethesda, MD, and from 1988-1995 research scientist at Mario Negri Institute in Bergamo, Italy. Since 1995 she is Head of the Unit of Tumor Angiogenesis, at Mario Negri Institute, in Bergamo. Research interests include tumor angiogenesis, endogenous inhibitors of angiogenesis (thrombospondin-1) and preclinical studies of antiangiogenic and vascular disrupting compounds, including tubulin-targeting agents. She is member of Metatasis Research Society (MRS, board of directors 2006-2008), American Association for Cancer Research (AACR), European Association for Cancer Research (EACR), and the Italian Society of Oncology (SIC, elected in the board of directors 2013). She is on the editorial board of European Journal of Cancer, TheScientificWorldJournal, and Current Cancer Therapy Reviews.
Selected publications


Paolo Ubezio got his B.Sc. degree in Physics at the University of Milan, in 1982, and the specialisation in Pharmacological Research Specialist at the Mario Negri Institute for Pharmacological Research in 1986.

Main activities are: i) Computer simulation of tumor proliferation during/after treatments using models based on the cell cycle; ii) Development of new methods and data analysis tools in flow cytometry and in time-lapse imaging of living cells; iii) Optimization of anticancer drug scheduling; iv) Cellular uptake of nanoparticles loaded with anticancer drugs.

Since 1991 is Head of the Unit of Biophysics at the Mario Negri Institute

Selected publications

- Ubezio P and Cameron D. Cell killing and resistance in pre-operative breast cancer chemotherapy. BMC Cancer (2008) 8:201

Massimo Zucchetti obtained his Chem. Pharm. Degree from the University of Milan in 1982. After specializing in Pharmacology at the Mario Negri Institute of Milan (1988), he worked in the Laboratory of Clinical Pharmacology of Department of Oncology at San Giovanni Hospital, Bellinzona, Switzerland (1988-1990). Since 1996 he has been Chief of the Cancer Clinical Pharmacology Unit at the Mario Negri Institute. He is member of the Pharmacology and Molecular Mechanisms Group of the European Organization for Research and Treatment of Cancer (EORTC) from 1988 up to date. His main field of interest are:

- Clinical pharmacology, phase I and Phase II studies
- Analysis of drugs, development of new analytical method by HPLC, HPLC-MS/MS, MALDI Imaging mass spectrometry
- Pharmacokinetic and pharmacodynamic studies in humans in GCP and GLP conditions
- Pharmacokinetic, toxicokinetic and metabolic studies in animals
- Pharmacokinetic drug interaction

Dr Zucchetti is author of more than 100 papers on pre-clinical and clinical cancer chemotherapy published in peer reviewed international journals.
Selected publications


ACTIVITIES

The Oncology Department comprises four preclinical experimental laboratories (Laboratory of Cancer Pharmacology, Laboratory of Molecular Pharmacology, Laboratory of Biology and Treatment of Metastases and Laboratory of Cancer Cachexia AIRC Start-Up) and two laboratories dealing with clinical research and clinical trials (Laboratory of Methodology of Biomedical Research and Laboratory of Clinical Research). The Oncology department hosts the coordination center of two networks of hospitals that carry on clinical research in gynecologic cancer (MaNGO: Mario Negri Gynecologic Oncology) and in cancer pain (CPOR-SG: Cancer Pain Outcome Research Study Group) and a center for cancer pain assessment and research (CERP: Center for the Evaluation and Research on Pain). In some cases research projects are carried out by single laboratories or research units, in other cases by collaborations between different laboratories of the Oncology Department or other departments, or other groups outside the Institute (see National and International Collaborations).

Preclinical laboratories focus on the discovery and development of new antitumor and antimetastatic drugs and their new combinations; on tumor biology, not only to acquire new scientific knowledge, but particularly as a base for more selective therapeutic approaches and to identify and evaluate experimental models for discovering and studying new drugs or treatments.

Clinical new drug development has been developed in collaboration with many oncological clinical centres and is based on the preclinical evidences obtained by the Laboratory of Cancer Pharmacology, the Laboratory of Molecular Pharmacology and the Laboratory of Biology and Treatment of Metastases. The laboratory of Methodology of Biomedical Research and the Laboratory of Clinical Research are involved in the evaluation of the effects of new therapeutic modalities in phase I/II and in phase III comparative and effectiveness outcome studies. Outcome Research implies organizing trials to clarify the results of certain health care practices and interventions in clinical practice. Observational (surveys) and outcome research (effectiveness) studies are carried out, in collaboration with regional and national health authorities and other scientific associations.

At the preclinical and clinical level there are studies of various human tumors, with particular emphasis on ovarian tumors and more recently on soft tissue sarcomas.
 MAIN FINDINGS

At nanomolar concentrations, trabectedin affects the regulatory mechanisms of the transcription. Cells that are deficient in Homologous Recombination DNA Repair -e.g. with mutations of BRCA1 or BRCA2 genes- are hypersensitive to the drug Nucleotide excision repair deficient cells that are hypersensitive to UV rays and to other DNA damaging drugs are resistant to trabectedin.

Exploiting a Mixoid liposarcoma cell lines resistant to trabectedin, we used an integrated approach based on miRNA-genes and proteins expression to shape the molecular pathways involved in trabectedin resistance.

The selective activity of trabectedin against human myxoid liposarcoma appears related to the drug ability to modulate the transcription of genes involved in adipocytic differentiation.

Trabectedin modulates the transcription of genes involved in pro-inflammatory mechanisms that are potentially relevant for tumor growth and progression and inhibits the production of cytokines and chemokines by macrophages that are tumor associated.

New sarcoma experimental models have been obtained. They will be useful to investigate new drugs for these diseases.

Use of mathematical models of tumor growth and anticancer treatment to interpret experimental data and to manage the complexity of underlying biological phenomena.

A new method enabling to perform dynamical measures of cell cycle checkpoint activities in response to anticancer treatments. Gene profiling analysis shows specific molecular signatures according to the histotype and prognosis of stage I ovarian carcinoma. Analysis of miRNA expression profile in a cohort of stage I patients gathered together from two independent tumor tissue collection revealed miR-200c as an independent prognostic factor of relapse and overall survival.

Zic2, a transcription factor involved in embryogenesis, was found upregulated in biopsies taken from epithelial ovarian cancer compared to its expression in borderline biopsies. Within stage I ZIC2 expression levels were associated with poor prognosis.

Patients with ovarian cancer have a different expression of genes involved in DNA repair that is dependent on the tumor stage and pharmacological response to treatment.

Through the screening of a siRNA library a gene (wee1) synthetically lethal with CHK1 has been identified. The simultaneous inhibition of CHK1 and wee1 strongly affects the in vitro growth of several cancer cell lines but not that of normal cells. These data are of potential interest.

The use of combinations of PI3K/akt/mTOR inhibitors acting at different sites of the same target, induces a pronounced antitumor effect. Mechanistically there is a selective inhibition of the translation of proteins involved in the cellular growth.

Mutations in the K-RAS gene have a different impact on the response to treatment that is dependent from the type of aminoacid substitution present at codon 12.
A new oncosuppressor gene, named DRAGO, cooperating with p53 in the control of tumor growth has been characterized.

A randomized, controlled clinical trial, in which more than 500 patients with non small cell lung cancer have been genotyped for the presence of mutations in the EGFR and K-RAS genes, has shown that patients in second line with a wild type EGFR have a better response to chemotherapy (docetaxel) compared to EGFR inhibitors (erlotinib).

The growth of breast cancer cells in the bones is slowed down by selective c-met inhibitors.

A population of potential stem cell origin has been characterised from ovarian cancer patients. These cells represent a unique tool to study new potential anticancer agents affecting these cells considered the most resistant cancer cells.

The vascular endothelial growth factor (VEGF) released by cancer cells modifies gene expression of the tumor microenvironment and response to treatment. In particular, the Regulator of G-protein signaling 5 (RGS5) was highly expressed by the stroma of VEGF rich tumors and its protein was selectively demonstrated in the vasculature of ovarian carcinoma specimens.

Genes preferentially expressed by vascular endothelial cells isolated from human cancer specimens were identified. We have discovered that PRSS3/TrypsinogenIV is induced by a pro-angiogenic environment and plays a role in tumor-endothelial cell motility mediated by such an environment.

A new antiangiogenic domain of thrombospondin (a physiological inhibitor of angiogenesis) that binds the angiogenic factor FGF-2 has been identified and characterized. Preliminary data indicate that this domain inhibits tumor angiogenesis and growth, and promotes tumor response to chemotherapy. Non-peptidic small molecules, mimetic of this domain, have been identified and are studied as potential inhibitors of angiogenesis.

Vascular Endothelial Growth Factor C (VEGFC, the main mediator in lymphoangiogenesis) promotes ovarian carcinoma progression through paracrine and autocrine mechanisms. Selective inhibitors of VEGF/VEGFRs pathway inhibit ovarian tumor growth and invasion.

A biobank of patient-derived ovarian cancer xenografts, which reflects the clinico-pathological-molecular features of this disease has been established (EOC-Xenografts); this is instrumental to study the biology and develop novel treatment modalities for ovarian cancer.

The response of ovarian cancer (EOC-Xenografts) to bevacizumab (an antibody anti-VEGF used for the treatment of ovarian cancer) is heterogeneous: bevacizumab added to the standard-of-care chemotherapy reduced tumor progression and improve survival.

The addition of chemotherapy counteracts metastasis augmentation caused by VEGF/VEGFR inhibitors in preclinical tumor models, thus highlighting the importance of ad hoc combination to limit unwanted effects and optimize combination therapy.

Preclinical studies have shown that bevacizumab combined with chemotherapy not only affects ovarian carcinoma progression, but when administered as maintenance regimen significantly prolonged mouse survival, reducing ascites and tumor dissemination.
The addition of chemotherapy counteracts metastasis augmentation caused by VEGF/VEGFR inhibitors in preclinical tumor models, thus highlighting the importance of testing ad hoc combination to abrogate unwanted effects.

Two International randomized phase III studies (AGO-OVAR 12 and 16 studies), with the participation of the MaNGO group coordinated by the Institute, have evaluated whether two different oral inhibitors of angiogenesis (respectively vargafet and pazopanib) added to the usual chemotherapy in patients with ovarian carcinoma would have guaranteed some clinical advantage. The two studies, that enrolled more than 2000 patients, demonstrated that both molecules determine a statistically significative advantage in terms of disease free survival when added to standard first line chemotherapy.

An Italian randomized phase III trial has assessed the role of systematic aortic and pelvic lymphadenectomy (SAPL) at second-look surgery in early stage or optimally debulked advanced ovarian cancer. This trial enrolled 308 patients and showed that the median operating time, blood loss, percentage of patients requiring blood transfusions and hospital stay were higher in the SAPL than in the control arm. In spite of this higher toxicity the SAPL did not improve either disease free survival of overall survival.

Results from a systematic review of literature and from a prospective epidemiologic study suggest that an important proportion of patients with cancer pain (up to 43%) receive an analgesic treatments that is not appropriate with the intensity of pain.

Results from a survey carried out on a national level on a sample of 1801 patients with cancer pain confirm that in Italy a relevant part of cancer patients does not receive an appropriate information about their prognosis: physicians reported that according to their knowledge only 31% received information about their prognosis. An independent survey carried out in a Northern Italian Region confirmed this finding: among 550 patients treated at home for cancer pain with palliative care, only 58% were classified to be fully aware of their prognosis.

An observational longitudinal study carried out in 110 Italian centers and involving about 1800 patients with metastatic cancer and pain have documented that that in terms of analgesics effectiveness, that each drugs prescribed by investigators (morphine, fentanyl, buprenorphine and oxycodone) were able to reduce the intensity of pain of about 2 points on a 11-eleven point numerical rating scale (p<0.001). The application of specific pe-planned algorithm identified about 30% cases who were classified as non-responders. Preliminary analyses documented some differences between drugs in terms of size of the analgesic effect, dosages required and side effects reported.

Furthermore it has been possible to report as the different opioid analgesics drugs have been able to ensure a substantially equi-analgesia but a different behavior in terms of other outcome and endpoints (as dose variations over time, use of switch, use of adjuvants co-treatments).

NATIONAL COLLABORATIONS

Age.Na.S. Agenzia Nazionale per i Servizi Sanitari Regionali, Roma
Agenzia Sanitaria Regionale (ASR), Bologna
Agenzia Italiana del Farmaco (AIFA), Roma
Alleanza Contro il Tumore Ovarico (ACTO), Milano
Associazione Italiana per lo Studio del Glaucoma (AISG), Torino
Azienda Sanitaria Locale, Rimini
Azienda Sanitaria Locale, Vercelli
Assessorato Sanità, Regione Emilia Romagna
Associazione Italiana di Oncologia Medica (AIOM)
Associazione Italiana di Ematologia Pediatrica (AIEOP)
Associazione Otorinolaringologoi Ospedalieri Italiani (AOOI), Roma
Associazione Volontari Assistenza Pazienti Oncologici (AVAPO)
Azienda Sanitaria Unica Regionale, Regione Marche
Azienda Ospedaliera di Reggio Emilia Arcispedale S. Maria Nuova
Azienda Ospedaliera San Gerardo, Università Milano-Bicocca, Monza
Azienda Ospedaliera “Guido Salvini”, Ospedale “di circolo” Rho, (MI)
Casa Sollievo della Sofferenza, San Giovanni Rotondo (IRCCS)
CNR IGBE, Pavia
Cochrane Collaboration
Fondazione Attilia Pofferi, Pistoia
Fondazione Centro San Raffaele del Monte Tabor, Milano
Fondazione Edmund Mach, Trento
Fondazione Filarete per le Bioscienze e l’Innovazione
Fondazione GISCAD (Gruppo Italiano per lo Studio dei Carcinomi dell’Apparato Digerente)
Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Milano
Fondazione IRCCS Istituto Neurologico, C. Besta, Milano
Fondazione Istituto FIRC di Oncologia Molecolare (IFOM), Milano
Fondazione Nerina e Mario Mattioli Onlus, Milano
Fondazione Piemontese Ricerca sul Cancro IRCCS, Candiolo
Fondazione Salvatore Maugeri, Pavia
Fondazione Edo ed Elvo Tempia, Cancer Genomic Lab, Biella
Gruppo Italiano Sarcomi
Istituti Ospitalieri di Cremona
Istituto Clinico Humanitas, Rozzano MI
Istituto Ortopedico Galeazzi, Milano
Istituti Ortopedici Rizzoli, Bologna
Istituto Europeo di Oncologia (IEO), Milano
Istituto di Fisica, Politecnico di Milano
Istituto di Genetica Molecolare CNR, Sezione di Istochemica e Citometria, Pavia
Istituto Nazionale Tumori Fondazione G. Pascale, Napoli
Istituto Regina Elena, Roma
Istituto Superiore di Sanità
Istituto Toscano Tumori, Firenze
Laboratorio Cell factory, Policlinico di Milano
LNCIB- Area Science Park & Dipartimento Scienze della Vita, Università di Trieste
Nerviano Medical Sciences Oncology
Ospedale Fatebenefratelli e Oftalmico, Milano
Ospedale San Matteo, Pavia
Ospedale Papa Giovanni XXIII, Bergamo
Politecnico di Milano
Rete Oncologica Lombarda (ROL), Milano
Spedali Civili di Brescia
Università Cattolica del Sacro Cuore, Roma
Università di Brescia
Università di Catania
Università degli Studi di Ferrara
INTERNATIONAL COLLABORATIONS

ARCAGY (Association de Recherche sur les Cancers Gynécologiques), France
AstraZeneca Ltd, UK
Barts and The London School of Medicine & Dentistry, Londra, UK
Breakthrough Breast Cancer Center, Institute of Cancer Research, Londra, UK
Cancer Biomarkers and Prevention Group, University of Leicester, UK
Cancer Research UK, Londra, UK
Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, USA
Cochrane Consumer network (via The Cochrane Collaboration), UK
EORTC, Bruxelles, Belgium
ETH Zurich, Institute of Chemical and Bioengineering
European Association for Palliative Care – research network (EAPC rn)
European Network of Gynaecological Oncology Trials groups (ENGOT)Eusoma – (European Society of European Palliative Care Research Network (PRC), Trondheim, Norway
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Breast Cancer Specialist Firenze, Italy
Executive Board of GCIG (Gynecologic Cancer Intergroup)
German Network of the Coordinating Centres for Clinical Trials U Koeln, Germany
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Gynecologic Cancer Intergroup (GCIG)
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Institute of Pathology, Friedrich Schiller University, Jena, Germany
Institut Villejuif, Paris, France
Istituto Oncologico della Svizzera Italiana, Switzerland
Johns Hopkins University, USA
Klinik und Poliklinik für Kinder- und Jugendmedizin, Muenster, Germany
Ludwig Institute for Cancer Research, Londra, UK
National Cancer Center, Singapore
Stichting VU-VUmc, The Netherlands
Swiss Federal Institute of Technology, Zurigo, Switzerland
Massachusetts General Hospital and Harvard Medical School, USA
MD Anderson Cancer Center, Houston, Texas, USA
Memorial Sloan Kettering, New York, USA
Metropolitan Hospital (MH), 1st Oncology Dept., AthensMRC, Londra, UK
National Cancer Institute (NCI), Bethesda and Frederick, MD, USA
Ospedale San Giovanni, Bellinzona, Switzerland
Paterson Institute for Cancer Research, Manchester, UK
SAKK (Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung)
EDITORIAL BOARD MEMBERSHIP

American Journal of Cancer Research (Maurizio D’Incalci, Massimo Broggini, Giovanna Damia)
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European Journal of Cancer (Massimo Broggini e Giulia Taraboletti)
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Frontiers in Pharmacotherapy of Neoplastic Diseases (Maurizio D’Incalci)
Journal of B.U.ON. (Maurizio D’Incalci)
Journal of Chemotherapy (Raffaella Giavazzi, Maurizio D’Incalci)
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Journal of Investigational New Drugs (Maurizio D’Incalci)
Molecular Cancer Therapeutics (Maurizio D’Incalci)
Oncology Research (Maurizio D’Incalci)
Open Cancer Journal (Maurizio D’Incalci)
The International Journal of Biological Markers (Raffaella Giavazzi, Valter Torri)
The Journal of Cancer Microenvironment (Raffaella Giavazzi)
TheScientificWorldJournal, (Maurizio D’Incalci, Giulia Taraboletti)
Tumori (Maurizio D’Incalci)
www.fondazionemattioli.it (Maurizio D’Incalci)
World Journal of Methodology (Irene Floriani)

PEER REVIEW ACTIVITIES

NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP

AACR-Women in Cancer Research Charlotte Friend Memorial Lectureship Award Committee, USA
Comitato Etico dell’associazione "La Nostra Famiglia" - IRCCS "E. Medea"
Comitato Etico Centro di Riferimento Oncologico, Aviano, PN
Comitato Etico Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Milano
Comitato Etico Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano
Comitato Etico Fondazione del Piemonte per l’Oncologia - IRCCS, Candiolo
Comitato Etico Istituto Clinico Humanitas, Rozzano, MI
Comitato Etico Ospedale Manzoni, Lecco
Comitato Scientifico, Associazione Italiana Ematologia e Oncologia Pediatrica, Monza, MI
Comitato Scientifico, Fondazione ABO (Application of Biotechnologies in Oncology)
Comitato Scientifico, Fondazione Buzzi Unicem Onlus
Comitato Strategico e di Studio per la Leucemia Linfoblastica Acuta (CSS - LLA)
Comitato Tecnico-Scientifico, Alleanza Contro il Tumorare Ovarico (ACTO), Milano
Comitato Tecnico-Scientifico, Associazione Italiana per la Ricerca sul Cancro, Milano
Comitato Tecnico-Scientifico, Fondazione Regionale Ricerca Biomedica, Milano
Comitato Tecnico-Scientifico, Fondazione Andrea e Libi Lorini, Milano
Comitato Scientifico, Fondazione Pezcoller, Trento
Comitato Tecnico-Scientifico, Mario Negri Gynecologic Oncology Group (MaNGO)
Consiglio di Amministrazione, Università di Trento
Consiglio Direttivo, Società Italiana di Cancerologia (SIC)
Consiglio Direttivo, Società Italiana di Citometria (GIC)
Consiglio Direttivo Fondazione Nerina e Mario Mattioli Onlus
Fondazione Attilia Pofferi, Pistoia
Developmental Therapeutics Program, National Cancer Institute (NCI)
Pezcoller Foundation-EACR Award

EVENT ORGANIZATION

Meeting with scientists “Studio clinico multicentrico, osservazionale sulla qualita’di vita del paziente glaucomatoso in Italia”. XXVIII Riunione AISG, Turin, March 7, 2013

Course: Moduli 3 (terapia del dolore) e 4 (trattamento dei sintomi). 13° Master di I° livello in cure palliative e terapia del dolore in collaborazione con l’Università degli Studi di Milano, Milan, April 8-10, 15-17, 22-23, 2013.


Meeting “La ricerca clinica in ginecologia oncologica”. 10° Assemblea MaNGO, Milan, June 21-22, 2013

Course: Corso di formazione sulla Terapia del dolore per gli specializzandi del 4° e 5° anno delle Scuole di specialità in medicina Interna e Geriatria dell’Università degli Studi di Milano, Milan, November 13, 20, 27 and December 11, 2013
CONFERENCE AND WORKSHOP CONTRIBUTIONS

Course: Giornata di aggiornamento sulla terapia dei Tumori Mammari, Back from San Antonio, Genova, January 11-12, 2013
“Building the Guide Lines”

“Round Table: New acquisitions from experimental studies”.
“Novel preclinical models for development of antiangiogenic agents”.

Meeting: Giornata sulle linee guida AIOM, Milan, February, 4-5, 2013

“Lettura magistrale: 5 anni di trabectedina: cosa abbiamo imparato”
“Tavola rotonda: Ottimizzazione del trattamento della malattia metastatica dei sarcomi dei tessuti molli”

Course “CANOA”: aggiornamento sulla terapia del carcinoma mammario, Negrar March 21-22, 2013

“MoA: chemioterapia multi target”

TMEO-Tumore Maligno Epiteliale dell’Ovaco: dai trattamenti consolidati all’ innovazione.
Turin, April 4, 2013
“Update Trials Clinici”

Confereence: Società Italiana di Farmacologia “Farmaci a brevetto scaduto: i problemi irrisolti e le soluzioni proposte”, Milan, April 5, 2013
“Revisione critica: gli studi clinici per la validazione dei biosimilari”

Meeting: AACR Annual Meeting, Washington (USA), April 05-10, 2013.
"Heterogeneous response to antiangiogenic therapy in ovarian cancer xenografts: a tool to investigate biomarkers of resistance”.
“Preclinical evaluation of ET-743 (trabectedin, Yondelis) in association with anti-IGF-1R therapies in Ewing sarcoma”

Course: Oncologia Hi-Tech nella ricerca e cura del carcinoma mammario. Pavia, April 12, 2013.
“Le nanotecnologie per nuovi approcci farmacologici”

Symposium: 5th Freiburg Symposium on Anticancer Drug Discovery. Freiburg (Germany), April 24-27, 2013.
"Targeting Angiogenesis in Ovarian Cancer: preclinical investigation”.

Course: AIOM sulla metodologia delgli studi clinici. Verona, May 3-4; Milan, May 10-11, 2013

Workshop: Workshop SIICA -Angiogenesi: basi molecolari ed implicazioni terapeutiche IV.
"Chemosensitizing effect of the thrombospondin-1 type III domain in ovarian carcinoma".
“Exploiting endogenous inhibitors of angiogenesis for antineoplastic therapy: the example of thrombospondin-1”

“Lettura magistrale: targeted chemotherapy o terapie biologiche: esiste una differenza?”

“Metodiche di misurazione dei livelli plasmatici di methotrexate: quali e quando utilizzarle?”

“AnPaldi protocol for anticancer drugs tumor uptake and distribution studies”.

“Recenti tendenze nella metodologia della ricerca clinica”

"Vascular endothelial growth factor C promotes ovarian carcinoma progression through paracrine and autocrine mechanisms".

Meeting: 2013 Summer FASEB Meeting "Matricellular proteins in development, health, and disease". Vermont Academy, Saxons River (USA), July 28-August 2, 2013.
"The TSP1 type III repeats in tumorigenesis and angiogenesis"

Meeting: Summer School on Endocrinology in Bregenz (Austria) July 29-August 1, 2013.
Invited lecture: “Mechanisms of muscle growth and atrophy/sarcopenia in mammals and Drosophila”. Interactive course: “How to build up a muscle: A tug of war between protein synthesis and degradation”.

"PRSS3/trypsinogen IV, a new player and target in tumor angiogenesis and vascular remodeling".

“Intra-tumour heterogeneity and the potential impact on drug sensitivity”.

“The p97 / VCP ATPase complex is critical in muscle atrophy and for the accelerated degradation of most muscle proteins”.

“MiRNA signature characterization in round cell myxoid liposarcomas treated with trabectedin”.
“Targeted resequencing approach to dissect the mutational spectrum associated to platinum resistance in EOC”

“MicroRNA profiling in metastatic colorectal primary tumor and STROMA”.
V BIAS Annual Congress. Milan, October 24-25, 2013
“Risk Based Monitoring & Statistical and Data Issues in Regulatory Submission: Risk Based Monitoring in Clinical Trials: a non-profit organization approach”

“The (EPOC) FP7-funded study of pharmacokinetics and pharmacodynamic of doxorubicin in children with cancer”.

“Collateral sensitivity to cisplatin of trabectedin-resistant cell lines”.
“Fsn0503h antibody-mediated blockade of cathepsin S as potential therapeutic strategy for the treatment of solid tumours”.

“MiRNA signature characterization in round cell myxoid liposarcomas treated with trabectedin”.

"Targeting angiogenesis in ovarian cancer: preclinical investigation”.

Meeting: CEUS e Linee Guida: PRO e CONTRO. Verona, November 9, 2013
“Sessione Fegato: Metanalisi e Revisione della Letteratura”.

“Trabectedin effects on transcription regulation and on tumor microenvironment”.

“The p97 / VCP ATPase complex is critical in muscle atrophy and for the accelerated degradation of most muscle proteins”

Workshop: European Society of Medical Oncology, Mian, December 8-11, 2013

“Una parola per il 2014, Stupore”.

**GRANTS AND CONTRACTS**

Actavis Italy SpA
Agenzia Italiana del Farmaco
Arcispedale Santa Maria Nuova di Reggio-Emilia
Agenzia Italiana del Farmaco
AIRC Associazione Italiana per la Ricerca sul Cancro
ArQule USA
Astra Zeneca SpA
AVAPO (Associazione Volontari Assistenza Pazienti Oncologici)
Azienda Ospedaliera Fatebenefratelli e Oftalmico- Milano
Eli Lilly Italia SpA
EOS SpA
FIRC Fondazione Italiana per la Ricerca sul Cancro
Fondazione Buzzi Unicem
Fondazione Cassa di Risparmio delle Province Lombarde
Fondazione Centro San Raffaele del Monte Tabor
Fondazione Nerina e Mario Mattioli Onlus
GISCAD (Gruppo Italiano Studi di Carcinomi Apparato Digerente)
Grunenthal Italia, Milano
Indena SpA
Istituto Nazionale dei Tumori, Milano
Istituto Regina Elena
Italfarmaco SpA
Marie Curie International Reintegration Grant
Medac
Merck Sharp & Dome
Ministero della Salute
Novartis
Novartis Farma SpA
Oncoethix
O.T.D. – Oncology Therapeutic Development s.a.r.l.
Pfizer Global Research and Development
Pharma Mar, SA
Regione Emilia Romagna
Regione Lombardia
Roche SpA
SAKK (Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung)
Sara Bet, Roma
Servier
SIA SpA
Sigma-Tau SpA
Unione Europea
University of Ulm
Università di Torino
Università Federico II – Napoli (Dipartimento di Endocrinologia ed Oncologia molecolare e clinica)

SCIENTIFIC PUBLICATIONS (2013)

Modeling of non-small cell lung cancer volume changes during CT-based image guided radiotherapy: patterns observed and clinical implications
Comput Math Methods Med 2013 2013 : 637181

Role of macrophage targeting in the antitumor activity of trabectedin
Cancer Cell 2013 23 : 249-262

Hoffmann E M, Miglior S, Zeyen T, Torri V, Rulli E, Aliyeva S, Floriani I, Cunha-Vaz J, Pfeiffer N
The Heidelberg retina tomograph ancillary study to the European glaucoma prevention study: study design and baseline factors
Acta Ophthalmol 2013 91 : e612-e619

A first in human phase I study of the proteasome inhibitor CEP-18770 in patients with advanced solid tumours and multiple myeloma
Eur J Cancer 2013 49 : 290-296

D'Angelo D, Borbone E, Palmieri D, Uboldi S, Esposito F, Frapolli R, Pacelli R, D'Incalci M, Fusco A
The impairment of the High Mobility Group A (HMGA) protein function contributes to the anticancer activity of trabectedin
Eur J Cancer 2013 49 : 1142-1151

Resistance to platinum-based chemotherapy is associated with epithelial to mesenchymal transition in epithelial ovarian cancer
Eur J Cancer 2013 49 : 520-530

Triple negative breast cancer have a reduced expression of DNA repair genes
PLoS One 2013 8 : e66243

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RESEARCH ACTIVITIES

Laboratory of Cancer Pharmacology

Mode of action of Ecteinascidins  
A project ongoing since several years is about the characterization of marine natural products possessing antitumor activity. In particular we carried on the studies on the effects of ET-743 in cells defective for some DNA repair mechanisms. Cells deficient for Homologous Recombination (HR) are very sensitive to the drug, while cells deficient for Non Homologous End-Joining (NHEJ) are only slightly more sensitive, but surprisingly cell lines defective for Nucleotide Excision Repair (NER) are less sensitive to ET-743. Flow cytometric analysis coupled to a software of computer simulation, developed in our laboratory, has demonstrated that NER defective cells showed, after ET-743 treatment, cell cycle perturbations different than those occurring in NER proficient cells, probably for the activation of different and more efficient repair mechanisms.
We study also a functional evaluation of the DNA repair mechanisms by the cell capacity to recognize and repair double helix breaks with a recently introduced test that is very sensitive to detect the phosphorylation of histone H2AX. An in vitro study is ongoing with flow cytometry and immunofluorescence techniques to evaluate in different tumor cell lines the phosphorylation level of histone H2AX in relation to the distribution of the cells in the different phases of the cell cycle and the cytotoxic effect induced after treatment with ET-743.

Studies are in progress on the mechanism of action of new ET-743 derivates compounds that have shown antitumoral activity on cell lines with different DNA repair mechanisms.

A new project is the study of the selective action of ET-743 on mixoid liposarcoma, a pathology representing 10% of all soft tissue sarcomas, trying to understand if the significative antitumor effect is due to a selective action of the compound on pathogenetic alterations characteristic of this pathology. In particular we are trying to evaluate how ET-743 interact with the transcriptional modifications of specific genes due to the translocation FUS-CHOP that characterizes mixoid sarcomas or those caused by the interaction host-tumor, modifying inflammatory and angiogenetic processes. A panel of myxoid liposarcoma xenografts able to reproduce the histological and molecular characteristics most frequently observed in the clinic was obtained directly from patient’s biopsies. These models have allowed us to demonstrate in vivo the mechanism responsible for the selective action of trabectedin in this particular tumor histology. In fact, the drug causes detachment of the chimeric protein FUS/CHOP from the promoters of its target genes, causing a functional inactivation that leads to the reactivation of adipocyte differentiation.

**Experimental models in human mesothelioma**

A new project is aimed at the study of malignant pleural mesothelioma, a highly aggressive cancer with a poor prognosis. Using cells from patients’s pleural effusion were obtained xenografts able to grow in a reproducible way in the nude mouse. These models have been characterized for the sensitivity to the main drugs of clinical use proving to be extremely resistant. Combination studies are in progress to identify new therapeutic strategies to improve the effectiveness of chemotherapy through a modification of the tumor microenvironment.

**Molecular profiling of epithelial ovarian cancer**

One of the main aim of the Translational genomic Unit is to draw a molecular map of the main genetic lesion affecting diagnosis and prognosis of epithelial ovarian cancer. By applying “-omic technologies” (miRNA and gene expression analysis, as well as target resequencing approaches) to human biopsies we have generated a large collection of molecular data that helped us in clarifying the role of some key determinant to relapse in stage I EOC. We have observed that relapsing patients with stage I EOC, are characterized by defects in their transcriptional programs. Of these we identified miR-200c as associated to poor survival. In particular, patients with low levels of miR-200c copared to those with haigh levels are characterized by poor prognosis and reduced overall survival. When we integrated miRNA and gene expression, we identified a specific regulatory loop in mucinous subtype, not present in the other histological subtypes, that seems to be involved in tumor growth. All these data have been generated by gathering together more than 200 tumor biopsies from three independent Italian tumor tissue collections.

Target resequencing approaches of tumor biopsies taken before and after chemotherapy are shedding new lights on the relationship among tumor tumor clones within each tumor masses, helping us in identifying key driving founder mutations involved in tumor resistance.
Combinations of natural products of marine origin with other anticancer drugs
We have observed additive or synergistic activity of ET-743 combined with other anticancer drugs such as cisplatin, doxorubicin, camptothecin, inhibitors of telomerase, bleomycin and varinostat.

Analysis of cell cycle data and interactions of different drugs
The Biophysics Unit is engaged in theoretical and methodological studies aimed at a critical evaluation of current techniques of investigation of drug effects on heterogeneous cell populations. Several computing tools have been produced to simulate the cell proliferation at different levels (from molecular interactions to in vivo growth of solid tumours) and the process of measure. Collaborations are ongoing with other research groups for design and data analysis of drug combination studies in vitro and in vivo. In this field, a number of computer programs have been developed, allowing comparative data analysis with the most common models of drug interaction.

Evaluation of the complexity of the response of cell populations to treatment with anticancer drugs
This project of the Biophysics Unit addresses the issue of establishing a connection between the intracellular drug interactions and the resulting cell cycle perturbations. It starts from the single-cell level of investigation to reach the cell-population level where the relevant end points of treatment efficacy are evaluated by flow cytometry and growth inhibition/cytotoxicity assays. The complexity of the experimental data can be deciphered by using a mathematical model able to rebuild the cell response to anticancer treatments. For this process we start with the reproduction of the unperturbed growth and we describe the response to the drug's challenge, using parameters measuring either the strength of cell cycle arrest, damage repair or cell death in every phase (G1, S and G2M). In this way, it is possible to reach an interpretation of the experimental results that overcomes the current qualitative and partial approaches to this problem, which are unable to resolve the overlapping of cytostatic and cytotoxic effects, and to establish a connection with phase-related events.

Recently, we focused our attention on the application of this method to the detailed description of the time and dose dependence of cell cycle perturbations induced on a pancreatic cancer cell line by treatments with erlotinib or gemcitabine. The information coming from these experiments, with the cells treated with the two compounds singularly, represents the base towards the comprehension of the origin of synergism or antagonism phenomena that can be observed in schedules of treatment with erlotinib and gemcitabine given together.

In silico rendering of the response to anticancer treatments integrating time-lapse imaging and flow cytometric techniques
We use flow cytometric (cell-population based analysis) and time-lapse imaging (single cell lineage based analysis) techniques to generate data that will be used to predict drug responses in term of the major components of cytostatic/cytotoxic actions of anticancer drugs: specific cell cycle perturbations (detecting accumulation or depletion of cells in G1, S and G2M phases) and the commitment to cell death (apoptosis). Time lapse data are currently integrated with those from single and multiparametric flow cytometric experiments, and univocally interpreted with a common computer program developed by the Biophysics Unit that renders in silico the proliferation process through the cell cycle and in the
cell generations during and after treatment. This kind of dynamic rendering establishes a connection between the available “macroscopic” data (time-lapse and flow cytometric) and the activity of molecular pathways which are in charge to the several functions that concur in the pharmacological response with individual timing and dose-dependence, and which are not otherwise measurable. Final aim is to achieve a quantitative level of understanding of the dynamics of response to anticancer treatment, enabling a full appreciation of the role and relative importance of the main cellular functions contributing to the overall response. Methods and computing tools with intuitive interface developed for these tasks are shared with the scientific community.

Use of nanotechnologies to design new therapeutic strategies for anticancer treatments
In these last years nanotechnologies have been largely used for biomedical purposes and the interest in this field and its application is still increasing.
The laboratory of Cancer Pharmacology is supporting a multicentre and multidisciplinary project focused on the use of polymeric, biodegradable and biocompatible nanoparticles or clusters of nanoparticles (eteronanoclusters) to design new therapeutic strategies for anticancer treatment of triple negative breast cancer.
In this contest, the Biophysics Unit performed preliminary in vitro studies to clarify some aspects of the interaction between cells and nanoparticles. The use of polymeric biocompatible and non-biodegradable nanoparticles labeled with Rhodamine-B allowed us to use flow cytometric techniques and fluorimetric measurements for the evaluation of the number of nanoparticles internalized in a cell population and its dependence on the environmental conditions or on the physical parameters characterizing the nanoparticles (labeling concentrations, dimension and Z potential). By joining the information from both platforms we obtained a reliable quantification of the mean number of nanoparticles in each cell, which represents an important preliminary step to optimize the design of these nanoparticles as potential drug delivery systems.
Then the nanoparticles selected in vitro for best characteristics of cellular intake and low toxicity are studied in preclinical tumor models in vivo..
The Cancer Clinical Pharmacology Unit perform analitycal measurements to monitor the distribution in the tumor and in other organs of the anticancer agents delivered by the nanoparticles. Preliminary results indicate that the anticancer drug paclitaxel can be efficiently carried by nanoparticles to the tumore site, achieving a better penetration and longer persistency into the tumor respect to the conventional formulation of the same drug.

Clinical pharmacokinetics of the novel inhibitor of angiogenesis Lucitanib
The Phase II clinical trial, began in late 2011, was completed in 2013 in patients with solid tumors carrying FGFR amplification. In the months before we developed the method for measuring the drug in human plasma by HPLC-mass- spectrometry and thanks to this we
studied the pharmacokinetic profile in patients who participated the Phase I. The definition of the pharmacokinetics within the phase I and within three expansion phases was performed on a total of 112 patients. Lucitanib, administered orally for 21 or 28 consecutive days, was well tolerated ensuring high plasma exposure. The drug achieves drug concentrations at steady state potentially pharmacologically active already after one week of therapy.

Also in the clinical setting we continued in 2013 a large multicenter study, in collaboration with the Italian association of pediatric hematology, for monitoring the asparaginase activity of Oncaspar (a new formulaion of pegilated asparaginase) used in children with Acute Lymphoblastic Leukemia and included in the poli-chemotherapic protocol AIEOP-BFM-ALL 2009.

Quality assurance program
During the year 2013 we completed the program and improved the quality of the GLP system inside the clinical Cancer Pharmacology Unit. Now we are operating the ordinary routine of the system with possible revision of some procedures.

In the clinical setting, we continued in 2012 and we will continue in 2013 a large multicenter study, in collaboration with the Italian Association of Pediatric Hematology, for monitoring the activity and tolerability of a new type of asparaginase, PEG asparaginase, used in children with lymphoblastic leukemia. This new treatment is included in the multi-chemotherapy protocol: AIEOP-BFM-ALL 2009.

Antitumoral activity and pharmacokinetic properties of new drugs and combinations
The antitumor activity, pharmacokinetic properties and toxicity of novel anticancer drugs with specific targets (e.g. different kinase inhibitors), conventional anticancer drugs (taxanes and trabectedin and its derivatives) and combinations is being investigated using rodent tumors and novel human tumor xenografts.

Life Science Informatics activity
The team in charge of Life Science Informatics initiative (LSI, http://lsi.marionegri.it) during the year 2013 has created for the departments of Oncology and Neuroscience several electronic case report forms for clinical studies and biobanks using Heavybase (eCRF management system), an internally developed integrated and multi-platform peer to peer database designed for the management of clinical data and the creation of randomized trials in accordance with FDA, 21 CFR part 11 directives. In particular, for the Department of Oncology have been started the following studies: B490, GLAUCOMA, TERAPIE-ORALI, BEVATRABE, ATREUS, and updated INOVATYON, ALC, ECT Vs ICT, PACT 18, MUCOSITIS, ATREUS and RER, are in preparation. For the Department of Neuroscience, the following clinical registries has been prepared: EURALS, ANACONDA, EL ESCORIAL. Two more registers are being making: Fatal Familiary Insomnia (FFI) and Evaluation of the Geriatric Care Needs (RF2009-1502045). The activity of the support group for the use of HeavyBase, in addition to the activities more closely associated with the IT aspects, covers any aspect of the remote assistance to the investigators for a proper use of the eCRF management system, and a technical follow up for a continuously software development.

From the bioinformatics point of view it has been preparated a computational cluster of about 500 elaborative units in cooperation with the banks Intesa San Paolo and Unicredit and thanks to the cooperation with SIA, for better handling the high-throughput analysis for microarray and deep-sequencing platforms, with the aim of starting a sensitivity analysis to deeply understanding these kind of data.
Laboratory of Molecular Pharmacology

Checkpoints proteins and cell cycle regulation

Chk1 and the synthetic lethality with Wee1 in lymphomas non Hodgkin

CHK1 is a key player of the signal transduction pathway activated in response to DNA damage which ensures maintenance of genomic stability. In the last years our laboratory has clarified the role of the Chk1 protein kinase in the cell cycle checkpoints induced by different chemotherapeutic drugs and also has deeply investigated the role of Chk1 under unstressed conditions, finding out that in some experimental conditions the lack of Chk1 may be deleterious depending on specific genetic background which characterizes some tumors. Recently a siRNA high-throughput screening performed in our laboratory by using a siRNA library against 719 human protein kinases identified WEE1 as in synthetic lethality with CHK1. WEE1 is another molecular player of the DNA damage checkpoint which regulates cell cycle transitions. They are indeed both involved in the control of the G2/M transition and in ensuring a faithful initiation and progression of DNA replication. Combined CHK1 and WEE1 inhibitor treatment showed a strong synergistic cytotoxic effect in human cancer cell lines from solid tumors and this effect could also be observed in an vivo setting with a tumor growth inhibition in xenotransplanted mice treated with the inhibitors. This year we have started to deeply investigate the role of of CHK1 and WEE1 as therapeutic targets in aggressive non-Hodgkin lymphomas. The project will aim in performing the investigation in mantle cell lymphoma (MCL) and in diffuse large B cell lymphomas (DLBCL). For the moment we have characterized the effects of Chk1 and Wee1 inhibitors in MCL, an aggressive, non curable lymphoma, characterized by a deregulated cell cycle, mainly due to the presence of the translocation t(11;14) which leads to constitutive expression of CyclinD1. The effects of a Chk1 inhibitor (PF-00477736) and a Wee1 inhibitor (MK-1775) alone or in combination has been investigated in a panel of 10 MCL cell lines and the results showed that these cells are much more sensitive to these drugs as single agents as compared to other hematologic cancer cell lines tested and different types of carcinoma cells. Possible involvement of the translocation t(11;14) in this sensitivity is hypothesized, since pharmacological inhibition of CDK4/6-CyclinD1 antagonizes the cytotoxic effect of PF-00477736 and MK-1775, and multiple myeloma cell lines bearing the t(11;14) are more sensitive to such inhibitors than the ones without the translocation. The combined inhibition of Chk1 and Wee1 is strongly synergistic in MCL cells, leading to complete deregulation of cell cycle, with an increased activity of CDK2 and CDK1, and activation of apoptosis. In vivo treatment of mice bearing Jeko-1 xenografts (MCL) caused marked antitumor effect with tumor regressions after the combination, at non-toxic doses. Interestingly, gene expression profile experiments suggest the involvement of apoptosis in the observed effect. A deeper investigation of the molecular markers responsible of such sensitivity is undergoing. We have also just started to study the cytotoxic effect of Chk1 and Wee1 inhibitors in a panel of 30 DLBCL cell lines belonging to different subtypes with different molecular features and we used an automated handle system to perform seeding and treatments. The analysis of the results is undergoing and will provide new information regarding the sensitivity of DLBCL cells to these inhibitors depending on specific molecular features, in order to possibly discover new genetic backgrounds in which such treatments could be more effective both as single agents and in combination. Taken together this investigation will help to define a new therapeutic tool to treat lymphomas.

Characterization of new potential oncosuppressor genes

DRAGO gene, identified and cloned in our laboratory is one of the most interesting projects of
the group. The characterization of the response of KO mice for DRAGO to ionising radiation is similar to normal mice.
Mice KO for DRAGO have been crossed with with p53 KO mice to evaluate the potential oncosuppressive function of DRAGO. The double mutants are viable and the genotypes arising from the crossing are at the normal Mendelian ratio, indicating that no specific genotypes (p53;DRAGO) are favoured.
In a p53KO background, removal of DRAGO gene accelerates tumor development suggesting a cooperative role of the two genes in the prevention of tumor formation. The analysis of the spectra of tumor formation did not show significant differences among the different genotypes. we are at present investigating the role of the gene as potential regulator of the p53-dependent immune response.

Molecular characterization of ovarian carcinoma
We have retrospectively characterised polymorphisms in genes participating in the response to damage such as mdm2, ERCC1 and XPG as possible predictors of response to treatment in patients with ovarian cancer. 420 patients have been genotyped and the allelic frequency found is the expected one for a Caucasian population. The data generated will be analysed together with the clinical parameters and with the follow-up data available for all the samples analysed. As for K-RAS gene, we have studied a polymorphism present in the coding region of the gene, called KRAS-LCS6, which is located in the region which binds the miRNA let7. The polymorphism determines the substitution of the more abundant T-allele to a G-allele which was observed to increase the KRAS expression and in turn to activate the downstream pathway at higher levels if compared to the T-allele. We assessed the role of the KRAS-LCS6 polymorphism in 97 early (stages I and II) and 232 advanced (stages III and IV) ovarian cancer patients. Our data indicate that KRAS-LCS6 polymorphism is not relevant in ovarian cancer, in fact, in our cohort of patients, is not associated to any outcome or physiopathological characteristic.

Expression of gene involved in DNA repair in human ovarian cancer
By Real Time PCR, the expression of genes involved in DNA repair has been evaluated in 150 samples of breast cancer: 70 cases of luminal A (estrogen receptor positive) and 80 cases of triple negative samples (negative for estrogen and progesterone receptors and HER2 negative). These two types of tumors represent two distinct breast cancer, characterized by a different biological behaviour and a different response to chemotherapy. A better understanding of the expression levels of genes involved in DNA repair might help in elucidate the biology of these tumors. The genes analysed include those belonging to the nucleotide excision repair, in the fanconi anemia repair, in the base excision repair. In addition, genes important for the cellular response to damage, such as chk1 and claspin have been studied.
Most of the gens involved in NER, FA and chk1 were significantly less expressed in triple negative breast samples than in luminal A samples. These data would suggest a DNA repair defect in triple negative breast cancers and these could have important clinical implication considering that most of the currently used anticancer agents are DNA damaging agents.

Inhibition of the signal mediated by PI3K/akt
Pi3K/akt axis represents one of the major altered pathway in human cancers and therefore is a good target for the development of new drugs. The laboratory has been involved in the pharmacological characterisation of new molecules able to inhibit the pathway.
We have characterised the molecular mechanism at the basis of the interaction between two molecules able to inhibit mTOR (the kinase downstream PI3K/akt) at two different portion of the protein. In vitro and in vivo data indicate that the strategy to inhibit the same target acting at different level could be an interesting strategy to shut down a transduction signal. The combination of the molecules, in fact, is able to inhibit tumor growth more than the single
drugs, even when these are used at doubled doses. The mechanism of activity of the combination is the ability to selectively inhibit one of the downstream effectors of mTOR leading to a selective inhibition of translation. The study combines cellular, molecular and proteomic analysis.

**Mechanisms of action of new antitumor drugs**

In collaboration with the laboratory of Biology and Therapy of Metastasis, we have characterised the mechanism of action and the antitumor activity of a new antiangiogenic drug, lucitanib. This drug is a small molecule able to inhibit receptors playing important roles in the tumor angiogenesis processes (VEGFR, FGFR). Our studies allowed us to define that the drug has a potent antiangiogenic activity, with a broad spectrum of activity in different human tumors transplanted in immunodeficient mice. We are currently investigating the antitumor activity and pharmacokinetics of the compound on human cancer implanted in immunodeficient mice and characterized by a de-regulation of the FGFR1 and FGFR2 pathway. The aim is to try to understand how important is the activity on FGFRs in determining the mechanism of antitumor activity of lucitanib.

**Generation of new cellular systems for in vivo imaging**

We have generated new cell clones derived from human cancer cells growing in vitro, which stably express fluorescent or luminescent probes which can allow us to follow in vivo the growth of primary tumors and metastasis in mice. These systems generated in human ovarian, breast and prostate cancer cell lines, can be implanted in nude mice and the growth and response to therapy followed by either optical and luminescent imaging or microTAC analysis. We have in particular set up models derived from human breast cancer, which are able to metastasise to the bone which can be evidentiated by optical imaging and microTC techniques in laboratory animals. Utilising different reporter genes, we have generated fluorescent and luminiscent human cancer cell lines which can be transplanted in immunodeficient mice. These cells can then be visualised in organs such as peritoneum and lungs were these cells were previously be observed only after sacrifice of the animal. The cells generated to be fluorescent or bioluminiscient will also have specific gene defects which will be useful for understanding the mechanism of action of new molecules. These systems will be particularly useful to study the antitumor potential of new drugs.

**Studies on the bone metastatic processes**

Using a model of human breast cancer cells metastatizing to the bones, we have characterised some molecular pathways involved in the colonisation and metastatic growth. In particular, we have evaluated the role of cMet receptor and of its activation both in vitro and in vivo. The in vivo model utilized develops bone metastasis following intraventricular injection of cancer cells. The bone metastasis can be visualized by optical imaging already after 10 days from cancer cell inoculum. By microTC analysis, bone osteolytic lesions can be evidentiated after 3-4 weeks from tumor cells injection.

In this model we have evaluated the response in vivo to a c-Met inhibitor, tivantinib, alone or in combination with a bisphosphonate, zoledronic acid, largely used in the clinical practice. The aim of the work was to determine whether combining drugs which hit different target, (cancer cells for tivantibi and host cells for zoledronic acid) we could have an enhanced response. The data obtained indicated that the combination is well tolerated and is able to increase the response and survival of animals with bone metastasis compared to the same drugs given as single agents. These effects were observed either when the drugs were given in the early phases of the metastatic process or when the bone metastisis were well detectable. We have now available models of murine breast cancer with different ability to metastatise. These tumors, transduced with the luciferase gene, grow in the breast and metastatize to the bones and allows the simultaneous evaluation of the antitumor and antimetastatic effects of different drugs.
Identification of cancer stem cells from ovarian cancer

The studies conducted in the last years in ovarian fresh tumor samples, obtained from the Gynecological Department of Ospedale San Gerardo di Monza, directed by Prof Mangioni, lead to the identification of a cell bearing the characteristic of tumor initiating cells. The pharmacological characterization we undertook revealed that these tumor initiating cells were more resistant to some drug than the corresponding differentiated much less tumorigenic cells, while sensitive to other drugs. These cells were not positive to markers described to identify the tumor initiating cell of the ovary by other groups, but we could demonstrate that they display a mesenchymal phenotype. This observation is very important in the light of the recent results linking a mesenchymal phenotype to an increased staminality and increased resistance to chemotherapy. These data generated a number of hypothesis on the role of epidermal to mesenchymal transition in the acquired resistance to a chemotherapy in ovarian cancer that we are addressing using a number of ovarian cancer xenografts, recently stabilized directly from patient’s tumor samples.

Determination of the impact of EGFR mutations in the activity of tyrosine kinase inhibitors in patients with NSCLC

The clinical study on the characterisation of the response of patients with NSCLC to therapy EGFR inhibitors is terminated. The data obtained so far indicate that patients not presenting mutations in the EGFR gene, respond less to treatment with the EGFR inhibitor erlotinib than to standard chemotherapy with docetaxel. The inferiority of erlotinib compared to docetaxel, is evident both in terms of response to treatment and in terms of progression free survival and overall survival. The trial, conducted with the collaboration of more than 50 centers, could impact on the clinical practice, where, at present, the EGFR inhibitor is registered for the second line treatment of NSCLC patients independently from the presence of mutations in the EGFR gene. We have clearly showed that in the absence of mutations the EGFR inhibitors is less efficacious. Finished the main trial, all the data and histological samples will be used to answer still open medical question.

Improvement of lung cancer therapies

Nowadays, some lung cancer subtypes have not a very effective therapy even if they have some peculiarities that could be investigated and exploited by the clinician. An example is the KRAS mutated non-small cell lung cancer. The K-RAS gene results mutated in significantly higher percentage of NSCLC patients (about 25%). The spectrum of mutation found in NSCLC is different from that observed in other tumor types such as colorectal cancer. The different mutations could explain the different impact of K_RAS on the selection of patients for therapies. In fact in colon cancer mutation in the K_RAS gene is an exclusion criteria for treatment with anti EGFR drugs such as cetuximab. In NSCLC the role fo K-RAS is more controversial. From the available clinical data we went back to the laboratory generating isogenic cellular systems differing for the type of K-RAS mutation. In particular we have generated in NSCLC cell lines clones overexpressing the wt K-RAS or mutants in which the glycine at codon 12 is substituted with aspartic acid, cysteine or valine. These mutants have indeed a different impact on the response to treatment of these cells with drugs such as cisplatin, sorafenib or taxol. Our data suggest that for the stratification of patients it is necessary to consider not only the presence of K-RAS mutation, but also the kind of mutation present which could modify the selection of the best therapeutic options. In this context new cellular models have been generated. These model have been obtained through the use of a new technology,(Zinc finger technology) which allows the insertion of the desired mutation directly in the gene locus. This has the big advantage of generating clones without overexpression and without perturbing other genomic regions. These models are now
under investigation with the aim of better understand and bypass the resistance to chemotherapy.

Another subtype of lung cancer named small cell, quickly incurs in resistance to chemotherapy making ineffective the chemotherapy treatments. Also for this type of cancer we are investigating which mechanisms are responsible for the chemotherapy resistance in order to bypass these phenomena with a new therapeutic approach.

Laboratory of Biology and Treatment of Metastasis

Physiologic regulation of angiogenesis

Angiogenesis - the neoformation of blood vessels from existing ones - has a critical role in tumor progression. A delicate balance between pro- and antiangiogenic factors finely tunes this process. We have extensively studied endogenous angiogenesis-regulatory factors, as a basis to develop new inhibitors. In particular, our studies focus on thrombospondin-1 (TSP-1), an endogenous inhibitor of angiogenesis. The ability to directly bind to angiogenic factors, in particular FGF-2 (Fibroblast Growth Factor-2) reducing its bioavailability and activity, is one of the manifold functions of this molecule. In a structure/function relationship analysis of different active domains of TSP-1, we have identified its binding site for FGF-2. This active sequence of TSP-1 is being used as a model to design new antiangiogenic and antineoplastic compounds. Moreover, we are investigating the possibility to develop pharmacological interventions or gene therapy approaches to upregulate the expression of TSP-1, as a strategy to block tumor angiogenesis and progression.

Angiogenesis and tumor-stroma interaction

We have observed that the release of vascular endothelial growth factors (VEGF) from tumor cells in the tumor microenvironment is accompanied by an altered response to some chemotherapeutics. Bevacizumab (Avastin®) – an antibody directed to VEGF – restores the response, suggesting that the tumor environment (stroma) might play a role in modulating the sensitivity to therapy. The transcriptional activity of the stroma microdissected from the tumor tissue was investigated. Two hundred ninety four gene transcripts were preferentially expressed by the stroma of tumors producing high levels of VEGF. For some of them it was demonstrated that the protein preferentially localizes in the stroma associated to the vasculature of VEGF-rich tumors. Specifically, the regulator of G-protein signalling 5 (RGS5) was demonstrated in the vasculature of ovarian carcinoma specimens, but not in human healthy ovaries and its expression by the tumor-derived endothelial cells (tum-EC) was sustained by a milieu of pro-angiogenic factors related to the tumor microenvironment (including VEGF). Furthermore we have identified gene expression differences between tumor derived endothelial cells (tum-EC) with respect to EC from healthy tissues; for some of these genes the expression was modified by an angiogenic-conditioned microenvironment. Studies are underway to understand the functional relevance of selected genes on tum-EC.

The bio-bank of epithelial ovarian carcinoma (EOC) preclinical models to investigate novel therapeutic modalities

The classification of epithelial ovarian cancer (EOC) has been recently revised based on distinctive morphologic and molecular genetic features. The whole laboratory has been involved since the 90’ in the characterization and continuous updated of preclinical models derived from EOC patients and transplanted in immudeficient mice (Xeno-EOC). The Xeno-EOC molecularly, biologically and pharmacologically characterized, which resemble the original patient tumor, together with a large bio-bank of Xeno-EOC derived biological materials, provide basis for the study of novel selective pharmacological interventions.
As an example, inhibitors of angiogenesis are being investigated on Xeno-EOC. We have shown that bevacizumab, the antibody anti VEGF, is active on Xeno-EOC in combination with chemotherapy, depending on the different XENO-EOC, their chemo-sensitivity and the schedule of treatments. The research is in progress aimed to understand the mechanisms of response/resistance, the relative involvement of the tumor and the host, the identification of biomarkers of response. The study of these classes of molecules in combination with chemotherapy is one of the main interests of our laboratory. Studies have been conducted and more are in progress to optimize the modalities of administration of the combinations accordingly to the mechanism of action of the drugs, the pharmacokinetics and pharmacodynamic profiles of the tumor.

**Lymphangiogenesis in ovarian carcinoma**

Lymphatic spread in epithelial ovarian cancer is an important predictor of outcome both in early and advanced stages of this cancer. We have developed preclinical tumor models derived from human ovarian cancer transplanted under the bursa (orthotopic xenograft), expressing luciferase and disseminating in the peritoneal cavities of immunodeficient mice. The levels of soluble VEGFC - the main factor stimulating the formation of lymphatic vessels (measured in plasma and ascites of mice bearing ovarian cancer) - correlates with tumor growth (measured through optical fluorescence) as well as lymphatic invasion. We found that tumor VEGFC promotes ovarian carcinoma progression through paracrine and autocrine mechanisms. The investigation of selective inhibitors of VEGF/VEGFRs pathway is underway to shed light on these mechanisms.

**Tumor biomarkers for early diagnosis and risk assessment of pancreatic cancer**

Pancreatic ductal adenocarcinoma (PDAC) has bad prognosis and is highly chemo-resistant. Early detection is the only means to substantially impact long-term survival, but screening methods are lacking.

PDAC is characterized by intense fibrotic reaction called dysplasia in which extracellular matrix reorganization occurs in terms of composition and structural organization. Studies are in progress to a) study extracellular matrix remodelling (synthesis, organization, composition) during PDAC progression; b) identify extracellular matrix related molecules in plasma of PDAC patients and of, in vivo, tumor models; c) validate plasma extracellular matrix related molecules as biomarkers predicting the risk of PDAC development and progression. In such a context we are developing patient derived PDAC preclinical models that resembling - molecularly, biologically and pharmacologically – the patient’s original tumor become a unique tool to study novel pharmacological interventions.

**Laboratory of Cancer Cachexia AIRC Start-Up**

Cancer cachexia is a very debilitating loss of muscle mass that affects up to 80% of cancer patients. Remarkably, 20-48% of cancer-related deaths are caused by respiratory failure due to loss of mass from the diaphragm muscle. Anti-cachexia therapies could thus increase the survival of cancer patients. The "Cancer Cachexia AIRC Start-up" lab is interested in dissecting the molecular mechanisms governing the cross-talk between muscle and cancer. Some of the questions we will try to address are:

How can we stop/delay the lethal muscle wasting associated to many forms of cancers?
Why are skeletal muscles exceptionally resistant to cancers?
Answering these questions may improve greatly the quality of life of cancer patients.
In 2013, we set up two independent murine models to study cancer cachexia: mice carrying Colon adenocarcinoma (C26) or Lewis Lung carcinoma (LLC). We performed gene expression analysis on their leg muscles obtained during early phases of cachexia (measured as body weight loss), in comparison with healthy muscles. The newly-identified pathways are the matter of our present research.

**Laboratory of methodology of biomedical research**

The laboratory was born out of the consideration that the advent of oncological drugs endowed with mechanisms of action different from those of traditional chemotherapics, introduces new treatment opportunities. At the same time, new problems arise concerning the choice of the most appropriate and effective design for research into the clinical activity profile of these new treatments.

The traditional paradigm where the choice of dose is based on the maximal tolerated toxicity, and the screening of therapeutic activity focus on tumor mass reduction, may not necessarily be suitable for the evaluation of new agents whose targets may include the extracellular compartment or specific molecular targets.

The clinical development of ‘non toxic’ anti tumor molecules requires a critical review of the existing models as well as of all the aspects relative to the conduction of clinical trials including: dose selection criteria, methods for determination and confirmation of pharmacological activity, and the validation of new technologies and laboratory methods. This is where the need for a profound integration of the ‘clinical screening’ and the preclinical research lies. It is a prerequisite for the construction of the pharmacological rationale for the identification of the most interesting molecules, the choice of dose, the hypotheses of combination with other drugs, and of the most appropriate indicators of clinical activity.

The acquisition of know how and the development and application of new designs for clinical activity studies, including the use of randomization, the introduction of groups of patients treated with placebo, and new discontinuation designs, proceed in parallel to the above.

Another fundamental issue in laboratory research is the recognition that the genomic characterization of any single tumor may now play a more relevant role in drug development and treatment identification.

This notwithstanding, numerous uncertainties remain regarding the role of biomarkers in drug development and in the implementation of genomic technologies in clinical trials. It is therefore necessary to improve the methodology and more biomarkers evaluation already in the early stages of research, thus shifting translational research from a simple process of correlation search to one producing knowledge regarding the predictive role of the clinical activity of the investigational treatments.

Therefore, the primary focus of the laboratory is to provide a methodological support for the activity of other laboratories of the Oncology Department, in order to optimize the methods for evaluating the activity of cytotoxic drugs, particularly for those therapies aimed at specific molecular targets, as well as the identification of factors predictive of therapeutic response. The laboratory carries out training activities and supports the methodological aspects of various projects managed within the department of oncology. In particular, it is involved in the conduction of various theoretical and practical courses, masters in clinical research methodology and systematic reviews and in the production of guidelines in oncology.

Since 2012 the laboratory supports methodologically the Italian Association of Medical Oncology (AIOM) in the production of its guidelines. These guidelines cover several areas such as prognosis, diagnosis and treatment of neoplasms (e.g elderly patients, support therapy).
First the revision and after the update of the guidelines has allowed to solve a big problem: the several ways of judging and interpreting the evidence extrapolated from the literature. The aim was that of aligning each guideline to a unique method. The Scottish Intercollegiate Guidelines Network (SIGN) was used for the old recommendations, and the GRADE approach for the new ones.

The laboratory is involved in the 2014 update of the “Guida all’uso clinico dei biomarcatori in Oncologia 2010” in collaboration with Professor Massimo Gion and the CRIBT (Centro Regionale Indicatori Biochimici di Tumore di Venezia).

Other activities are several systematic reviews on non small cell lung cancer and on biosimilar drugs.

The Laboratory improved its computational skills; in the era of personalized medicine a better efficiency is requested to the applied methodology, from observational studies to meta-analysis; adaptive and bayesian techniques were identified as necessary tools for the clinical research; data simulation was used for the estimation of statistical parameters (e.g.: hazard ratio) and for the study of the Survival-Post-Progression endpoint.

**Laboratory of Clinical Research**

Laboratory of Clinical Research, formerly Laboratory of Clinical Trials until September 2012, has been active since 2006 and has inherited the nearly thirty-year experience, acquired by the Institute in oncological clinical trials. It has expanded over the years and adapted its structure to plan, organize and coordinate experimental and observational clinical studies, keeping mission and identity of a not-for-profit organization, and working with high standards of quality. Staff includes statisticians, study coordinators, data managers, informatics and local monitors. A Unit of Quality Assurance has been created and an electronic system for registration/randomization and data collection has been developed in-house and validated by an external structure.

Studies are conducted in cooperation with networks of researchers. Main oncological research areas are gastric, lung, colorectal, and head & neck. Besides these areas, the Laboratory has gained long-standing experience in ophthalmology field.

**Oncological diseases**

**Breast cancer**

Breast cancer affects one of eight women throughout their lives. In the female gender it is the most common cancer accounting for 29% of all tumors and it is the leading cause of cancer mortality, with a mortality rate of 16% of all deaths due to cancer. Every year in Italy 48,000 new cases are diagnosed.

The risk factors for developing breast cancer include age (more than 75% of cases occur in women over 50 years old), familiarity (about 5-7% of women with breast cancer have more than one close relative with this disease), high level of estrogen, obesity and smoking.

Mutations in the BRCA1 and BRCA2 genes are responsible for about 50% of hereditary forms of breast cancer.

Surgery is the treatment of choice; currently conservative procedures are adopted in all cases where it is possible. Studies have shown that there is no increase in mortality in the case of conservative surgery, when combined with radiotherapy and adjuvant therapy. Pharmacological treatments include hormone therapy in patients with estrogen receptor-positive tumor (70% of cases), chemotherapy, treatment with the anti-Her2 monoclonal antibody trastuzumab, in patients with tumors positive for Her2. The triple negative breast carcinoma which does not express estrogen receptor, progesterone and Her2, is an aggressive form of cancer, unresponsive to standard therapies.
During 2013 two studies conducted in patients with triple-negative breast cancer, the PAINTER study and the TRIPLE NEGATIVE study, were in the activation step.

TRIPLE NEGATIVE - A multicenter, single-arm, phase II study to evaluate the activity of pre-operative zolendronate in triple negative breast cancer patients, according to p53 level

It is a multicenter, Italian, single-arm, phase II study, investigating the antitumor activity of zolendronate administered before surgery in patients with favorable and unfavorable prognostic features, defined according to p53 expression.

The triple negative breast cancer is a particularly aggressive tumor, unresponsive to conventional therapies, for its treatment is necessary to identify potential new molecular targets to which the therapy can be addressed. The development of new treatments is also possible through molecular analyses on tumor tissue samples that allow to find the tumor features useful to select responding patients.

The bisphosphonate zolendronate is currently used to prevent bone complications in cancer patients and clinical studies suggest that it may have antitumor activity in breast carcinoma, through inhibition of the mevalonate pathway, which is involved in tumor progression.

The antitumor activity of zolendronate is evaluated in terms of reduction of expression in the tumor tissue of the molecule Ki67, used as a surrogate marker of treatment efficacy. Secondary objectives are the evaluation of treatment response according to the RECIST criteria and the assessment of treatment compliance and the safety and tolerability of the drug.

STUDIO PAINTER - Multicenter, interventional, single-arm, phase IV study evaluating tolerability of Eribulin and its relationship with a set of polymorphisms in an unselected population of female patients with metastatic breast cancer

Therapies which offer a survival benefit for treatment of women with metastatic breast cancer already heavily treated are worldwide solicited. Eribulin mesylate is a novel inhibitor of microtubule dynamic, synthetic analogue of the natural marine macrolide Halicondrina B present in many kinds of Halicondria and Axinella sponges. Eribulin binds tubulin filaments at a different site from that all other drugs interfere with, blocking the microtubule growth and polymerization and promoting the raising of not functional aggregates within tumor cells. The molecule has been shown to improve the overall survival in patients already pretreated for metastatic breast cancer, compared with the standard treatment; based on these results Eribulin has received the marketing authorization for the third-line treatment of locally advanced or metastatic breast cancer.

The project, in collaboration with the Fatebenefratelli Oncology Hospital in Milan, foresee a phase IV, multicenter, interventional, single-arm study assessing the tolerability of Eribulin and its association with a set of polymorphisms in an unselected patients population of women with metastatic breast cancer. The study will evaluate the incidence, severity and duration of all adverse events occurring during the treatment with Eribulin, with particular attention to the most common events reported in previous clinical studies (asthenia / fatigue, neutropenia, alopecia, nausea, peripheral neuropathy and constipation). In particular, using pharmacogenetic analysis, it will be performed an assessment of the association between a number of selected polymorphisms and peripheral neuropathy of any grade, in patients who developed neurotoxicity. The quality of life during treatment, the intensity of the dose, duration of treatment and overall survival will be evaluated as well.

The enrollment of 200 patients with metastatic breast cancer treated with Eribulin according to the approved indications is planned.

The study was approved by the Coordinating Ethic Committee during the session of December
2013. The administrative part of the project is now under definition and the protocol submission in the Italian centers (22) is expected during February-March 2014.

Gastric cancer
Despite the decline in incidence, gastric cancer is one of the most common malignancies in Western countries, and Gastric cancer is the second leading cause of cancer mortality worldwide accounting for 12.1% of cancer-related deaths. The prognosis is poor if the cancer is not diagnosed at a very early stage. In Western countries, the 5-year survival is less than 20%, but in countries like Japan, where screening is widespread this is higher than 60%. Surgical resection remains the only potentially curative treatment, although after surgery, the recurrence rate remains high. The prognosis of patients undergoing radical surgery is mainly due to the extension of the disease and the pathological stage is currently the only prognostic factor for discriminating patients at different risk of recurrence. The benefit of post-operative chemotherapy is still under debate, despite five meta-analysis of randomized trials comparing chemotherapy versus surgery alone have confirmed a statistically significant, although moderate benefit in favor of chemotherapy. Currently four types of cytotoxic drugs are used for gastric cancer: fluoropyrimidine, platinum-based compounds, taxanes, and irinotecan. Based on potential role of radiotherapy in improving regional control, several studies have explored the association radio-chemotherapy, but an inadequate surgery has not allowed to obtain robust results. In fact, over the last decade, several studies have shown the negative influence of an inadequate surgery on survival. During 2013 two phase III studies on gastric cancer have been concluded.

**ITACA-S - Intergroup Trial in Adjuvant Chemotherapy for Adenocarcinoma of the Stomach**

This was a multicenter, randomized, open-label, active-controlled, superiority, phase III trial. It was designed to evaluate whether a post-operative polichemotherapy of 5-fluorouracil with CPT-11 and docetaxel plus cisplatin improves the outcome in patients who underwent radical resection with extended lymph-nodes dissection in comparison to a regimen with 5-FU/LV. The study, sponsored by IRCCS- Istituto di Ricerche Farmacologiche Mario Negri, involved 11 oncological collaborative groups and more than 110 Italian experimental centers. From February 2005 to August 2009, 1106 patients have been enrolled. At a median follow-up of 57 months, a total of 483 patients died and 581 patients recurred or died. Our study confirmed the high survival rates observed in previous studies in adjuvant setting, but it failed to show a significant benefit in terms of disease free and overall survival of a more intensive regimen vs. 5-FU/LV.

**ITACA-S 2 - Intergroup Trial in Adjuvant Chemotherapy for Adenocarcinoma of the Stomach: Comparison of the efficacy of a pre-operative versus a post-operative chemotherapy treatment in patients with operable gastric cancer and assessment of the benefit of a post-operative chemo-radiotherapy**

The same collaborative group launched in 2008 a new study supported by a grant of AIFA. This study, called the ITACA-S 2, is a multicenter, randomized, open-label, with a 2x2 factorial design, superiority, phase III study in patients with histological confirmation of localized gastric adenocarcinoma, which is considered operable. According to factorial design, the project consisted of two independent randomizations, which gave rise to two substudies, corresponding to two different questions and different endpoints: to compare the efficacy in terms of overall survival of a peri-operative chemotherapy (3 cycles administered before surgery and 3 cycles after surgery) with a post-operative treatment, and to compare the efficacy in terms of local recurrence free survival of post-operative chemo-
radiotherapy operatively to no further treatment after surgery. Approximately 1100 patients were needed in the first study and approximately 500 in the latter study. Unfortunately, the study was prematurely discontinued in December 2013 due to insufficient enrollment rate with only 55 patients randomized.

Lung tumors
With more than 1.6 million new cases diagnosed each year, lung cancer is the leading cause of cancer death worldwide. In Italy the estimated annual incidence of lung cancer is approximately 34,000 new cases in people aged up to 84 years and the annual mortality from lung cancer amounts to approximately 27,500 persons (of which 22,000 men and 5,500 women), representing the leading cause of cancer death in men and the second in women, after breast cancer. In fact, these tumors are often diagnosed when the disease is at an advanced stage and it is associated with very low rates of survival. Lung carcinomas are classified for therapeutic and prognostic purposes into two broad categories as follows:
- Small Cell Lung Carcinoma (SCLC) originating from neuro-endocrine cells
- Non-Small Cell Lung Cancer (NSCLC) representing approximately 85% of all lung carcinomas can be further divided into four histological sub-classes with differing prognoses. Tobacco smoking is the risk factor implicated in the genesis of approximately 85% of all malignant tumors of the lung. Other causes of lung cancer include: passive smoking, asbestos exposure, air pollution with particulate materials and radioactive radiations. It has been also assumed the role of several genes (such as those encoding for the epidermal growth factor) that have become targets for new drugs. With the development of targeted anticancer agents, the therapeutic strategy previously based on standard protocols for all patients will be substituted by an approach which takes into account the molecular characteristics of the tumor, aiming at the choice of the best treatment regimen for each patient.

In the year 2013, two clinical studies in patients with non-small lung cancer cells in advanced stage, the Tailor study and the Acetylcarnitine study, were closed.

TAILOR STUDY - Optimization of erlotinib for the treatment of patients with advanced non-small cell lung cancer: an Italian randomized trial

The Tailor study is a multicentre, randomized, Italian study which started on September 2007. The aim of this trial is the optimization of the second line therapy in patients with advanced NSCLC. The development of target-therapy suggested to evaluate the treatment’s efficacy according to molecular features of the tumoral cell. In particular the epidermal growth factor receptor (EGFR) is a promising target for anticancer therapy. A "tailored therapy" based on individual molecular features may result in better responses and optimization of resources and costs. Erlotinib, an EGFR tyrosine kinase inhibitor, has been registered for the treatment in EGFR mutated NSCLC patients, but at the start of this study there was not yet firm evidence of its effectiveness in the general population and in patients without mutations of the epidermal growth factor and the best therapy for these patients has not been established. The aim of the study was to compare the efficacy in terms of overall survival of erlotinib and docetaxel as second-line treatment in NSCLC patients without mutations in EGFR exons 19 or 21.

The study, which ended in February 2013, showed that the standard chemotherapy with docetaxel is more effective than treatment with erlotinib in terms of both overall survival and progression-free survival. The median overall survival was 8.2 months with docetaxel and 5.4
months with erlotinib, while the median progression-free survival was 2.9 months with docetaxel and 2.4 months with erlotinib. This study is so far the only one conducted with tyrosine kinase inhibitors addressing which is the best therapeutic option in a population of patients which is entirely EGFR wild-type for exons 19 or 21.

**ACETYLCARNITINE STUDY** - Randomised, double-blind, placebo-controlled, phase III, superiority trial to assess the efficacy and safety of acetyl-L-carnitine in combination with a cisplatin-containing chemotherapy as first line treatment of advanced or metastatic non small cell lung cancer

On July 2011 a multicentre, double-blind, placebo-controlled, phase III study, investigating the combination of acetyl-L-carnitine (ALC) with a cisplatin-containing chemotherapy as first line treatment of advanced or metastatic NSCLC started recruiting. ALC facilitates the uptake of acetyl CoA into the mitochondria during fatty acid oxidation, the production of energy in the form of ATP, through the Krebs cycle and oxidative phosphorylation, it is also involved in the acetylation of proteins such as tubulin and stimulates acetylcholine and membrane phospholipid synthesis. Recent studies show that alterations in energy metabolism are a hallmark of cancer cells and its modulation may constitute a new therapeutic approach for improving the effectiveness of pharmacological treatments. Preclinical data suggest that acetylcarnitine may have a neuroprotective role by reducing the toxicity of platinum-based compounds and may enhance their activity. The aim of the trial is to assess whether acetyl-L-carnitine prolongs toxicity free survival in patients with advanced or metastatic NSCLC and reduces neurotoxicity due to platinum compounds. In fact, in patients receiving chemotherapy administered with legitimate “curative intent” many toxicities can be justified to accomplish this goal, while in patients with metastatic cancer, for whom the goal is to “palliate symptoms” and optimise the quality of life, toxicity is less acceptable and justified. Unfortunately, in November 2012, the enrollment of the study was terminated early, after 107 randomizations, because of the difficulties encountered by the experimental centers. In 2013, the follow-up of patients continued. The closure of the study is scheduled for April 2014.

**Malignant pleural mesothelioma (MPM)**

This tumor is a relatively rare and very aggressive form of cancer originating from the mesothelium. Among all forms of malignant mesothelioma, MPM is the most frequent, accounting for approximately 80% of all mesotheliomas. The incidence of this cancer is on the rise worldwide with approximately 2.2 cases per million inhabitants. The single identified risk factor for the development of mesothelioma is exposure to asbestos. Asbestos in itself is not a mutagen, but is able to promote self-phosphorylation of EGFR activating the proliferative RAS-MAP kinase pathway. The crystalline forms, also containing iron (crocidolites), are able to catalyze the synthesis of reactive oxygen species that are carcinogenic. Unfortunately, MPMs are most often diagnosed at an advanced stage. The delay is probably due to the unspecific clinical picture and the considerable length of time from exposure to the onset of clinical disease.

**ATREUS: A phase II study on the Activity of TRabectedin in pretreated epithelioid or biphasic/sarcomatoid malignant pleural mesothelioma (MPM)**

There is no active second-line treatment for MPM recurring after first-line treatment, except for patients who respond to the standard platinum-based plus pemetrexed regimen for at least 6 months; in such cases re-challenge with the same therapy may be effective.
Biphasic and sarcomatoid MPM are generally resistant to the aforementioned standard chemotherapy, there is not a standard first line treatment for this histological type, which represents an unmet medical need.

Trabectedin binds in the minor groove of DNA, alkylating the N2 of guanine and affecting transcription regulation in gene- and promoter-dependent fashion. Considering the unique features of the mechanism of action of trabectedin and the preclinical and clinical evidence that the drug can be effective against tumours that are poorly responsive to conventional chemotherapeutics, its activity was tested in patients with MPM. The mechanism of action of Trabectedin presents some peculiar features, such as the activity on inflammatory processes, which play a fundamental role in the pathogenesis of MPM. These characteristics have been evaluated in a series of translational analyses.

Atreus study is a phase II non-randomized multicentre study conducted in patients with unresectable MPM with epithelioid subtype previously treated with pemetrexed plus platinum-based chemotherapy, or patients with biphasic and sarcomatoid histotypes who are either chemo naïve or previously treated with pemetrexed plus platinum-based chemotherapy. The study shall enrol 79 patients, of which 62 with epithelioid sub-type MPM in progression notwithstanding a previous course of treatment with pemetrexed and platinum derivatives and 17 with sarcomatoid or biphasic MPM irrespective of treatment history.

The primary objective of the study is to assess the activity of trabectedin in patients with epithelial MPM relapsing after treatment with pemetrexed plus platinum-based drugs. Additional aims include the assessment of trabectedin activity in patients with biphasic or sarcomatoid either as first line treatment or following a previous course of platinum derivatives and pemetrexed, and the evaluation of its safety and tolerability profile. In addition, the performance of trabectedin with respect to some biological features of MPM, shall be evaluated.

The study started enrolling patients in July 2013. Notwithstanding the rarity of this tumour, 18 patients have already been included in the two active centres. In parallel to the initiation of the research activities, an amendment to the protocol has been submitted to the single opinion ethics committee, widening the translational part. This now foresees the evaluation of the effects of trabectedin on circulating levels of miRNA, HMGB1 protein and blood monocytes which act as precursors to tumour macrophages. In consideration of the anti-inflammatory activity of trabectedin a form investigating the effects of treatment on pain and use of analgesic therapy has been added to the study as well.

Colorectal cancer

Colorectal cancer is a cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine), or in the appendix. Symptoms of colorectal cancer typically include rectal bleeding and anemia which are sometimes associated with weight loss and changes in bowel habits.

In western countries this neoplasm is the third malignant tumor after lung cancer for men and breast cancer for women.

Most colorectal cancer occurs due to lifestyle and increasing age with only a minority of cases associated with underlying genetic disorders. It typically starts in the lining of the bowel and if left untreated, can grow into the muscle layers underneath, and then through the bowel wall. Screening is effective at decreasing the chance of dying from colorectal cancer and is recommended starting at the age of 50 and continuing until a person is 75 years old. Localized bowel cancer is usually diagnosed through sigmoidoscopy or colonoscopy.

There are three open studies on this disease:

**CETUXIMAB - Prognostic factors for patients with advanced colorectal cancer treated with Cetuximab**

This is an Italian multi-center, single arm, phase II clinical trial.
The aim of the study is to assess the prognostic value of PTEN mutation in patients with metastatic colorectal cancer and histologically documented KRAS wild-type, treated with Cetuximab plus Irinotecan, Fluorouracil, Leucovorin (FOLFIRI regimen) as first line therapy, in terms of Progression Free Survival (PFS).

The secondary end-points are: overall survival; response rate assessed by RECIST criteria and Quality of Life assessed by QLQ-C30 questionnaire, toxicity, frequency and nature of serious adverse reactions. Sample size is approximately 50 KRAS wild type patients.

This study, sponsored by Regione Lombardia, has foreseen the involvement of 8 centers and about 50 patients enrolled in 36 months starting in April 2009. Publication is ongoing.

*TOSCA - A randomized trial investigating the role of FOLFOX-4 or XELOX (3 versus 6 months) regimen duration and bevacizumab as adjuvant therapy for patients with stage II/III colon cancer*

On June 2007 started the accrual of this study, a multicenter, open label, randomised, phase III clinical trial of not inferiority aimed at identifying the best therapeutic adjuvant strategy in radically resected colon cancer (stage II/III) patients. The study is sponsored by “Fondazione Giscad per la Cura dei Tumori” and supported by the “Agenzia Italiana del Farmaco” (AIFA) - Bando per la ricerca Indipendente 2005.

According to the factorial design, this project consists of two independent substudies, following specific eligibility criteria and different randomisation schemes studies, called DURATION study and BEV study. Once randomised in the duration study, patients fulfilling eligibility criteria for BEV study may also be randomized to receive BEV or no BEV, in addition to FOLFOX-4 chemotherapy only.

Duration study is designed to optimize FOLFOX-4 treatment duration, evaluating the efficacy and safety of a 3-month FOLFOX-4 treatment vs. a 6-month FOLFOX-4 treatment.

Bev study is designed to assessing the benefit of the addition of BEV to the FOLFOX-4 regimen.

Since 2010, after substantial amendment, it was given the possibility to use XELOX regimen (12 vs 24 weeks) as an alternative to FOLFOX-4 treatment, for patients that not participating in the sub-study with bevacizumab.

The objective and the primary endpoints are:
- To assess whether a 3-month (6 cycles) FOLFOX-4 treatment or 12-week (4 cycles) XELOX treatment is at least not inferior to a 6-month (12 cycles) FOLFOX-4 treatment or 24-week (8 cycles) XELOX treatment in terms of RFS in patients with radically resected stage II/III colon cancer
- To assess whether the combination of BEV and FOLFOX-4 is superior to FOLFOX-4 alone in terms of RFS in patients with radically resected high-risk stage III (T4, N+, M0, or any T, N2, M0) colon cancer

This is an event driven study. The study will continue until approximately 1270 and 390 events have occurred in patients enrolled in the DURATION study and BEV study, respectively. In order to achieve this targeted number of events in the DURATION study, it will be necessary to randomise 2860-4100 patients, based on the observed case-mix of the patients, while in the BEV study it will be necessary randomise 430-620 patients.

BEV study was prematurely closed in December 2010 incorporating the recommendation of Data Safety Monitoring Committee following the negative results of the NSABP C-08 and AVANT trials. Patients are still being followed-up.

The duration of study’s enrollment is finished on April 2013, 3759 patients have been randomised.

Out of these, 534 was recruited, following signature of dedicated and further informed consent, in an ancillary pharmacogenetic study aimed at evaluating the association between 17 polymorphisms on 11 genes involved in the action mechanism of 5-fluorouracil and oxliplatini...
or in the detoxification mechanism and clinical outcomes, for identifying patients who are likely not gaining optimal results in terms of disease free survival and patients more prone to suffer from side-effects.

**COMETS** – Open-label randomized, parallel group, phase III, multicenter trial comparing two different sequences of therapy (irinotecan/cetuximab followed by fluorouracil/leucovorin with oxaliplatin (FOLFOX-4) vs. FOLFOX-4 followed by irinotecan/cetuximab) in metastatic colorectal patients treated with fluorouracil/leucovorin with irinotecan (FOLFIRI)/bevacizumab as first line chemotherapy

On September 2009 the accrual of COMETS study was started, a randomised, phase III clinical trial aimed at comparing the efficacy and safety of two different sequences of chemotherapeutic agents in order to optimize the treatment of patients with metastatic colorectal cancer progressed to a first line chemotherapy with FOLFIRI and Bevacizumab.

The study is sponsored by “Fondazione Giscad per la Cura dei Tumori” and supported by the “Agenzia Italiana del Farmaco” (AIFA) - Bando per la ricerca Indipendente 2006.

The primary objective is to compare the efficacy of FOLFOX-4 followed by Irinotecan + Cetuximab versus Irinotecan + Cetuximab followed by FOLFOX-4 in terms of progression free survival.

The secondary objectives are overall survival, quality of life, toxicity, health resource utilization and economic evaluation.

This is an event driven study. The study will continue until approximately 101 events have occurred in patients enrolled. In order to achieve this targeted number of events, it will be necessary to randomise 110 patients.

The study enrollment is ongoing and up to January 2014, 107 patients have been randomized.

**Head & Neck cancer**

In Italy, Squamous cell carcinoma of the head and neck accounts for 5% of all cancers in adult patients. More than 12,000 new cases per year are projected, whereas worldwide they amount to more than 500,000.

It is a potentially curable malignancy when diagnosed at an early stage. Unfortunately, 60% of the patients present with advanced inoperable locoregional disease and a considerable proportion of the patients relapse either locally or at distant site.

Concomitant chemo-radiotherapy is the standard treatment for locally advanced squamous cell carcinoma of the head and neck, while, for resectable patients, standard treatment is surgery plus post-operative radiotherapy with or without adjuvant chemotherapy.

The laboratory has activated three trials on head and neck cancer:

**H&N07- Neoadjuvant docetaxel plus cisplatin and 5-fluorouracil (TPF) followed by radiotherapy plus concomitant chemo or cetuximab versus radiotherapy plus concomitant chemo or cetuximab in patients with locally advanced squamous cell carcinoma of the head and neck. A randomized phase III factorial study**

Randomized multicentre (60 Italian sites participating) open label, phase III factorial trial is the implementation of a previous phase II randomized trial and it is sponsored by AVAPO-Ricerche Venezia. Patients with locally advanced squamous cell carcinoma of the head and neck are eligible for the study. The total study period is approximately 6 years (4 years of recruitment + 2 years of follow-up); the total number of patients enrolled is 421. According to factorial design, the trial aims to compare the efficacy in terms of overall survival of a neoadjuvant chemotherapy on TPF regimen (docetaxel, cisplatin, 5-fluorouracil), followed by a concomitant chemo-radiotherapy or radiotherapy plus Cetuximab. This study also compares the tolerability...
of the concomitant chemo-radiotherapy vs. radiotherapy plus Cetuximab treatment, irrespective of the prior neoadjuvant chemotherapy. The accrual of the study started in March 2008 and ended on April 2, 2012, whereas the follow-up period is still active and it will continue until the reaching of expected events. It is planned to publish the final results of the study by 2014.

TPF HN10/01 - Phase II study of preoperative TPF chemotherapy in locally advanced resectable oral cavity squamous cell cancer in order to improve the rate of pathological complete response

Single-arm, multicentre (16 Italian sites participating), phase II trial, sponsored by the IRCCS – Fondazione “Istituto Nazionale Tumori” di Milano, conducted in patients with resectable locally advanced squamous cell cancer of oral cavity, clinically suitable to receive a preoperative chemotherapy treatment and who present the predictive factors of complete response to therapy (functional p53 protein status and/or low expression of beta-tubulin II). Patients will be treated with 3 cycles of chemotherapy on TPF regimen (docetaxel, cisplatin, 5-fluorouracil), after that they will undergo surgery for excision of the tumour. In case of histological features, a post-surgical radiotherapy will be performed. The aim of the study is to evaluate the proportion of complete pathological responses after induction chemotherapy and surgical removal. It will also assess whether the molecular profile of the tumour could provide indication on the appropriateness of association of a cytoreductive chemotherapy to standard surgical therapy.

The accrual of the study has started in April 2013 and the number of patients required is 67 with a period of accrual of 12-18 months and a follow-up period of 6 months. To date 7 patients have been recruited.

B490 – Cetuximab and Cisplatin with or without Paclitaxel in recurrent/metastatic head and neck cancer

On June 2012 started the accrual of B490 study, a randomised, phase II-B clinical trial aimed at assessing whether a treatment based on Cetuximab and Cisplatin is at least not inferior to a treatment based on Cetuximab and Cisplatin and Paclitaxel. The primary endpoint is the progression free survival. The secondary endpoints are the overall survival, response rate, toxicity profile and the study of predictive and prognostic markers in tumor tissue.

The study is sponsored by “Istituto Nazionale dei Tumori di Milano”.

The study will continue until approximately 164 events have occurred. In order to achieve this target number of events it will be necessary to randomise approximately 200 patients.

The study is ongoing and up to January 2014, 49 patients have been randomized.

MUCOSITIS DUE TO CHEMO-RADIOThERAPY – Double-blind, randomised parallel trial comparing morphine mouthwashes to placebo mouthwashes.

The study, which is promoted by A.O. Santa Maria di Terni, is supported by AIFA. Study population is represented by head and neck opioid naïve cancer patients receiving chemoradiotherapy both as exclusive and postoperative intent and developing painful mucositis due to treatment. Patients will be randomized to receive topical morphine as mouthwashes plus rescue doses of normal release oral morphine if needed or topical placebo as mouthwashes plus rescue doses of normal release oral morphine if needed. A number of 140 enrolled patients is expected. The primary objective will be to assess the analgesic efficacy of morphine mouthwashes versus placebo mouthwashes in terms of difference in total dose requirement of systemic opioids (as rescue morphine medication or continuous opioids administration) via oral (morphine),
transdermal (fentanyl patch) or parenteral (morphine) routes, expressed as equivalent oral morphine dose during the treatment.

The secondary objectives will be to evaluate:

- mean intensity of pain during the entire period of study and number of days spent with a level of pain intensity ≥ 4, assessed daily by means of numerical rating scale 0-10 (0=no pain; 10= the worst pain) during the previous 24 hours;
- opioid related adverse effects (drowsiness, nausea, vomiting, constipation and confusion) are assessed by means of a verbal scale with four grade intensity (No, a little, much, very much);
- total number of doses of NSAIDs required in case of failure of rescue opioids;
- quality of life, evaluated weekly through EORTC QLQ-C30 and EORTC QLQ-HN35 questionnaires;
- the need for nutritional support, expressed as number of days spent with feeding tube; percentage of weight loss from randomization;
- number of days of hospitalization and day hospital required for support therapy due to oral mucositis.

Twenty patients will take part in the pharmacokinetic study. Venous blood samples will be taken for measurement of plasmatic morphine concentrations. The trial is completing its activation procedures and the patient enrolment is expected within the first months of 2014.

Activities in onco-gynecological

The Mario Negri Gynecologic Oncology group (MaNGO) is a new name for a collaborative group that has been active in clinical gynecologic oncology for several years. Infact, this group consolidated its network and logistics while running the ICONs studies which were conducted in very close partnership with researchers at the Medical Research Council, Clinical Trial Unit, UK. MaNGO was formally set up in May 2006 and is mainly representative of the northern part of Italy, although there are important sites in the central and southern part of the country too. Participating centers are either general public and private hospitals or university clinics. One of MaNGO’s main statutory objectives was to foster an active collaboration with the Gynecologic Cancer Intergroup (GCIG), and the European Network of Gynaecological Oncology Trials groups (ENGOT) that represent two International Forum circulating the scientific proposals from many national collaborative groups. MaNGO group is actively involved in many international phase III trials.

MaNGO has been coordinating the Italian participation to the PORTEC 3 study: this is an academic randomized phase III trial in endometrial cancer promoted by the Dutch collaborative group. MaNGO received government funds from the Italian Agency for Drugs (AIFA) supporting its national coordinating role. International accrual was stopped in December 2013 as the targeted sample size of 686 patients was reached. MaNGO network was represented by 14 active clinical sites throughout Italy and globally randomized 103 patients into the trial.

In 2010 MaNGO launched the TAUL study, a randomized phase II trial aimed to evaluate the efficacy of trabectedina in the treatment of patients with uterine leiomyosarcoma. As of December 2013, the number sites activated in Italy was 24 and 110 patients suffering from this rare disease have been enrolled into the study out of a calculated final sample size of 150-160 patients. During 2013 a total of 40 the tissue samples have been collected and the expected translational data of putative prognostic/predictive markers will soon be analysed.

During 2013 the MaNGO reactivated the INOVATYON protocol. This is an academic, international, phase III, randomized clinical trial aimed at comparing the combination of
pegylated liposomal doxorubicin+carboplatin with the combination of pegylated liposomal doxorubicin (PLD) and trabectedin in partially platinum sensitive ovarian carcinoma relapses. During all 2012, this trial was kept “on hold” due to the worldwide shortage of PLD. This happened because of the shutdown of the only site of PLD manufacturing in the USA. Most of the Italian sites of the INOVATYON network are fully operative (2 new patients were enrolled) while the European sites will be open up in Q1 2014.

During 2013, MaNGO in partnership with the other onco-gynecologic group named MITO, activated a phase III randomized study (MITO-16b / MaNGO-Ov2b) aimed at comparing the disease free and overall survival of patients undergoing II line chemotherapy plus bevacizumab or chemotherapy only. Eligible for this trials are patients with ovarian cancer patients who had already received bevacizumab in first line treatment.

In 2013 MaNGO launched a phase II randomized but non-comparative trial aimed to assess the efficacy and safety profiles of the therapeutic regimens (trabectedin and bevacizumab +/- carboplatin) in partially platinum sensitive ovarian cancer patients. The planned sample size is about 80 patients and the trial will be implemented in about 5 site of the MaNGO network. Since July 2013, 9 patients have been involved in this trial. Enrolment is still ongoing.

During 2013, MaNGO’s Technical-Scientific Committee met quarterly while MaNGO affiliates were conveyed at the 10° General Assembly that was held in June.

**Pain Unit activities**

The activities at the Research in Pain and Palliative Care Unit aim to further research and knowledge in the area of pain and its management in the field of palliative care as part of the more general program of activities promoted by the Institute. In addition, the unit conducts several clinical trials on cancer pain and systematic reviews of the literature.

During 2011, was started the clinical study CERP, a multi-center, open-label, prospective study evaluating the effects of different pharmacological strategies to treat pain in cancer patients. This study also includes an ancillary pharmacogenomic project: evaluation, in parallel to the main project, of the genetic profiles of patients and their potential correlations to observed clinical effects. During January 2013 we carried out an investigator meeting with researchers from the 45 centers that collaborate actively in the study. At the end of 2013 the number of patients recruited was 406. The end of study is scheduled for 2014.

During 2013, was started the observational study RER, a prospective longitudinal observational study to evaluate clinical characteristics and treatments using opioids in patients with breakthrough cancer pain (BtCP). The coordination of the research project on BtCP, to be implemented at the oncology and oncohematology network centers of the Emilia-Romagna, has been entrusted to the CERP. The main objective of this study is to evaluate the clinical characteristics of BtCP (number and duration of episodes, time to reach the peak of pain, maximum intensity, trigger mechanisms) and the related patterns of care, in a sample of cancer patients suffering from pain of moderate-severe intensity based, already in therapy or in the beginning phase of treatment with opioids of 3rd-step, and with episodes of BtCP, treated with rescue therapy with opioids, followed longitudinally for a period of 28 days (visits on days 0, 7, 14, 21, 28). In this year we carried out an investigator meeting. At the end of 2013 the number of patients recruited was 6.

In this year we also defined the drafting of the protocol of one observational study, named as GREAT (Good REsponse with Appropriate Treatment): “Factors affecting the analgesic response association oxycodone-naloxone in the treatment of pain in cancer patients”. In December we organized a first advisory board meeting to discuss a draft of protocol.
In addition, the Unit is responsible for the assessment of the quantity and quality of available evidence on the epidemiology of pain, its characteristics and effectiveness of pharmacological analgesia. It was published a systematic review of studies that have assessed the frequency of pain incident documenting the extreme variability of the frequency of this phenomenon in clinical trials. A further review of the literature published in December, has allowed us to assess the pharmacokinetics/pharmacodynamics and clinical properties of all formulations of fentanyl transmucosal on the market for this type of cancer pain.

In March 2013, in the context of formative activities organized by CERP, at Mario Negri Institute were held lessons of the clinical module of the 13th “Master in Palliative Care at the End of Life” in collaboration with the University of Milan. Continued collaboration with the European network for research in the field of palliative care that has produced some scientific papers (published or in press).

Other

TERAPORA – Oral anticancer drugs: nurse interventions to improve therapy management and patient safety

Recent published data suggest the benefit of an active monitoring to improve the efficiency and safety of anticancer oral therapy administration, confirmed by the Italian monocentric pilot study experienced by Sacro Cuore Don Calabria Hospital of Negrar. These results show the potentiality of nurse active monitoring on patients in decreasing the improper accesses into first aid and in controlling the toxicity trough: 1) accurate information given to the patient 2) administration of a daily record on which the patients will take note of taken drug dose and symptomatology eventually occurred 3) a telephone monitoring that means two phone interviews during first month of therapy and one during the second month. The experience of Negrar produced the reduction of proportion of graded 3 toxicities among the patients from 12% to 6% and the number of improper accesses into first aid from 17% to 7% compared with obtained data in the same hospital in the previous year.

An observational, multicentre randomized study is in progress; study is sponsored by the Associazione Italiana di Oncologia Medica (AIOM). Patients will be randomized in the “active” intervention arm or in the “control” arm. “Active” intervention consists of giving an accurate information given by nurse to the patients before starting therapy, a survey on toxicity by nurse according to CTCAE grade, a daily record to the patients and phone interviews to check out the presence of toxicities during therapy. Patients enrolled in the “control” group will be followed according to standard organizational and informative ways of each centre. The observation will last the first two cycles of therapy independently from single cycle duration (3, 4 or 6 weeks). Is expected the enrollment of 430 patients in 28 centers. The first objective is to assess the proportion of patients with improper accesses into first aid, whereas the secondary objectives are to assess the proportion of patients with severe toxicity, the concordance between toxicity observed during the medical examination and the toxicity deduced from phone interviews and the adherence to nurse intervention protocol. To date 215 patients have been enrolled.

Retroperitoneal sarcomas

Retroperitoneal soft-tissue sarcomas (R-STSs) are rare neoplasms, accounting for 10% to 15% of STSs, which represent 1-3% of all cancers. They may show different histological types, but the predominant ones in the retroperitoneal region are: leiomyosarcoma, liposarcoma.
Liposarcoma comprises three distinct histological subtypes: well differentiated/dedifferentiated, myxoid and pleomorphic. The most commonly encountered in the retroperitoneum is the well differentiated/dedifferentiated Liposarcoma. Surgery is the mainstay of treatment in localized disease. Indeed, in all primary sarcomas, local control is critical and largely depends on the extent of resection; since anatomic constraints limit the achievement of wide resection margins, local recurrence is much more frequent than at any other anatomic site and is the leading cause of death. Patients with unresectable or metastatic disease usually receive chemotherapy. Chemotherapy may also be reasonable in patients with a recurrent local regional disease having a short previous free interval. First-line chemotherapy usually consists of doxorubicin and/or ifosfamide. These two drugs are the most active agents in adult STSs with a dose-response relationship and response rates between 20% and 50%. However, the sarcoma community is currently doubtful as to the activity of ifosfamide in the subgroup of leiomyosarcomas. Trabectedin is an anticancer agent derived from a natural marine product. This drug has been found to be mainly active in leiomyosarcoma and liposarcoma and is approved by EMA as second-line chemotherapy for STSs. Trabectedin binds in the minor groove of DNA, alkylating the N2 of guanine and affecting transcription regulation in gene- and promoter-dependent fashion.

TRAVELL is a phase II, non-randomized, multicentre study conducted in patients with leiomyosarcoma and well differentiated/dedifferentiated liposarcoma. The study will be conducted in Italy in approximately 20 investigational centres, in order to recruit 95 patients over a 4 years period. This study is aimed at confirming the activity of trabectedin as second/further line treatment in retroperitoneal leiomyosarcoma and well as differentiated/dedifferentiated liposarcoma. Another objective of this study is to investigate the peculiar benefit provided by trabectedin in typical retroperitoneal sarcomas, in order to help multidisciplinary clinical decision-making. The primary end point of the study will be the proportion of responders to trabectedin, based on the ratio, in each single patient, between PFS under trabectedin (PFS) and time to progression after previous chemotherapy treatment (TTP1).

Secondary end points will be:
- Objective response (OR) in the overall sample
- Pathological tumour response in the two eligible histological types, in patients undergoing surgery after treatment
- PFS and OR in the two eligible histological types
- PFS in patients who undergo surgery after, or during, medical therapy and those who do not
- Safety profile
- Efficacy of trabectedin in reducing cancer related pain

Translational studies will be performed with the aim of characterising the tumour biological features associated with different response patterns to trabectedin. These assessments will be done in 15-20 patients who will undergo surgery after trabectedin, comparing tumour tissue specimens collected before and after treatment. The study is concluding the authorization procedures.

Glioblastoma
Glioblastoma is the most common and most aggressive malignant primary brain tumor in humans, involving glial cells. Glioblastoma occurs mostly in adults (median age of 64 years at diagnosis) with an estimated incidence of 2–3 cases per 100,000 people in Europe and North
America with 1- and 5-year overall survival (OS) rates of 29% and 3%, respectively, the prognosis of glioblastoma is poor and the development of more effective therapeutic approaches is imperative. Although important progress has been made in the last few years, the treatment of glioblastoma is still one of the greatest challenges in the field of oncology. The management of glioblastoma requires a multidisciplinary approach including repeat surgery, stereotactic radiosurgery, combinations of repeat surgery with local/second line chemotherapy, anti-angiogenic treatment with Bevacizumab, treatment with Fotemustine. In Europe, Fotemustine, a third generation nitrosourea, is one of the most practiced options in the setting of glioblastoma relapse. All of these treatments, however, ultimately fail, due to a number of factors, among which failure to achieve persistent tumoricidal concentrations of the drug in the tumor is one of the most relevant.

One clinical trial is open on this disease.

**ORTATAXEL - Multicenter, randomized, non-comparative, open-label phase II trial on the efficacy of Ortataxel and Fotemustine in recurrent glioblastoma**

This study is a multicenter, Italian, non-comparative, randomized, phase II clinical trial aiming to assess the Ortataxel efficacy in recurrent glioblastoma. Ortataxel is the experimental treatment, a second-generation taxane that crosses the blood-brain barrier and which is distinguished from the currently approved taxanes because it is not a substrate for the P-glycoprotein (Pgp-170); Fotemustine is the calibration arm treatment. Patients fulfilling the eligibility criteria will be randomized to receive Ortataxel or Fotemustine with a 2:1 ratio, respectively.

The study is divided in two stages: 50 patients will be enrolled in the first stage and the number of patients will reach 87 at the end of the second stage. At each stage the results of the calibration arm will be evaluated to assess the adequacy of the enrolled sample; only if the results of the calibration arm (Fotemustine) are consistent with those expected, the analysis of experimental arm (Ortataxel) will be performed. The study primary objective is to evaluate the efficacy of Ortataxel in terms of progression free survival at 6 months (PFS-6) from the randomization. The secondary objectives are the objective response, safety and treatment compliance. The study is on-going and up to December 2013, 1 patient has been randomized.

**DENDR-STEM – A phase I study of Immunotherapy with GSC-loaded dendritic cells in patients with recurrent glioblastoma**

Cancer immunotherapy strategies pointed to re-education of the immune system to eradicate the tumour met some recent success in prostate carcinoma and melanoma. However, the capacity of escaping immune responses, now considered one hallmark of cancer, limits the efficacy of immunotherapy. One example has been provided in glioblastoma (GBM), the deadliest of primary brain tumours with average survival of 15 months. A number of studies have shown that in GBM and other cancers a sub-population of cells, defined as cancer stem-like cells, express stem cell programs and is responsible for tumour perpetuation. The reacquisition of stem cell features may be critical for tumour survival under environmental challenges like hypoxia. Thus, targeting GBM stem-like cells (GSC) is one strategy to increase the potential efficacy of GBM immunotherapy. DENDR-STEM is a phase I study aiming to test for the first time in patients with recurrent glioblastoma (GBM) the biological activity, safety and feasibility of a novel target for dendritic
cell (DC) immunotherapy: GBM stem-like cells (GSC). Data on the immunological evidence of response, and hints on the potential survival gain provided by the treatment, will be collected. The study, which is undergoing approval will enrol approximately 20 patients with recurrent GBM in order to reach 12 patients with measurable response. A preliminary consent for processing the surgically removed tumour tissue will be obtained from patients prior to diagnostic- ablative surgery. Patients with diagnosis of GBM and a positive laboratory reply will then be enrolled in the clinical study and subjected to leukapheresis and treatment with seven doses of GSC-loaded homologous dendritic cells. The activity of this immune therapy protocol will be evaluated measuring the variation in the levels of several immune system variables from baseline to the second vaccine administration. The safety outcome includes the evaluation of auto-immune reactions. The feasibility assessment will evaluate the proportion of cases with GSC positive growth.

Non-oncological diseases

Studies in ophtalmology

Glaucoma is one of the main causes of visual impairment and is now recognized as the second most frequent cause of blindness in industrialized countries. In patients with glaucoma there is a progressive increase in intraocular pressure resulting in damage to the optic nerve. It causes visual field defects, which are usually asymptomatic until the macula (the anatomical site of central vision) is affected. The aim of the therapy currently available for glaucoma treatment is to reduce the intraocular pressure up to a level considered safe, to preserve the visual quality of patients and their quality of life. The treatment options are represented by topical drugs, followed by more invasive procedures such as laser trabeculoplasty and incisional surgery. However such kind of therapies, because of their side effects, are playing an important role on the patient quality of life, particularly because it is sometimes required to start treatment before development of appreciable visual defects.

On this disease there are two studies ongoing in our laboratory:

**IRFMN-OG1** - Multicenter, observational study on quality of life (QoL) in patients with glaucoma in Italy.

Multicenter, observational study on QoL in patients with glaucoma in Italy. The project includes a substudy in a longitudinal cohort of patients at first diagnosis of glaucoma followed prospectively for one year. The population consists of patients aged ≥18 years with instrumental diagnosis of primary open-angle glaucoma. The objective of the transversal phase is to assess the QoL by a single visit; moreover the longitudinal phase evaluate the change in QoL in relationship with the disease progression (subjects at first diagnosis should be followed in two subsequent visits at 6 and 12 months). The assessment of QoL is performed by the administration of two validated questionnaires (the National Eye Institute Visual Function Questionnaire -NEI -VFQ -25 and the Glaucoma Symptom Scale - GSS).

Patients enrollment started in March 2012 in 21 Italian centers and it was closed, in line with expectations, at the end of July 2013, when the forecast number of patients (3000 in total and 200 at the first diagnosis) was reached. In the study were actually enrolled a total of 3226 patients (224 patients at first diagnosis which will be followed prospectively for one year after baseline). An abstract with preliminary study results has been submitted at ARVO 2014 and the drafting of papers is currently ongoing.
**PEDIATRIC GLAUCOMA - Experimental study, single-arm, phase II trial, on pediatric population suffering from congenital glaucoma, treated with prostaglandin analogues and / or carbonic anhydrase inhibitors**

Experimental study, single-arm, phase II trial, conducted in pediatric population suffering from congenital glaucoma, treated with prostaglandin analogues and / or carbonic anhydrase inhibitors.

The population consists of children aged from 0 to 12 years with a diagnosis of congenital open-angle glaucoma refractory to surgical treatment. The protocol is designed to evaluate the effectiveness (in terms of hypotensive effect) and safety of prostaglandin analogues and carbonic anhydrase inhibitors (dorzolamide and latanoprost) administered topically.

The study is supported by AIFA, the first patient was enrolled in July 2009 and in January 2014 the recruitment phase ended.

**Otorhinolaryngology**

Obstructive sleep disordered breathing (OSDB) is a common and serious cause of metabolic, cardiovascular, and neurocognitive morbidity in children. The spectrum of OSDB ranges from habitual snoring to partial or complete airway obstruction, termed obstructive sleep apnea (OSA). The etiology and pathophysiology of OSA in children are multifactorial, however adenotonsillar hypertrophy plays a major role in its pathogenesis. The volume of lymphoid tissue in the upper airway increases from about six months of age up to puberty, with maximum proliferation occurring in the preschool years, which coincides with the peak incidence of obstructive sleep apnea syndrome (OSAS) in children. The traditional surgical procedure for treating OSAS in children is extra-capsular tonsillectomy (ECT) that involves the complete removal of the tonsil by anatomic extra-capsular dissection. The widespread use of classic ECT is decreasing in our days despite the development of several new techniques, aimed at reducing post-operative morbidity. Among those, the intra-capsular tonsillotomy (ICT) or partial tonsillectomy has been advocated in the last few years as an effective and safer than ETC method in OSDB treatment. The rationale to perform ICT is that tonsillar capsule and pharyngeal muscles are not violated, preventing them from sustained injury and inflammation, thus resulting in lower post-operative pain and a more rapid recovery.

Evidences of the efficacy of the two different surgical procedures derive from not randomized, retrospective, mono institutional studies, carried out on a restricted number of patients and involving a single instrumental technique. There is one trial open on this disease:

**ECT VS ICT - Extra-capsular tonsillectomy (ect) vs intra-capsular tonsillotomy (ict) in children with symptomatic tonsillar hypertrophy**

This is an Italian, multicenter, randomized, open-label study comparing two different surgical techniques (Extra-capsular tonsillectomy - ECT vs. Intra-capsular tonsillectomy - ICT) in children with symptomatic tonsillar hypertrophy.

The primary aim of the study is to compare and evaluate the safety of ICT versus ECT in terms of postoperative hemorrhage risk in children with symptomatic tonsillar hypertrophy. This study, sponsored by Associazione Otorinolaringologi Ospedalieri Italiani, foresees the involvement of 10 centers and 3 are going to start-up. Due to complexity to enroll young patients, this study has been stopped in December 2013.

**Other activities**

**Quality Assurance**

Our quality management system is on-going and it is applied in the Laboratory of Clinical Research. Our Quality Assurance System is designed to ensure the quality of execution of
clinical trials that are developed in the Laboratory. To reach this goal we proceed with the writing of standard procedures (SOP), the maintenance of documentation of clinical studies and the development of validation processes. This will ensure that the conduct of clinical trials be done in accordance with the requirements of Good Clinical Practice and Regulations in force. During 2013, the maintenance activities of the validation of our electronic data collection (HeavyBase) is continued and has also started the development of procedures in order to align the management and oversight of clinical data (Data Management) to international standards. This activity has been put in place to meet the quality standards required by Ecrin (European Clinical Research Infrastructures Network) to act as their Data Management Center, and, in this way, collaborating with clinical trials coordinated by this network.

Monitoring
The laboratory carries out monitoring activity for clinical trials sponsored by other research institutions or collaborative groups, in particular, it is working with the Italian Group for Breast (GIM) for a phase III trial called “First Adjuvant Trial on All aromatase inhibitors in early breast cancer” (FATA). The aim of this study is to compare anastrozole, letrozole and exemestane used upfront (for 5 years) to sequentially (anastrozole, letrozole and exemestane administered for 3 years after 2 years of tamoxifen) as adjuvant treatment for postmenopausal patients with endocrine-responsive breast cancer. The laboratory is also involved, for monitoring activities, in a clinical trial with the European collaborative group "Swiss Group for Clinical Cancer Research" (SAKK). This project is an international phase II trial with rituximab or rituximab plus lenalidomide monotherapy for patients with follicular lymphoma. The laboratory is also part of another international project sponsored by the Department of Neonatology of the Rigshospitalet in Copenhagen, with the aim to assess the feasibility of an instrument for monitoring the brain tissue oxygenation in premature infants.

Given the growing importance of monitoring as part of the Clinical Research it was established a Competition Announcement to select the admissions to the "Course of Clinical Monitor" which will take place at the Institute starting from February 2014. The purpose of the course, which will last eighteen months, is to train certified Clinical Monitors who will have fully autonomous in carrying out monitoring activities, as fixed in Annex 1 of the Ministerial Decree of 15th July 1997.

Systematic reviews and meta-analyses
The laboratory performs systematic reviews and meta-analyses, mainly in the oncologic, diagnostic and ophthalmologic areas.

The systematic reviews completed or ongoing in 2013 were about:
- Performance of different diagnostic techniques in use in clinical practice in the diagnosis of pancreatic adenocarcinoma.
- Performance of contrast-enhanced ultrasonography (CEUS) in the diagnosis of pancreatic lesions
- Efficacy in terms of tumor local control of single- and multi-session (hypofractionated) radiotherapy in patients with meningiomas.
- Effects of acromegaly on bone metabolism
- Efficacy and safety of non-penetrating surgical techniques, compared to the standard technique (trabeculectomy), in reducing intraocular pressure and incidence of complications in the treatment of open-angle glaucoma.
- Efficacy and safety profile of pre/post surgery thromboprophylaxis pharmacological treatment in major surgery of the hip and knee.
- Efficacy and safety profile of a concomitant chemo-radiotherapeutic treatment compared with a radiotherapeutic treatment in patients with head and neck carcinoma.
Statistic
The laboratory, through a dedicated unit, offers methodological support and statistical analysis cooperating with clinicians developing research projects. These activities lead to many publications regarding techniques for glaucoma diagnosis and the accuracy of diagnostic modalities for tumour stadiation.

Informed consent project
In October 2013, thanks to collaboration between the Laboratory of Clinical Research and the Laboratory of Medical Research and Consumer Involvement (Mario Negri Institute) a new project is started, aimed to investigate and examine the structure of informed consent and privacy policy through the years. The objective of the study is to evaluate how the informed consent and the note attached has changed, in particular the number and type of information given to patients involved in phase II and III clinical trials in oncology.
In order to examine these documents, was asked to the main pharmaceutical companies and more independent structures involved in clinical research, to provide a copy of the informed consent and protocols of two randomized trials with drugs in oncology as older over time, and two recent studies (2013).
In the meantime, with the help of lay people and experts in Ethics Committees, we are preparing a tool for assessing the clarity and completeness of the material with the terms of reference, in order to have parameters as objective as possible during this review.
The final analysis provides a qualitative assessment on the contents of the documents analyzed and it will be shared through a meeting with those who have agreed to the project.
DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES

STAFF

Head Roberto FANELLI, Biol.Sci.D.

Laboratory of Analytical Biochemistry
Head Chiara CHIABRANDO, Biol.Sci.D.

Laboratory of Environmental Chemistry and Toxicology
Head Emilio BENFENATI, Chem.D.

Industrial and Environmental Health Unit
Head Marco LODI, Chemist

Laboratory of Food Toxicology
Head Ettore ZUCCATO, M.D.

Environmental Biomarkers Unit
Head Sara CASTIGLIONI, Biol.Sci.D.

Laboratory of Mass Spectrometry
Head Enrico DAVOLI, Anim.Sci.D.

Laboratory of Molecular Toxicology
Head Luisa AIROLDI, Pharm.D.

Protein and Gene Biomarkers Unit
Head Roberta PASTORELLI, Biol.Sci.D

Department’s Units

Environmental Pollutants’ Risk Assessment Unit
Head Elena FATTORE, Biol.Sci.D

Analytical Instrumentation Unit
Head Renzo BAGNATI, Chem.D.
CURRICULA VITAE

Roberto Fanelli, Head of the Environmental Health Sciences Department since 1997, Laboratory Head 1978-97, Researcher 1975-78, Research fellow 1969-74 at the Mario Negri Institute.

Doctoral Degree in Biological Sciences (University of Milan, 1973), Assistant Professor in Biochemistry at Baylor College of Medicine (Houston, Texas). Member of the Commissione Consultiva Prodotti Fitosanitari (Ministero Salute), Member of the Scientific Panel on Contaminants in the Food Chain (European Food Safety Authority, 2003-2006), Certified Italian Toxicologist.


Selected publications:

Luisa Airoldi, Head of the Molecular Toxicology Laboratory since 1994, Unit Head 1987-94, Researcher 1978-87, Technician 1967-75 at the Mario Negri Institute.

Doctoral Degree in Pharmacy (University of Milan, 1975), Postdoctoral fellow at the Massachusetts Institute of Technology (Cambridge, MA, 1976) and at the Northwestern University Medical School (Chicago, IL, 1977), Researcher at the Yale University Medical School (New Haven, CT, 1980-81).

Research areas: Proteomics in toxicology with particular interest on the study of proteome changes in tissues and biological fluids from animals and humans after exposure to toxic compounds; clinical proteomics aimed at the identification of protein biomarkers as diagnostic tools; molecular epidemiology focused on the identification and measurement of biomarkers of exposure to environmental carcinogens and disease susceptibility.

Selected publications:

Research areas: Computer-based models for chemistry and toxicology; Molecular descriptors; QSAR; Toxicity prediction; Metabolism studies; Characterization and assessment of wastes, industrial effluents, emissions from landfill and incinerator; Integration of chemical analysis and eco-toxicological data; Chemical analysis of organic compounds by mass spectrometry.

Selected publications:


Selected publications

Doctoral Degree in Animal Sciences (University of Milan, 1983), Postdoctoral fellow at the University of Nebraska (Lincoln, NE, 1987) and at the University of Colorado Health Sciences Center (Denver, CO, 1988). Postgraduate degree in Pharmacological Research, Mario Negri Institute (1988). Member of the American Association for Mass Spectrometry (ASMS) of the Environment and Energy Commission, of the Safety Commission of IGQ and of the ETS (Emission Trading System) commission. Member of the National Biomass Research Center Scientific Committee. Environmental Applications Interest Group (ASMS).

Research areas: Development of methodology, instrumentation and software for environmental research. Studies of urban air pollution and characterization of environmental odor annoyance.

Selected Publications


Doctoral degree in Medicine (University of Milan, 1986), Postdoctoral degree in Human Nutrition (1999), Postdoctoral fellow at the King’s College School of Medicine (London, UK, 1988-89).

Member of the ANSISA, EMEA expert, member of the Commissione Consultiva per i Prodotti Fitosanitari, and expert for the evaluation of plant protection products for registration within the EU.

Research areas: Food safety, including the study of dietary chemical contaminants, safety assessment of GMO in human nutrition, food allergens and toxicants, emerging issues in food toxicology, risk perception and risk communication to the consumers, and evaluation of plant protection products for registration within the European Union. Environmental pollution by pharmaceuticals, and monitoring of illicit drugs in surface waters to estimate community drug abuse.

Selected publications


Doctoral degree in Chemistry (University of Turin, 1985), Postgraduate degree in Pharmacological Research, Mario Negri Institute (1989).

Research areas: Mass spectrometry applied to the analysis of biological and environmental relevant substances (proteins, peptides, hormones, pharmaceuticals, drugs of abuse, pesticides).

Selected Publications
3. Selected publications


Doctoral Degree in Biological Sciences (University of Insbsria, Varese, 2000). Postdoctoral Degree in Environmental Analysis, Management and Protection of Biodiversity (University of Insbsria, Varese and Mario Negri Institute, 2002-2006). Postdoctoral Fellowship at University of New South Wales, Sydney, Australia (2004).

Research Areas: Sewage Epidemiology – use of wastewater analysis to study habits and consumption of some selected substances (i.e. illicit drugs) in the population producing wastewater. Monitoring occurrence and fate of several classes of emerging contaminants in the environment and evaluation of their biological and environmental effects.

Selected publications:


Doctoral Degree in Biological Sciences (University of Milan, 1991), Postgraduate degree in Pharmacological Research, Mario Negri Institute (1994), Postdoctoral fellow at the National Institute of Environmental Medicine, Karolinska Institutet, Stockholm (1998-2000). Member of the Working Group of External Scientific Experts to externally review the quality of the scientific outputs of the European Food Safety Authority (EFSA) in the area of activity of chemical risk assessment and connected fields (2010-2012).

Research areas: Environmental chemistry, toxicology, assessment of human exposure and risk from environmental pollutants with emphasis on dioxins and dioxin-like compounds.

Selected publications:


6. Grassi P, Fattore E, Generoso C, Fanelli R, Arvati M, Zuccato E. Polychlorobiphenyls (PCBs), polychlorinated dibenzop-dioxins (PCDDs) and dibenzofurans (PCDFs) in fruit and vegetables from an industrial area in northern Italy. Chemosphere 2010; 79: 292-298

Marco Lodi, Head of the Industrial and Environmental Unit since 2002, Consultant 1997-2002 at the Mario Negri Institute.

General Certificate of Education in Industrial Chemistry (Milan, 1974).

Member of AIDII (Italian Industrial Hygiene Association), certified by ACGIH (American Conference of Governmental Hygienist).


Selected publications:


2. Boriani E, Benfenati E, Baderna D, Thomsen M. Application of ERICA index to evaluation of soil ecosystem health according to sustainability threshold for chemical impact. Sci Total Environ 2013 443 : 134-142


Research areas: Proteomics-Metabolomics-System Biology. Investigations of global protein/metabolite expression profiles and their modulation in different biological compartments as a mean for biochemical and mechanistic studies (e.g. for understanding the onset and progression of human diseases, or for detailing regulatory modules in cells or subcellular compartments).

Selected publications:


ACTIVITIES

The Department works to investigate environmental factors and their effects on human health. The main research lines focus on the survey of environmental contaminants, the assessment of human exposure with related health risks, and toxicity mechanisms of pollutants. The assessment of environmental contamination is carried out not only for well-known and widespread compounds, like dioxins and PCBs, but also for new classes of "unconventional" pollutants, e.g., endocrine disruptors, potentially toxic "natural" compounds, and drugs entering the environment after human or veterinary use. The identification –for the first time– of illicit drugs in urban waste and river waters, led to a new original tool for the evidence-based monitoring of community drug abuse. For all these survey activities sophisticated analytical methods based on advanced mass spectrometric techniques are developed. The Department is active in the assessment of human exposure to toxic compounds in the atmosphere and the diet, which is the main source of priority pollutants (PCBs, dioxins and other endocrine disruptors). Assessment of the risk associated to contamination in real-life scenarios has recently gained much importance. In order to respond to the growing demand for information, the Department is more and more involved in toxicological and ecotoxicological risk analysis, based on studies in field and predictive models of toxicity. The activities on predictive models are done in collaboration with the US EPA, and public authorities of some European countries, such as Italy and UK. This produced a platform, VEGA (Virtual models for property Evaluation of chemicals within a Global Architecture), which is open to the public via the internet, for the prediction of toxicological and environmental properties. The nanomaterials have been also modeled with QSAR methods. The toxic effects of environmental contaminants on neurodevelopmental mechanisms of environmental contaminants are investigated in animal models in vivo and in vitro. Molecular epidemiology studies are used to identify genetic and/or environmental factors posing risks to human health. By this approach, we search for new useful “biological markers” to identify susceptible subjects, in view of finding appropriate preventive strategies.

The Department has implemented an advanced technological proteomic platform, in order to identify proteins differentially expressed in biological compartments in various experimental and clinical conditions. This approach is particularly relevant in toxicology, since it can contribute to find new biomarkers of toxicity or pathology, and to identify molecular targets and toxic effect mechanisms of pollutants and drugs. To integrate our proteomic studies, we have now introduced among our activities metabolomics, i.e., the study of small molecules, such as amino acids, carbohydrates, lipids, hormones etc., the final products of protein expression and activity which contribute to define the biochemical phenotype of a biological system. Mass spectrometry (MS) is a central analytical technique at the Department, where a complete set of state-of-the-art instrumentation is available, from GC-MS and LC-MS to MALDI-TOF-MS. These instruments are provided with modern solutions for sample introduction (chip-based nanoLC), sample ionization (ESI, DESI and MALDI), tandem MS (MSn) by triple quadrupole and TOF-TOF instruments, high mass resolution analysis (hybrid ion trap/orbitrap).

FINDINGS/MAIN RESULTS

We demonstrated in a fully translational investigation that the kynurenine pathways is activated

early following resuscitation from cardiac arrest in rats, pigs, and humans, and might have contributed to post-resuscitation outcome.

Untargeted and targeted metabolomics reveals perturbations in specific metabolic pathways involved in outcome of cardiopulmonary resuscitation in experimental animal models of cardiac arrest and thus potential mechanisms accounting for outcome of cardiac arrest.

Plasma proteome analysis identifies clusterin as a pre-diagnosis biomarker of colorectal cancer risk in a cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC) study.

Proteomic analysis of mouse brain in different ischemia models suggests metabolic down-regulation as a general feature of ischemic preconditioning, playing a pivotal role in neuroprotection.

Importance of NDL-PCBs as a risk factor in developmental neurotoxicity in laboratory rodents. Evidence of brain proteome alterations with detrimental consequences on cognitive functions in the offspring.

Evidence of new molecular players in the effects of TCDD on bone development provided by proteomics coupled to networks analysis.

Bone protein profile in a murine model of osteoporosis.

Identification of novel protein targets responsive to the effects of estrogens in bone.

TCDD's effect on the liver proteome profile of exposed rats. Determination of a subset of rat hepatic proteins indicative of differences in dioxin susceptibility.

The presence of 4-aminobiphenyl-hemoglobin adducts may help identify nonsmokers at high risk of cancers related to environmental tobacco smoke exposure.

Reference values of allele and genotype frequency of several metabolic genes in 15,000 control subjects.

CYP1A1 polymorphism affects lung tumor risk.

Identification of CYP2C9 genetic polymorphism as a determinant of severe adverse reactions to phenytoin.

Read across and in silico models to predict NOAEL for cosmetics.

New in silico models, freely available on-line, to predict toxicity and ecotoxicity of chemicals for the REACH European legislation. The tools have been used to predict properties of 4 millions chemicals.

A tool to assess if a chemical is bioaccumulaive, with a high rate of accuracy, avoiding the use of the experimental fish model.

The VEGA models for mutagenicity resulted to be the most predictive, in a comparison among 8 different models, achieving accuracy similar to that of the experimental methods.

There are almost one thousand of VEGA users world-wise.

A new index integrating risk assessment for human and ecotoxicity endpoints.

A method aimed at characterizing environmental odors to identify odor sources in complex environments.

Proteomic/bioinformatic workflow for comparative secretome analysis in cancer cell lines.

Global proteomic profiles of secretomes (different pancreatic carcinoma cell lines; pancreatic cell lines with or without oncogenic K-RAS transfection), with identification of perturbed functional networks. Accurate quantitative evaluation of protein dysregulation in the secretome by stable isotope labeling by amino acid in cell culture (SILAC) and mass spectrometry.

In depth structural characterization of gamma-conglutin, a bioactive legume seed glycoprotein by a glycoproteomic approach based on mass spectrometry and bioinformatic tools.

Illicit drug residues and their metabolites were found in urban waste and river waters. Environmental levels can be used as a new tool to estimate illicit drugs consumption in the population.

In Milan, between 2008 and 2009 we observed a significant decrease of heroin and cocaine consumption, and an increase of methamphetamine.

The distribution of dietary intake values of dioxins, dioxin-like PCBs and non dioxin-like PCBs
was characterized for the general Italian population. The higher intake of PCBs due to consumption of farmed fish vs. wild fish is mainly due to the higher fat content in farmed fish.

Development of novel mass spectrometric methods for odour characterization in environmental samples, for odour pollution and its toxicity.

We characterized the pro-inflammatory and neurotoxic effects (activation of glial cells, release of inflammatory cytokines, and motor neurons death) mediated by the activation of TLR4 in primary cultures from mouse spinal cord. Both in this in vitro setting and in an in vivo model of spontaneous motor neuron degeneration (the Wobbler mouse), a TLR4 antagonist extracted from cyanobacteria showed anti-inflammatory and neuroprotective properties.

We characterized the neurotoxic effects of two different environmental pollutants, polybromodiphenyl ethers (PBDE) and methylmercury (MeHg), in a mouse model of prenatal exposure to the contaminants. We found that both the contaminants—at low concentrations corresponding to documented human exposure—were able to induce significant alterations on key proteins and molecules regulating the nervous system development.

NATIONAL COLLABORATIONS

AMA Roma
ARPA Emilia Romagna
ARPA Veneto
ASL Bergamo
ASL Brescia
ASL Cagliari
ASL Como
ASL Cremona
ASL Lecco
ASL Lodi
ASL Milano
ASL Milano 1
ASL Milano 2
ASL Monza Brianza
ASL Napoli
ASL Vallecacmonica-Sebino
ASL Varese
Centro Reach Srl
CLIR Spa Lomellina
CNR – IRSA
Comune di Peschiera del Garda (BS)
Comune di Rosignano Marittimo (LI)
Comune di Sant’Urbano (PD)
CSRA-Asti
Dipartimento delle Politiche Antidroga, Presidenza del Consiglio dei Ministri
Federchimica
Fondazione ‘S. Maugeri’
INRAN-Istituto Nazionale di Ricerca sugli Alimenti e la Nutrizione
ISPO, Firenze
Istituto Clinico Humanitas, Milano
Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano
Istituto Superiore di Sanità
I.Z.S.L.T - Istituto Zooprofilattico Sperimentale del Lazio e Toscana
Metropolitana Milanese
Mineracqua
Ministero dell’Ambiente
Ministero della Salute
Ministero dello Sviluppo Economico
Politecnico di Milano
Politecnico di Torino
Provincia di Vercelli
Provincia Pordenone
Rotary Club Sirmione (BS)
Stazione Sperimentale dei Combustibili, Milano
Università Bocconi
Università degli Studi del Piemonte Orientale
Università degli Studi di Cagliari
Università degli Studi di Genova
Università degli Studi di Milano
Università degli Studi di Napoli "Federico II"
Università degli Studi di Palermo
Università degli Studi di Parma
Università degli Studi di Pavia
Università degli Studi di Perugia
Università degli Studi di Roma "La Sapienza"
Università degli Studi di Siena
Università degli Studi di Torino
Università dell’Insubria, Varese
Università degli Studi di Verona

INTERNATIONAL COLLABORATIONS

BASF Agricultural Centre, Limburgerhof, Germania
CEFIC, European Chemical Industry Council, Bruxelles, Belgio
Centre for Environmental Policy, Imperial College, Londra, Gran Bretagna
Danish Institute of Agricultural Sciences, Research Centre Foulum, Tjele, Danimarca
Department of Analytical and Pharmaceutical Chemistry, The Royal Danish School of Pharmacy, Danimarca
Department of Computer Science and Engineering, University of Galati, Romania
Department of Electrical and Computer Engineering, University of Patras, Grecia
Department of Environmental Science, Faculty of Science and Technology, Aarhus University, Aarhus, Finlandia
Department of Epidemiology & Public Health, Imperial College, Londra, Gran Bretagna
Department of Inland Fisheries, Institute of Freshwater Ecology and Inland Fisheries, Berlino, Germania
Department of Molecular Biology, University of Bergen, Bergen, Norvegia
Department of Organic Chemistry, Universidad de Cadiz, Cadice, Spagna
Environmental Chemistry, IIQAB-CSIC, Barcellona, Spagna
Environmental Hygiene and Chemistry Department, Institute of Environmental Medicine and Hospital Epidemiology, University of Freiburg, Germania
Environmental Protection Agency, US EPA - National Risk Management Research Laboratory (NRMRL), Cincinnati OH, USA
European Chemicals Agency, ECHA, Helsinki, Finlandia
European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Lisboa, Portogallo
Faculté de Médecine et de Pharmacie, Université de Mons-Hainaut, Mons, Belgio
Faculty of Veterinary Medicine, Utrecht University, Utrecht, Olanda
Food and Environment Research Agency, York, Gran Bretagna
Forschungszentrum Jülich GmbH, Jülich, Germania
Helmholtz-Zentrum für Umweltforschung UFZ, Lipsia, Germania
In Vitro Testing Industrial Platform, Tres Cantos (Madrid), Spagna
Institute of Environmental Assessment and Water Research (IDAEE-CSIC) Barcellona, Spagna
Institute of Environmental Medicine, Karolinska Institute, Stoccolma, Svezia
Institute of Pharmaceutical Chemistry, University of Pécs, Pécs, Ungheria
Institute of Phytomedicine, Biological Control, Horticulture and Nematology, Vienna, Austria
Institute of Soil Science and Plant Cultivation, Pulawy, Polonia
Interdisciplinary Nanotoxicity Center, Department of Civil and Environmental Engineering, Jackson State University, Jackson, Mississippi, USA
Interuniversitaeres Forschungsinstitut fuer Agrarbiotechnologie, Tulln, Austria
Istituto di Chimica di Säo Carlos, Università di Säo Paulo, Brasile
KnowledgeMiner Software, Berlino, Germania
KWR Water cycle Research Institute (KWR) Utrecht, Olanda
Laboratory of Chemometrics & Bioinformatics, University of Orléans, Orléans, Francia
Laboratory of Neurobiology, Centro de Investigation Principe Felipe, Valencia, Spagna
Lithuanian Institute of Agriculture, Vilnius, Lituanìa
Liverpool John Moores University, Liverpool, Gran Bretagna
National Institute of Chemistry, Kemijski Institut Ljubljana, Lubiana, Slovenia
Natural Resources Research Institute, University of Minnesota, Duluth, USA
National Institute for Public Health and the Environment (RIVM), Bilthoven, Olanda
Norwegian Institute for Water Research (NIVA), Oslo, Norvegia
Pesticide Safety Directorate, York, Gran Bretagna
Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Ungheria
PublicSpace Ltd, Lancaster, Gran Bretagna
Research Institute for Pesticides and Water, University Jaume I Castellón, Spagna
Rudjer Boskovic Institute, Zagabria, Croazia
School of Biomedical Sciences, University of Ulster, Coleraine, Gran Bretagna
SETAC Europe, Bruxelles, Belgio
Symlog, Parigi, Francia
Syngenta Crop Protection AG, Basilea, Svizzera
Technische Universität Dresden, Dresda, Germania
TNO, Delft, Olanda
Toxicological Centre, Department of Pharmaceutical Sciences, University of Antwerp, Anversa, Belgio
Unit of Environmental Risk and Health, Flemish Institute for Technological Research, Boeretang, Belgio
Universitat Politècnica de Catalunya, Barcellona, Spagna
Universitat Rovira i Virgili, Tarragona, Spagna
University of Bath, Bath, Gran Bretagna
University of Paris - Sud 11, Parigi, Francia
University of Santiago de Compostela, Santiago de Compostela, Spagna
University of Tartu, Tartu, Estonia
EDITORIAL BOARD MEMBERSHIP

Journal of Environmental Science and Health, Part B (Emilio Benfenati), Journal of Environmental Science and Health, Part C (Emilio Benfenati), Chemistry Central Journal (Emilio Benfenati), Frontiers (Emilio Benfenati), The Open Toxicology Journal (Emilio Benfenati), The Open Biomarkers Journal (Luisa Airoldi), Journal of Waste Management (Enrico Davoli).

PEER REVIEW ACTIVITIES


NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP

CCPF - Commissione Consultiva Prodotti Fitosanitari (Ministero della Salute, Ministero dell'Ambiente)
CEFIC - External Scientific Advisory Panel
ECCO - European Commission Coordination
EFSA - European Food Safety Authority
IGQ - Environment and Energy Commission, Safety Commission

EVENT ORGANIZATION

Conference “New Drugs, update sulle nuove sostanze psicoattive, December 12, 2013, Milano, Italy

CONFERENCE AND WORKSHOP CONTRIBUTIONS

Keynote presentation: “Uncertainties associated with the determination of illicit drug use through sewage analysis: results from an international study” Sara Castiglioni. Testing the Water Conference, Lisbon 6-8 May 2013.
“Research needs and information gaps: conclusions from the conference rapporteur” Sara Castiglioni, Testing the Water Conference, Lisbon 6-8 May 2013.
Meeting: ITN SEWPROF Project Training Course: human health and the environment. 22-24 April 2013, Bath, UK.
ITN SEWPROF Project Supervisory Board Meeting, 25 April 2013, Bath, UK.
Meeting ITN SEWPROF Project Training Course: Sampling, sample handling, storage and sample preparation. CIENS, Oslo Science Park, Oslo, Norway (9-10th September 2013)
ITN SEWPROF Project Supervisory Board Meeting 11th September 2013, Oslo Science Park, Oslo, Norway.
Nutrition: Research, Innovation and Markets, Università di Montréal, Montréal, October 8, 2013.

GRANTS AND CONTRACTS

A2A Brescia
ACEGAS S.p.A, Trieste
AIDEPI (Associazione delle Industrie del Dolce e della Pasta Italiane)
AIIPA (Associazione Italiana Industrie Prodotti Alimentari)
AMA, Roma
ASL Cagliari
ASL Como
ASL Mantova
ASL Napoli 2
ASSOFOODTEC/UCIMAC (Costruttori Italiani Macchine per Caffè Espresso ed Attrezzature per Bar)
BASF Italia S.r.l.
Bergamo Pulita S.r.l.
Bluegreen Biotech
Bracco Imaging Spa
Cambrex, Paullo (MI)
Catanzaro Costruzioni S.r.l.
Chemservice S.r.l.
CLIR S.p.A.
COGEIDE S.p.A.
Commissione Europea
Comune di Gorla Maggiore (VA)
Comune di Lomello (PV)
Comune di Mazzano e Rezzato (BS)
Comune di Rosignano Marittimo (LI)
Comune di Sant’Urbano (PD)
Consorzio Quadrifoglio S.p.A.
COOP Italia
CSRA
Dipartimento delle Politiche Antidroga, Presidenza del Consiglio dei Ministri
ECODECO S.r.l.
Elior SpA

EnergyGreen S.r.l.
European Commission (ANTARES, ORCHESTRA, OSIRIS, RISKCYCLE, ToxBank)
Federchimica, Milano
FIAT Auto S.p.A.
Fondazione CARIPLO, Milano
Fondazione “AQUALAB”
Fondazione Italo Monzino, Milano
Gruppo CSA, S.p.a. Rimini (RN)
HERA S.p.A. (Holding Energia Risorse Ambiente)
INDENA S.p.A.
Istituto Superiore di Sanità, Roma
I.Z.S.L.T - Istituto Zooprofilattico Sperimentale del Lazio e Toscana
Lachifarma, Zollino (LE)
Ministero dell’Ambiente, Italia
Ministero della Salute, Italia
Nufarm S.A.S., Francia
Oxon Italia S.p.A., Pero (MI)
NIVA, Oslo, Norvegia
Politecnico di Milano
Provincia di Pordenone
Provincia di Vercelli
Regione Lombardia
SO.GE.NU.S. S.p.A
Tenacta Group
TM.E. S.p.A.
Umweltbundesamt, Dessau, Germania
Università Bocconi
Università degli Studi di Milano
Università di Zurigo
Veolia Servizi Ambientali S.p.A.

SCIENCeFULClcATIONS (2013)


Boriani E, Benfenati E, Baderna D, Thomsen M. Application of ERICA index to evaluation of soil ecosystem health according to sustainability threshold for chemical impact. Sci Total Environ 443: 134-142 (2013)


**RESEARCH ACTIVITIES**

**Laboratory of Molecular Toxicology**

**Proteome Analysis**
Proteome analysis includes protein separation by one- and two-dimensional gel electrophoresis, protein excision from the gel, their digestion with proteolytic enzymes and their identification by mass spectrometry (MALDI-TOF-MS, LC-ESI-MS/MS) coupled to the use of existing databases. Alternatively, peptides resulting from the digestion of protein mixtures with specific proteases are separated by two-dimensional liquid chromatography. Relative and absolute quantitative analyses of proteins differentially expressed are performed respectively by label-free mass spectrometry (e.g. Spectral counts), and Stable Isotope Labeling AminoAcids in Culture (SILAC), or Selected Reaction Monitoring-Mass spectrometry (SRM-MS).

**Toxicoproteomics**
Studies are ongoing on the characterization of changes in the proteome profile induced by environmental toxic compounds, with the aim of obtaining protein biomarkers with the ability to differentiate two or more biological states. Proteome changes in tissues and target organs of animals, and cells treated with endocrine disruptors, estrogens, or environmental carcinogens, are related to functional changes during toxicological processes.

**Clinical Proteomics**
Qualitative and quantitative proteome changes resulting from the exposure to environmental toxic compounds or in pathological conditions are monitored in human biological plasma and urine. Ongoing studies aim at the characterization of protein biomarkers for early diagnosis of diseases and for the identification of therapeutic targets.

**Interactome**
Identification and characterization of protein networks by combining SILAC (stable isotope
labeling by/with amino acids in cell culture) strategy coupled to mass-spectrometry as powerful method to study in vitro protein-protein interaction.

Metabolomics

Metabolomics research focuses on the analysis of metabolites in biological fluids to link human metabolic profile variations to endogenous or exogenous pathophysiological stimuli and to genetic modifications. The study of small molecules (amino acids, carbohydrates, fatty acids, hormones, etc), which contribute to define the biochemical phenotype of a biological system, is addressed by two different basic mass spectrometry based approaches: (i) untargeted metabolomics as the comprehensive analysis of all measurable metabolites in a sample without any a priori knowledge of their chemical structure; (ii) targeted metabolomics as the measurement of a defined group of chemically characterized metabolites. These techniques are applied to human samples (biofluids) in different pathological conditions (e.g. cardiovascular dysfunctions, cancer, rare diseases), and to in vivo or in vitro models.

The integration of proteomic and metabolomic studies will provide information that can help to better understand disease development and to identify preventive interventions.

Pathways analysis

An integrated data-mining platform such as MetaCore (GeneGo Inc., USA) is used in order to map the proteomics and/or metabolomics data into biological networks and for their functional interpretation.

Molecular Epidemiology

The laboratory works mainly on the measurement of biological markers used to assess human cancer risk or human exposure to environmental toxic compounds. Our most recent studies include comprehensive mass spectrometry based analysis of plasma protein expression changes in cancer patients, followed by semi-quantitative analysis of putative protein biomarkers of cancer risk. Carcinogen plasma levels, DNA- and blood protein-adduct formation by several environmental carcinogens are also studied. The laboratory participates in an international cooperation study aimed at the collection of reference values on allele and genotype frequency of the most common metabolic enzyme polymorphisms in control populations.

Laboratory of Analytical Biochemistry

Identification and characterization of proteins by mass spectrometry

Our laboratory is developing different analytical and instrumental techniques –based on mass spectrometry– for the identification and characterization of proteins and peptides in biological samples. This activity is mainly aimed at 1) global proteomic characterization and comparison of secretomes from human cancer cell lines; 2) profiling proteins in biological fluids for discovery and identification of biomarkers of physiopathological and toxicological relevance, 3) identifying and characterizing endogenous degradation products of proteins, 4) identifying proteins produced by cells in vitro in response to given stimuli, 5) identifying and characterizing biologically relevant proteins isolated from biological samples by immunoaffinity-based techniques.

Proteomics in oncology

This activity is mainly aimed at discovering –among the proteins we find abnormally secreted by human cancer cell lines or oncogene-transfected cell lines– novel candidate therapeutic targets or diagnostic/prognostic biomarkers. The complex alterations observed in the cancer
secretome are rationalized and interpreted by using “systems biology” tools that are able to highlight the functional networks most significantly perturbed. Ongoing projects focus on pancreatic cancer, and in particular on the perturbations induced by oncogenic K-Ras in the secretome of pancreatic ductal epithelial cells.

Glycoproteomics
Glycoproteomic characterization (amino acid sequence, glycosylation site(s), and type of bound saccharides) of plant proteins of pharmaceutical/nutraceutical interest by gel electrophoresis, enzymatic degradation and mass spectrometry.

Neurotoxicity by environmental pollutants on the developing Central Nervous System
We are studying the effects of environmental contaminants (PBDE and methylmercury) on neuronal cell primary cultures and in a mouse model of prenatal exposure to the contaminants. Alterations on the most important proteins and molecules regulating the nervous system development are studied by biochemical and immunochemical methods in vitro, and by histological and immunoblotting analysis in vivo.

Laboratory of Environmental Chemistry and Toxicology
Development and use of analytical methods to evaluate contamination in water bodies, soil, biota, human samples in exposed population
Analytical methods are developed to study environmental pollutants in water ecosystems, landfills, contaminated sites. Qualitative and quantitative analyses of organic pollutants are done by mass spectrometry (GC-MS, LC-MS, LC-MS/MS). Typical analyses include PCDD/F, PCB, PAH, polybrominated diphenylethers, pesticides, endocrine disruptor chemicals, and industrial pollutants.

Studies on environmental, toxicological and ecotoxicological properties of chemicals
Research is carried out on pollutant properties, exploring a broad range of toxicological and environmental properties in order to get safer chemicals. The use of computational models allows processing millions of chemicals. This involves searching literature data, comparing and evaluating different sources, and mainly developing predictive models to cope with the lack of experimental data. Thus, we develop models starting merely from the chemical structure. The research addresses the different kinds of chemical descriptors and chemical fragments, obtained with different software. Then, we develop models using algorithms such as neural network, fuzzy logic, genetic algorithms, classifiers, multivariate analysis, etc. Different methods are compared and integrated within a structured ensemble. Standardized methods for pesticides were developed and validated according to OECD guidelines. Innovative tools to evaluate the applicability domain of the models have been developed, to get predictions useful for regulatory purposes, such as REACH, biocide, pesticides, and other regulations.

Risk assessment of pollutants
Studies are aimed at assessing the risk of pollutants for human population and environment. For this we model transport and diffusion of pollutants, to obtain a predicted concentration on given space and time scales. Such an activity is integrated with those above described on chemical analyses and toxicity prediction, to achieve a continuous transfer of data and research.

Research on pollutants emitted in the atmosphere (Unit of Industrial and Environmental Hygiene)
Studies address different aspects of atmospheric pollution. Research deals with: sampling areas around the pollution source, chemical analyses, transport modeling depending on meteorological conditions and orography, risk assessment for population and environment. Qualitative and quantitative analyses are done by gas chromatography-mass spectrometry using high resolution for PCDDs/PCDFs, and negative ion-chemical ionization for PCBs.

**Laboratory of Mass Spectrometry**

**Mass Spectrometry Imaging**

Mass spectrometry imaging is one of the latest, rapidly growing innovative technique in mass spectrometry. It is used to visualize molecular distribution in a two dimensional space of a sample. A mass spectrometry imaging protocol has been developed in collaboration with the Analytical Instrumentation Unit, based on nano-particles assisted laser desorption-ionization, that allows distribution studies of anticancer drug, in tumor tissues of mice, revealing differences of drug penetration, related to the dosage-schedule.

**Method development in environmental sciences**

Methods, analytical methodologies, instrumentation and software for data acquisition and reduction, are developed for environmental studies. High-sensitivity instrumentation, mainly based on mass spectrometry, is developed for trace and ultra-trace analysis. Also, transportable instrumentation is developed for field studies or continuous monitoring.

**Characterization of environmental odor annoyance and its toxicity**

Characterization of odors poses several analytical problems because they result from a complex mixture of compounds (odorants) stimulating receptors in the nasal cavity. Most odorants are volatile organic compounds (VOC) generated by bacterial degradation of organic matter. They are often present at trace levels, while numerous sources can contribute to the total odor. Using sampling techniques specifically developed for olfactometry, solid phase microextraction and GC/MS analysis, we can detect traces (low ppb to high ppt) of a wide polarity/volatility range of airborne VOC odorant compounds. With a chemometric approach, we can characterize the sources of emissions, assess odor control methods, and identify emissions that contribute to odors in ambient air.

**Laboratory of Food Toxicology**

**Nutrition studies: Chemical contaminants in food. Nutrition and Health**

We are studying human exposure to dietary PCBs and dioxins in Italy. In particular, contaminants were measured in samples of human milk collected from mothers living in highly contaminated areas. Further studies were aimed at measuring PCBs and dioxins in samples of fish caught in Italy and in food items from an Italian area at high risk of contamination. Other studies will investigate the relationship between dietary sodium in intake and health. In particular this activity will set up and apply practical methodologies to reduce sodium content of the daily diet in groups of volunteers.

**Therapeutic and illicit drugs in the environment**

Pharmaceuticals are a class of emerging environmental pollutants. We have organized a campaign to detect the presence of pharmaceuticals and their metabolites in Italian rivers and sewage treatment plants and in samples of drinking water, with the aim of characterizing the contamination and assessing related risks.
Further ongoing studies are aimed at investigating a possible relationship between antibiotic occurrence and resistance in environmental bacteria. The possible presence of illicit drugs in water samples from sewage treatment plants and rivers was investigated, starting with cocaine and its metabolites. Their levels, used to estimate drug abuse in the local population, revealed that cocaine consumption greatly exceeds official estimates. This approach has been subsequently extended to include other common drugs of abuse such as cannabis, opiates (heroin, morphine), and amphetamines (amphetamine, methamphetamine, ecstasy). Our evidence-based method allows monitoring of patterns and trends of drug abuse in local communities, and is able to detect qualitative and quantitative consumption changes in real time. This tool can therefore complement survey methods in more realistically describing the drug abuse phenomenon. Ongoing studies are focused to assess consumptions at national scale, in collaboration with the National Agency for Drug Policy, at regional scale in collaboration with Regione Lombardia, and locally, in collaboration with Metropolitana Milanese.

Further ongoing studies, carried out in collaboration with several research groups in Europe and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), are aimed to study illicit drug consumption in Europe. We will simultaneously measure consumptions in 19 cities in 14 different nations and will compare our results with consumptions estimated by traditional epidemiological methods.

**Unit of Environmental Pollutants Risk Assessment**

**Toxicological risk assessment**
The activities of the unit focus on risk assessment related to specific environmental conditions, or human activities, which pose a risk for human health. These activities include risk assessments related to atmospheric pollution, contamination of soil, surface- and ground-waters, and transfer of contamination through the food chain. In particular, during 2013, studies of risk assessment due to toxic emissions from wastes disposal into landfills and indoor pollution from ozone have continued. In addition the activity of the unit also focused on food safety: a survey on the contamination of acetaldehyde in alcoholic and non-alcoholic beverages, in order to assess the risk of health effects for consumers, has been carried out. Finally an investigation concerning the effect of palm oil on blood lipid related markers of cardiovascular disease has been performed by a systematic review and meta-analysis.

**Unit of Analytical Instrumentation**

**Development and application of analytical methods for compounds of biological and environmental interest.**
Research activities include the analysis of biological fluids and environmental samples using solid phase extraction (SPE) and liquid chromatography - mass spectrometry (LC-ESI-MS/MS). Available instruments include liquid chromatographs and mass spectrometers equipped with different analyzers: time of flight (TOF), triple quadrupoles, ion traps and high resolution LTQ-Orbitrap XL, with conventional and nanoElectroSpray sources.
Substances of interest include: proteins, peptides, steroids, hormones, pharmaceuticals, drugs of abuse, other environmental and food contaminants (pesticides, perfluorinated compounds, mycotoxins) and small polymers (MW < 5000 Da).
DEPARTMENT OF NEUROSCIENCE

STAFF

Head Gianluigi FORLONI, Biol.Sci.D.

Laboratory of Biology of Neurodegenerative Disorders
Head Gianluigi FORLONI, Biol.Sci.D.

Genetic of Neurodegenerative disorders Unit
Head Diego ALBANI, Biol Sci. D.

Acute Spinal trauma and regeneration Unit
Head Pietro VEGLIANESE, Ph D

Laboratory of Cell Death and Neuroprotection
Head Tiziana BORSELLO, Biol.Sci.D.

Laboratory of Epidemiology and Social Psychiatry
Head Barbara D’AVANZO, Philos.D.

Laboratory of Experimental Neurology
Head Annamaria VEZZANI, Biol.Sci.D.

Physopathology of glia-neuron communication Unit
Head Teresa RAVIZZA, Biol. Sci.D

Laboratory of Experimental Psychopharmacology
Head Luigi CERVO, Ph.D.

Laboratory of Geriatric Neuropsychiatry
Head Ugo LUCCA, MSc

Geriatric Epidemiology Unit
Head Mauro TETTAMANTI, Biol.Sci.D.

Geriatric Pharmacology Unit
Head Emma RIVA, M.D.

Laboratory of Inflammation and Nervous System Diseases
Head Maria Grazia DE SIMONI, Biol.Sci.D

Cell therapy and Acute Brain Injury Unit
CURRICULA VITAE

Gianluigi Forloni, obtained the Degree of Biological Science at the University of Milan in 1985. After two years of post doc at the Department of Neuroscience and Psychiatry at Johns Hopkins University in Baltimore, USA, he came back to the Mario Negri Institute and between 1992 and 1996 he was the head of the Neurobiology of Alzheimer’s disease Unit; since 1996 he is the Head of the Biology of Neurodegenerative Diseases Lab and since 2002 the Head of the Neuroscience Department. His scientific interest is focused on the biological and genetic bases of aging-related disorders in particular Alzheimer’s disease, Prion-related encephalopathies and Parkinson’s disease. He has been member of several European committees for the examination of projects in the neuroscience field. He is now member of the coordination group of the European IMI Consortium PharmaCog. He is President of the Italian Association on Brain Aging Research (AIRIC), member of the Scientific Committee of the Dementia section of the Italian Society of Neurology (SINDEM) and member of the European Academy of Sciences. He is the author of more than 240 peer-reviewed scientific articles and about 30 reviews or book chapters.

Selected publications


Ettore Beghi graduated in Medicine in 1972 and received his specialty in neurology in 1976 at the University of Milan. He trained in epidemiology with a fellowship at the Department of statistics and Epidemiology of the Mayo Clinic in Rochester, MN (USA). He is Head of the Laboratory of Neurological Disorders at the Mario Negri Institute, Director of the Neurophysiology/Epilepsy Unit and Professor of Neuroepidemiology at the University of Milano-Bicocca, Monza. He is member of the editorial board of the journals Epilepsia, Neuroepidemiology, Inpharma, Drugs in R & D, Clinical Drug Investigation, Neurological Sciences and is a referee of several national and international medical journals. The main areas of interest and research include studies on the descriptive, analytic, and experimental epidemiology in the field of epilepsy, peripheral neuropathies, headache, and amyotrophic lateral sclerosis.

Selected publications


• Neurology 2013:14:397-405.


Caterina Bendotti got her degree in Pharmacy at the University of Milano in 1984; In 1986 -1988 she was post doc at the Genetic developmental Lab, Dept. of Physiology of the Johns Hopkins University, Baltimore, USA. In 1988-1992 she was research fellow in the laboratory of Neuropharmacology and in the 1992, she became head of the Molecular Neurobiology Unit in Institute, since 1998 she is head of laboratory. The major research interest is the study of pathogenetic mechanisms of familial Amyotrophic Lateral Sclerosis.
Since 2002 she is a member of the editorial board of Journal of Neurochemistry. In 2002-2003 has been Member of Scientific Committees of the International Symposia on ALS held in Milano, 17-19 November, 2003. In 2003-2007 has been member of the Italian Ministry of Health Committees for the diagnosis, cure, care and assistance of patients with ALS. Since 2005 is member of the Board of Directors of the Italian Society of Neuroscience. Since 2006 is member of the Research Advisory Panel of the MND Association, UK. Scientific reviewer of 11 international scientific journals. In 2007 she has co-organised the first international meeting on “Mutant SOD1 and familial ALS: from the molecule to man” held in Milan (13-16 September). She is author and co-author of 140 articles with peer-review. Rapporteur of many communications in national and international meetings.

Selected publications
- Pizzagella C, Caron I, Daleno C, Ronchi A, Minoia C, Carri MT, Bendotti C. Treatment with lithium carbonate does not improve disease progression in two different strains of SOD1 mutant mice. Amyotrophic Lateral Scler. 10(4):221-8, 2009

Tiziana Borsello
got her Degree in Biological Science at the University of Turin in 1990 and she then obtained a PhD in Neuroscience at the University of Turin Medical School. She won a 1 year fellowship from the European Science Foundation to work at the Netherlands Research Institute of Amsterdam. From 1997 to 1999 she was a Researcher at the CNR, CNR, Rome Italy. In the period 1999-2003 she was Premier Assistant at the Département de Biologie Cellulaire et de Morphologie, Université de Lausanne, Switzerland, and then became Maître Assistant and group leader in the same institute in 2004. In 2004 joined the Biol. Neurodeg. Disorders Lab at the “Mario Negri” Institute. In 2005 won the Prize of the Pfizer Foundation, Neuroscience and Diseases Nervous System. Since 2006 she is the Head of the Unit: Neuronal Death and Neuroprotection. Her main scientific interests focus on understanding the role of signalling pathways in neuronal death after different stress-stimuli and neuroprotection. In particular, the present research is focused on the study of mechanisms leading to excitotoxic stress, ischemia, Traumatic Brain Injury and cell death pathways in neurodegenerative diseases such as Alzheimer, with the challenge to design more specific methods of neuroprotection.

Selected publications
Luigi Cervo, Ph.D. (Open University, Milton Keynes, U. K.), since 2006 is the head of the Experimental Psychopharmacology Laboratory. From 1978 to 2001 he was a research fellow and then chief of the Behavioural Pharmacology Unit in the Laboratory of Neuropsychopharmacology and in 1981 he was awarded the degree in Biochemical Research from the “M. Negri” Institute. Between 1981 and 1983 he spent two years as a research fellow in the Department of Psychiatry at the Chicago University, Illinois, U.S.A (Prof. Charles Robert Schuster). His main research interests concern drug dependence and drug craving, depression, anxiety. Author and co-author of several peer-review articles, author of communications in international meetings, he is reviewer of several international peer-reviewed scientific journals. He is member of the Society for Neuroscience, European Behavioural Pharmacological Society, Italian Society for Neuroscience and Italian Society of Neuropsychopharmacology.

Selected publications


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IRFMN
Roberto Chiesa graduated in Biological Sciences with major in Genetics at the University of Pavia in 1991, and obtained a Ph.D. in Pharmacology at the Mario Negri Institute for Pharmacological Research of Milan in 1994. From 1996 through 2000 he was Research Associate at the Department of Cell Biology and Physiology of Washington University in St. Louis, MO, USA. In 2001 Dr. Chiesa moved back to the Mario Negri Institute where he is currently head of the Prion Neurobiology lab in the Department of Neuroscience. He also holds an Associate Telethon Scientist position (Dulbecco Telethon Institute, Mario Negri Institute) and an Associate Professor of Pharmacology (University of Pavia). He received the James L. O’Leary Prize (1998) and Bruno Ceccarelli Prize (2000) for the research in neuroscience area. He is member of editorial board of PlosOne and Biochemical Journal.

Selected publications


Barbara D’Avanzo obtained her master in philosophy at the University of Milan in 1989. Her main field of interest is epidemiological research in mental health and quality evaluation of the mental health services. First involved in the analysis of the implementation of the psychiatric reform in Italy, then addressed the quality and the role of residential facilities and treatment and continuity of care in the community services network. She works at the effectiveness evaluation and implementation problems of the most common psychosocial and psychological interventions for severe mental illness. More recently, she is implementing a monitoring system of suicide attempts and self-harm episodes in various areas of Italy, in the framework of suicide mortality monitoring and suicide prevention study and implementation, and is also working on issues related to recovery-oriented services, consumers’ empowerment, methods of consumers participation to service evaluation, and acknowledgment of the value of consumers’ knowledge and perspective about mental health services and treatments. She is head of the Laboratory of Epidemiology and Social Psychiatry since 2011, and is member of the Scientific National Board of the World Association for Psychosocial Rehabilitation.

Selected publications

Maria Grazia De Simoni got the Doctoral Degree in Biological Sciences in 1977 at the University of Milan, Italy. 1981: Research Specialist in Pharmacology (PhD), Mario Negri Institute, Milan, Italy. 1981-1982: European Community fellowship for "Advanced Professional Training", INSEERM U 171, Université Claude Bernard, Lyon, France; 1984 Department of Histology, Karolinska Institute, Stockholm. Working experience: 1987-1997: Chief of the Neurochemistry Unit, Mario Negri Institute, Milan; 1998-present: Chief of the Laboratory of Inflammation and Nervous System Diseases, Mario Negri Institute. Scientific interests: pathogenesis of cerebral ischemia/reperfusion and traumatic brain injury; inflammatory response and apoptotic mechanisms as targets of therapeutic strategies; animal models and clinical studies. She is member of the board of “Master in Tecnologie Avanzate Applicate alle Patologie Neurodegenerative”, University of Milan. Author of about 120 peer-reviewed papers in international journal

Selected publications
Roberto W. Invernizzi started his career in the laboratory of Neuropharmacology of the “Istituto di Ricerche Farmacologiche “Mario Negri” in 1976, where, at present, he heads the Laboratory of Neurochemistry and Behavior. In 1986 he got his degree in Biological Sciences at the Università Statale di Milano and in 1996 he was nominated head of the Intracerebral Microdialysis Unit. Of particular interest to Invernizzi’s research team is the study of the neurochemical mechanisms and neuronal circuitries involved in the pathology of the main psychiatric diseases, such as depression and schizophrenia and in the mechanism of action of psychotropic drugs. Since 1987 he applied the intracerebral microdialysis technique to study the in vivo release of monoamines. Using this technique, Invernizzi’s team first contributed to clarifying the role of serotonergic and adrenergic autoreceptors in the effect of antidepressant drugs suggesting new hypotheses on their mechanism of action. Currently, Invernizzi’s laboratory is involved in two main collaborative projects aimed at clarifying the neurochemical mechanisms involved in the “resistance” to antidepressant drugs and the role of glutamatergic and serotonergic mechanisms in attentional processes. Reviewer for various international journals in the field of pharmacology and neurochemistry. Author and co-author of more than 70 peer-reviewed articles. Member of the Italian Society of Neuroscience and the Italian Society of Pharmacology.

Selected publications
- Agnoli L, Mainolfi P, Invernizzi RW, Carli M. Dopamine D1-like and D2-like receptors in the dorsal striatum control different aspects of attentional performance in the five-choice serial reaction time task under a condition of increased activity of corticostrital inputs. *Neuropsychopharmacology*. 2013;38:701-14.
- Carli M, Calcagno E, Mainini E, Arnt J, Invernizzi RW. Seridolone restores attentional performance and suppresses glutamate release induced by the NMDA receptor antagonist CPP. *Psychopharmacology (Berl)*. 2011;214(3):625-37


- Baviera M, Invernizzi RW, Carli M. Haloperidol and clozapine have dissociable effects in a model of attentional performance deficits induced by blockade of NMDA receptors in the mPFC. *Psychopharmacology* 2008;196:269-280.


**Ugo Lucca** got his Master of Science, University of Aberdeen - UK, 1999. At the Mario Negri Institute he was investigator from 1986-1995, head of the "Clinical Evaluation of Antidementia Drugs Unit" (1995-1996) and, since 1996, head of the "Laboratory of Geriatric Neuropsychiatry". The main areas of interests include epidemiology and clinic features of dementia; natural history of dementia; neuropsychiatric disorders of the elderly; instruments for the screening diagnosis and clinical course assessment of dementia; clinical evaluation of anti-dementia treatments and CNS active drugs (phase I, II, III, IV and observational studies).

### Selected publications


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Main areas of interest Methodology of Randomized Clinical Trials; Pharmacoepidemiology and post-marketing surveillance research; Drug utilization studies; Quality assessment of geriatric services; Qualitative studies on caregiver role in the care of patients with dementia; Methodological evaluation of the Special Care Unit for Alzheimer Disease patients; Methodology of drug information. Employment and research experience Chief of the Unit of Quality Assessment of Geriatric Services Chief of the Drug Information Services for the Elderly, Laboratory of Geriatric Neuropsychiatry, Istituto di Ricerche Farmacologiche “Mario Negri”, Milan. Editorial Board of the MICROMEDEX Inc., Englewood, Colorado 80111-4740 USA. National Expert accredited by Italian Ministry of Health for The Italian (AIFA) and European Agency for the Evaluation of Medicinal Products (EMEA). Head of the Laboratory of the Quality Assessment of Geriatric Services at the Mario Negri Institute since 2007.

Selected publications


Annamaria Vezzani got her Degree in Biological Science at the University of Milan in 1978 and she specialized in Neuropharmacology at the Mario Negri Institute in 1982. She spent her post-doctoral period in Baltimore at the University of Maryland in 1983-1984 working on the mechanisms of epileptogenesis in experimental models of epilepsy. She spent additional post-doctoral periods at the University of Stockholm and at the Karolinska Institute between 1985 and 1999. She was on sabbatical at the Albert Einstein College of Medicine in 2002 in the laboratory of Developmental Epilepsy. She is involved in studies on the biochemical and molecular mechanisms involved in the etiopathogenesis of seizures disorders using experimental models of epilepsy. The present research is focused on the functional role of neuroactive peptides and inflammatory mediators in the modulation of neuronal excitability and seizure-related neurodegeneration. Focus of the research is also on the mechanisms of pharmaco-resistance. Since 1997 she is the Head of the Laboratory of Experimental Neurology at the Mario Negri Institute. She is member of the Editorial Board of various scientific journals and Associate Editor for basic science of Epilepsia, the official journal of the International League Against Epilepsy (ILAE). She has been appointed of the Chair of the Commission on Neurobiology of ILAE which is promoting initiatives for improving translational research in epilepsy. She has been awarded of the prestigious Epilepsy Research Recognition Award for translational research in 2009 by the American Epilepsy Society.

Selected publications

Diego Albani graduated in Biological Sciences in 1996 with full marks and he has been working at the “Mario Negri” Institute since 2002, after a 3-year post-doc experience in the laboratory of Prof Renato Dulbecco, CNR-ITBA in Milan, Italy. His is head of the Unit of Genetics of Neurodegenerative Disorders since 2011. His present interests deal with the biological basis of neurodegenerative disorders including Alzheimer’s (AD) and Parkinson’s disease (PD), with a particular focus on genetics, oxidative stress and recombinant proteins as innovative drugs. Dr Albani is actively involved in ongoing research projects focused on pharmacogenomics of AD, the genetic basis of aging and the activation of neuronal enzymes (sirtuins) by natural phytoproducts as novel strategy against AD and PD. He is currently member of the Editorial Board of three international journals.

Selected publications

Mirjana Carli started his scientific career in the laboratory of Neuropharmacology of the “Istituto di Ricerche Farmacologiche Mario Negri” Milan in 1977, where, at present, she is head of the Pharmacology of Cognitive Behaviour Unit. She spent a few years in the laboratory of Cognitive Neuroscience, Dept. of Experimental Psychology, University of Cambridge (UK) directed by Prof. Trevor W. Robbins. Here she took interest in the role of brain monoamines in attention, and for this purpose developed several behavioral tests for rats. In 1986 she returned to the laboratory of Neuropharmacology of the “Istituto di Ricerche Farmacologiche Mario Negri”. Here she devoted her efforts to the study of the role played by neuronal mechanisms in cognitive processes such as memory, attention and executive functions. Her work has improved the knowledge of the role played by some serotonin receptors in cognitive processes.

Selected publications

Teresa Ravizza got her Doctoral Degree in Biological Sciences in 1996 at the University of Milano. Then she got a Master in “Research Specialist in Pharmacology” at Mario Negri Institute in 2000. She spent her post-doc training at the Albert Einstein College of Medicine of New York in 2000-2001, where she studied the mechanisms underlying epileptogenesis in experimental models of pediatric epilepsy. She spent additional post-doc periods at the Academic Medical Center of Amsterdam and at University of Irvine (UCI), California (USA) between 2005 and 2009. Since 2010, she is the head of the Unit of Pathophysiology of Neuron-Glia Communication. Her scientific interest is to characterize changes in the expression of molecules produced by astrocytes and microglia in various pathological conditions, such as...
epilepsy, trauma, excitotoxicity and inflammation. A special focus is given to the pro- and anti-inflammatory molecules, and to the role played by these mediators in mediating functional and biochemical alteration in the brain (neuronal cell loss, neuronal excitability, alteration in blood-brain barrier permeability).

Selected publications

- Maroso M, Balosso S, Ravizza T, Liu J, Aronica E, Iyer AM, Rossetti C, Molteni M, Casalgrandi M, Manfredi AA, Bianchi ME, Vezzani A. Toll-like receptor 4 (TLR4) and High Mobility Group Box 1 (HMGB1) are involved in icterogenesis and can be targeted to reduce seizures (2010) Nature Medicine, 16, 413
- Akin D, Ravizza T, Maroso M, Caracè N, Eryigit T, Vanzulli I, Aker RG, Vezzani A, Onat FY. IL-1β is induced in reactive astrocytes in the somatosensory cortex of rats with genetic absence epilepsy at the onset of spike-and-wave discharges, and contributes to their occurrence. Neurobiol Dis, 2011; 44:259-69

Emma Riva, Medical Doctor degree in 1984 University of Milan, PhD in 1990 in Cardiovascular Pathophysiology at the University of London (UK) Training: Research Assistant, Department of Pharmacology, Medical School, University of Ottawa, Canada; Internship in Internal Medicine, Ospedale Luigi Sacco, Milan; Cardiac Fellow, St Thomas’ Hospital, London, UK. Field of interest: Prevalence and effects of anemia on cognitive, functional and clinical variables in the elderly; Problem behaviors in dementia; Burden for care-givers of Alzheimer Disease patients; End of life care. Present and past roles: Head of the Geriatric Pharmacology Unit, Istituto "Mario Negri", Milan; Scientific Director of the hospice “Via di Natale Franco Gallini”, Aviano, Italy; Consultant Istituto Geriatrico “Pio Albergo Trivulzio”, Milan. Project member of PREDICT (Policy Review and Evaluation of Dementia and Institutional Care Trends): a Transnational Comparison.

Selected publications

Researches in the geriatric field: Phase I, II, III and observational studies on the efficacy of drugs on neurologic disorders, with special emphasis on dementia; Effects of multi-disciplinary interventions on geriatric/dementia patients; Epidemiology and risk factors of dementia; Care of patients with terminal illness; Association of anemia with prevalence of diseases and cognitive problems Scholarship between Italy's Lombardy Region: a large population-based study. Diabetes Research and Clinical Practice 2011 DOI: 10.1016/j.diabres.2011.05.004


Mauro Tettamanti

Mauro Tettamanti got his Biology Degree at the Università degli Studi di Milano in 1986, and the specialisation in Epidemiology and Medical Statistics in 1993, at the Università degli Studi di Pavia. Teaching experience Introduction course to statistics, Master in Ergonomy, Politecnico di Milano, years 2001-2004 Areas of interest: Planning, conduction and analysis of clinical trials and epidemiologic researches in the geriatric field: Phase I, II, III and observational studies on the efficacy of drugs on neurologic disorders, with special emphasis on dementia; Effects of multi-disciplinary interventions on geriatric/dementia patients; Epidemiology and risk factors of dementia; Care of patients with terminal illness; Association of anemia with prevalence of diseases and cognitive problems Scholarship between 1989 and 1998, Senior Researcher since 1999 and Head of the Unit of Geriatric Epidemiology at the Mario Negri Institute since 2001.

Selected publications

- Lucca U, Nobili A, Riva E, Tettamanti M. Cholinesterase inhibitor use and age in the general population. Arch Neurol 2006; 63:154-155

Pietro Vegliansese

Pietro Vegliansese got the degree in Chemistry and Pharmaceutical Technology at the University of Milan in 2000. In 2005 he got the specialization in Pharmacological Research at the Mario Negri Institute in Milan. In 2007 he got the PhD from the Open University of London. Since January 2014 he is the head of the Unit of Acute Spinal Injury and Regeneration at the Mario Negri Institute. The scientific interests of dr, Vegliansese are related to the characterization of biomaterial for the drug release inside the area of spinal injury. In 2013 his findings have shown that the use of biomaterial, nanoparticles and hydrogel, could release and increase the efficacy of different drugs also in combination, this evidence indicating new therapeutic strategy. Furthermore some smart delivery tools can be selectively target microglial cells, opening new perspective in the treatment of brain inflammation (cell-target therapy). With a multifactorial approach directly in the injury site the Unit of dr Vegliansese is exploring the possibility to combine drug delivery and staminal cells through the support of idrogel. These project are developed in multidisciplinary way in close collaboration with Politecnico Institute of Milan and SUPSI of Lugano. Pietro Vegliansese act as referee for several scientific journal including Brain, Journal of Control Release, Cell Biology and British Journal of Pharmaceutical Research

Selected publication

Elisa R Zanier. 1998, Medical Doctor degree (110/110) at the University of Milano, Italy. 1998/2001: Residency in Anesthesiology and Critical Care Medicine at the University of Milano. 2 years Postdoctoral fellowship at the Neurotrauma Laboratory-Neurosurgery Division, University of Los Angeles, California (UCLA), USA. 2003-2008 Assistant physician in the Neurosurgical Intensive Care Unit, Department of Neuroscience, Fondazione IRCCS Ospedale Maggiore Policlinico, Milano. Since 2007: Teaching assignment into postgraduate school of Critical Care Medicine, Policlinico, Milano. Since 2008: Associate researcher at the Laboratory of Neuroinflammation and Nervous System Diseases, Mario Negri Institute. Since 2012 Head of the Unit of Cell Therapy and Acute Brain Injury, Mario Negri Institute, Milano. Present interests include: experimental models: traumatic brain injury and stroke. Scientific fields: pathophysiology of brain ischemia/reperfusion injury and traumatic brain injury; inflammation as target of therapeutic strategies; the protective mechanisms of stem cells. Publications in PubMed: 32.

Selected Publications:


activities

The Department of Neuroscience is formed by twelve Laboratories; the activities of research are devoted to the study of neurological and psychiatric diseases, evaluated by the biological point of view, clinical and epidemiological aspects and the quality of care. Together with these activities, in the Department other more general expertise are present, drug information service and preparation of protocols for clinical trial and epidemiological studies are activities in charge
of the Neuroscience Department. Traditionally part of the Department was devoted to the creation of experimental models for the pharmacological, neurochemical and pathogenetic studies in Alzheimer or prion's diseases, epilepsy, depression and cognitive impairment. More recently, consolidated expertise were created in the pathogenesis of amyotrophic lateral sclerosis (ALS), cerebral stroke and drug abuse. Some of these disorders, like epilepsy, ALS and Alzheimer's disease, are investigated from the clinical and epidemiological points of view for the evaluation of drug and care efficacy. The activities of the Department are aimed to an integration of the different expertise to develop multidisciplinary approaches. The purpose is to address at different levels, knowledge, therapy and clinical practice to the numerous questions, largely unresolved, proposed by the disorders of nervous system.

**MAIN FINDINGS**

The intracerebral application of synthetic β amyloid 1-40 e 1-42 in oligomeric form is associated with a cognitive damage is partially due to inflammatory mechanism mediated by non-neuronal cells.

α-synuclein, essential component of intracellular aggregates, named Lewy bodies, found in Parkinson disease brain and in other neurodegenerative diseases, injected, in oligomeric form, intracerebraventricularly induced a cognitive decline similar to that caused by β amyloid oligomers

The comparative MRI analysis of different experimental models of Alzheimer’s disease (AD) showed similar reduction of brain regions volume associated to aging, only partially superimposable at the AD condition. At the striatal level the reduction of volume is particulary relevant and it has been associated to a synaptic loss

Doxycycline, a tetracycline that pass the blood brain barrier with anti-amyloidogenic activity not only reduced the β amyloid aggregates but also antagonize the neuronal dysfunction induced by β amyloid oligomers.

A polyphorphism in the gene coding for SIRT-2, a protein member of the sirtuin family, proteins with deacetylase activity, has been associated with development of chronic diseases in elderly,

A Αβ peptide with transmembrane sequence TAT (TAT 1-6 A2V) including the mutation that in homozygosis is associated to the Alzheimer’s disease antagonized the toxic effect induced by β amyloid in vitro and in vivo models

It has been shown in animal models that the peptide D-JNK-TAT, capable to inhibit the phosphorylation mediated by JNK, can control the production of β amyloid indicating new therapeutic strategies.

In vitro and in vivo approaches demonstrated that JNK can be activated postsynaptically in response to Αβ amyloid oligomers inducing synaptopathy through two different targets. The specific inhibition of JNK prevented the excitatory synapse degeneration.

It has been shown an interaction between JNK and syntaxin 2 a protein involved in the vesicle docking, this interaction is sensible to the action of D-JNK-1 peptide opening new perspectives in the intervention of synaptic activity modulation and glutamate release
New peptides has been synthetized capable to selectively control the kinase MKK7, responsible of the JNK activation following cellular stress as the excitotoxicity.

Studies in different cell models have shown that activation of endoplasmic reticulum stress or alterations in the proteasome are not responsible for the neurodegeneration in prion diseases of genetic origin.

The PrP molecules carrying deletions encompassing the conserved central region (PrP ΔCR) are strongly neurotoxic this toxicity is inhibited by the wild-type form of PrP. we found that while ΔCR-dependent toxicity is cell-autonomous, the rescuing activity of wild-type PrP can be exerted in trans from nearby cells.

Mutated PrP interacts with the voltage-gated calcium channel α2δ-1 subunit which promotes the anterograde trafficking of the channel. Owing to ER retention of mutant PrP, α2δ-1 accumulates intracellularly, impairing delivery of the channel complex to the cell surface.

In a prospective population-based study in the oldest old (Monzino 80-plus Study), the presence of an APOE ε4 allele continued to be associated with an increased risk of dementia. In advanced age, also APOE ε2 carriers showed a higher risk of dementia.

In the same prospective population-based study (Monzino 80-plus Study), the risk of dementia in the oldest old was not associated with a history of head trauma with loss of consciousness.

In a prospective ambulatory population of non-demented individuals 60 years or older seen consecutively at the Memory Clinic of the Ospedali Regionali of Mendrisio and Lugano, Switzerland (Canton Ticino Study), elevated homocysteine and low B-vitamin concentrations were cross-sectionally associated with worse cognitive performance but did not predict an increased rate of cognitive decline in this large ambulatory population of elderly with subjective memory complaint but normal cognition or mild cognitive impairment.

Prevalence of reduced kidney function can vary greatly depending on which equation is used to estimate GFR in the oldest old (between 22.7% and 86.0%). In this age segment, after two years, risk of all-cause mortality was significantly higher for reduced GFR estimated with the C-G and BIS-1 equations. After five years increased mortality in the oldest old was associated with reduced GFR estimated with most equations, but only the MDRD equation showed a significantly graded increase in all-cause mortality with decreasing eGFR estimates.

In the Creutzfeldt-Jakob disease study 55 patients were randomized and followed up in the Italian part of the study. The data from these patients were pooled with 66 patients of a similar study performed in France. At variance with the results of previous observational studies, no significant differences between patients treated with doxycycline and placebo with regard to survival times were found (hazard ratio 1.1, 95% CI 0.8–1.7, p=0.50). Serious adverse events were judged not to be related to treatment. This experience could be useful in the design of large multinational controlled trials of potential anti-prion molecules in this rare disease.

The Fatal Familial Insomnia study started in 2012. The recruited subjects underwent a baseline medical, neurological, laboratory, imaging and neuropsychological assessment. In the meantime, it was decided to create a database for entering data that will be collected over the years.
In a population of patients admitted to medicine and geriatric wards, impaired cognition was associated with in-hospital mortality (odds ratio = 3.1; 95% CI = 1.1-8.6) but not with mortality at follow-up (3 months); increased severity of cognitive impairment was associated with higher odds of mortality. Adverse events may represent an important target of prevention due to their high association with mortality and cognitive impairment.

In the same hospitalized population, drugs with anticholinergic properties were found to be associated with worse cognitive and functional performance, measured by cognitive test and functional scales, evidencing the need to check prescriptions in elderly patients for this effect, in view of the high number of drugs to which they are exposed.

From the prospective survey conducted between 2003 and 2011 in 1,080 hospice patients, we have found that younger age, higher education, cancer type, being previously informed about the diagnosis/disease and previous treatments significantly increased the awareness of patients of being at the end of life. The information to the patients in term of diagnosis was delivered by an oncologist (n=145; 33.1%) or hospital specialist (n=264; 60.3%) and, in a small number of patients, by MMG (n=6; 1.4%), family member (n=9; 2.1%) or others (n=14; 3.2%).

Patients with dementia resident in Alzheimer’s special care units (ASCU) had a lower rate of hospitalisation and use of physical restraints than those in traditional nursing homes. In ASCU, 60% of patients with dementia were taking at least one antipsychotic, 49% typical and 51% atypical. More than 50% of patients exposed to antipsychotics at baseline, were still taking the drug after 18 months of follow-up. The use of antipsychotic agents was strongly related to the presence of agitation, irritability, delusions, anxiety, night-time behaviour and aberrant motor behaviour.

In the Lecco Local Health Authority 16% of elderly patients were exposed to potential severe drug-drug interactions; age and number of chronic drugs were associated with an increasing risk of DDIs. Since physicians still have some difficulty in managing this topic, it is essential to provide them with adequate information on which factors raise the risk of DDIs.

Age, local health unit (LHU) of residence, number of drugs and co-prescribed PIDs were predictors of hospitalization for hemorrhage.

During 2005 in Lombardy Region, 76% of the elderly aged 65 years or more (76% women and 75% men) received at least one chronic drug, 46% were exposed to polypharmacy (46% women and 45% men) and 20% to chronic polypharmacy (18% women and 22% men). Elderly in the age groups of 75-79, 80-84 and 85-89 years had the highest risk to be exposed to chronic polypharmacy (OR 2.25; 95%CI: 2.23-2.27, OR 2.68; 95%CI: 2.65-2.71, and OR 2.84; 95%CI: 2.79-2.89 respectively).

During 2005, 34% of the population living in Lombardy Region received at least one antibiotic drug prescription. The highest prescription prevalence was observed in the 0-17 and 80 or more year age ranges (41.6% and 41.9%, respectively). Patients aged <18 years (OR = 1.73; 95% CI 1.73, 1.74), aged 65 or older (OR = 1.64; 95% CI 1.63, 1.65), and those that live in Brescia (OR 1.66, 95% CI 1.65, 1.66) had a statistically significant higher risk of antibiotic drug exposure.

In a large population sample of subject living in Lombardy Region, the use of paroxetine and fluoxetine peaked in 2002 and then decreased. The prescription rates of mirtazapine gradually increased all through the study period: from 0.07% in 2000 to 0.13% in 2006. On the contrary,
the prescription rates of reboxetine showed a different trend and progressively decreased from 0.20 in 2000 to 0.04 in 2006.

In a sample of 38 internal medicine and geriatric wards, at hospital admission 52% of 1332 elderly patients aged 65 years or older taken five or more different drugs (polypharmacy) and were in the ward for a mean of 11 days. At hospital discharge there was an increase in the rate of patient with polypharmacy (+13%) and with multiple disease (+16%).

Among elderly patients admitted with a diagnosis of AFF to internal medicine wards, an appropriate antithrombotic prophylaxis was taken by less than 50%, with an underuse of VKAs prescription independently of the level of cardio-embolic risk. Hospitalization did not improve the adherence to guidelines.

After multiadjustment, the diagnosis of dementia was associated with in-hospital death (OR = 2.1; 95% CI = 1.0 - 4.5). Having dementia and at least one adverse clinical event during hospitalization showed an additive effect on in-hospital mortality (OR = 20.7 ;95% CI = 6.9 – 61.9).

The strongest association between clusters of diseases and polypharmacy was found for diabetes mellitus plus CHD plus CVD, diabetes plus CHD, and HF plus atrial fibrillation (AF).

The prescription of typical antipsychotics has been associated with an increased risk of CVEs. After stratification, persons prescribed with AChEI did not show any association with CVEs. Nineteen percent of patients admitted to internal medicine and geriatric hospital wards are re-hospitalized at least once within 3 month after discharge. Adevrse events during hospitalization, previous hospital admission, and vascular and liver diseases were significantly associated with likelihood of readmission.

We found a significant association with an increased risk of mortality at 3 months follow in patients exposed to at least 2 potentially severe DDIs (OR=2.62; 95% CI, 1.00-6.68; p=0.05). Hospitalization was associated to an increase in potentially severe DDIs. Careful monitoring for potentially severe DDIs, especially for those created at discharge or recently generated, is important to minimize the risk of associated harm.

We found that there were geographical differences in the prevalence of elderly people with chronic polypharmacy, only partly explained by health indicators. These findings highlight the need for targeted efforts on prescription practice to reduce polypharmacy.

In elderly hospitalized, severely reduced eGFR at the time of admission was associated with in-hospital mortality (OR 3.00; 95 % CI 1.20-7.39, p = 0.0230), but not with re-hospitalization (OR 0.97; 95 % CI 0.54-1.76, p = 0.9156) or mortality at 3 months after discharge (OR 1.93; 95 % CI 0.92-4.04, p = 0.1582). On the contrary, an increased risk (OR 2.60; 95 % CI 1.13-5.98, p = 0.0813) to die within 3 months after discharge was associated with decreased eGFR measured at the time of discharge.

REPOSI patients represent a population at high cardio-embolic and bleeding risks: most of them were at high cardio-embolic/high-intermediate bleeding risk (70.5% combining CHADS2 and HEMORR2HAGES, 98.3% combining CHA2DS2-VASc and HAS-BLED), and 50-60% of patients were classified in a cardio-embolic risk category higher than the bleeding risk category. In univariate and multivariable analyses, a higher bleeding score was negatively associated with warfarin prescription, and positively associated with aspirin prescription. The cardio-embolic scores were associated with the therapeutic choice only after adjusting for bleeding score or age.
Drugs with anticholinergic properties identified by the ACB scale and ARS are associated with worse cognitive and functional performance in elderly patients. The ACB scale might permit a rapid identification of drugs potentially associated with cognitive impairment in a dose-response pattern, but the ARS is better at rating activities of daily living.

The use of INTERCheck(®) was associated with a significant reduction in PIMs and new-onset potentially severe DDIs. CPSSs combining different prescribing quality measures should be considered as an important strategy for optimizing medication prescription for elderly patients.

The increasing number of drugs prescribed at hospital discharge is correlated to non-adherence and a high percentage of patients did not understand the purpose of their medications. Simplification of drug regimens and reduction of pill burdens should be targets for intervention.

Prevalence of inappropriate prescription of allopurinol remained almost the same at admission and discharge. Inappropriate use of this drug is principally related to asymptomatic hyperuricemia. Careful assessment of clinical conditions and stricter adherence to evidence-based guidelines are essential for a rational use.

In the GISAS trial 301 patients with schizophrenia have been randomized and evaluated. The rate of interruption at 12 months was 52%, 32% and 37% in those randomized to aripiprazole, olanzapine and alopeidole respectively; the differences between aripiprazole and olanzapine and aripiprazole and alopeidole were statistically significant. Those assuming aripiprazole interrupted earlier, and the difference between aripiprazole and olanzapine in time to interruption was significant. Metabolic syndrome onset was 37%, 47% and 42% in aripiprazole, olanzapine and alopeidole respectively.

The prevalence rates per 10 000 of subjects treated with lithium in Lombardy in 2000 and in 2010 increased from 7.2 to 9.9 and the incidence decreased from 2.8 to 1.9.

The synthesis of the surveys conducted in the area of Como found that 342 doses of cocaine were consumed every day, similar to the 386 found in the waste waters. HoNOSCA showed a good overall reproducibility on the 13 items with a intraclass correlation coefficient of 0.71, although four items were <0.40.

There is a direct correlation between ALS and mechanical trauma as a result of the following observations: The risk of ALS increases with the number of traumatic events and the severity of injuries. There is an inverse correlation between ALS and coffee intake. The prevalence of extrapyramidal signs in patients with ALS is higher than that expected in the general population. Early onset differs from late onset ALS for the higher exposure to lead, solvents, electromagnetic fields, and professional physical activity.

There is an inverse correlation between physical exercise and ALS. However, among affected individuals the disease tends to occur at a younger age is the patient practiced physical exercise. Data on the 10-year mortality of ALS show a 12% survival rate with significant differences according to the phenotype at diagnosis. Predictors of long-term survival include younger age, possible/suspected ALS, spinal onset, and disease duration longer than 12 months. Survival of a male patient diagnosed at 75 years or older overlaps that of the general population.

L-acetylcarnitine associated to riluzole is more effective than riluzole alone in patients with ALS. Patients receiving the drug present slowing of functional impairment and reduction of short-term mortality In patients with traumatic spinal cord injury erythropoietin was not found
to be unequivocally superior to methylprednisolone in terms of efficacy and tolerability; however, some results favored the experimental treatment.

The study on medication overuse headache supports the efficacy and safety of sodium valproate vs. placebo.

A comprehensive rehabilitation program does not reduce the risk of falls in Parkinson disease when compared to usual care. In patients with epilepsy, an active monitoring of adverse events and drug interactions reduce significantly these events without addictive monetary costs. Treatment of chronic inflammatory demyelinating polyradiculoneuropathy with immunoglobulins for 6 months is less frequently discontinued because of inefficacy, adverse events, and/or intolerance than treatment with intravenous methylprednisolone.

The Italian population and, more specifically, Italian teachers show a satisfactory knowledge of epilepsy but they have still negative attitudes towards the disease and an inadequate approach to its manifestations.

The attitudes of Italian specialists toward epilepsy surgery are heterogeneous and reflect the cultural background and the number of surgical candidates commonly seen.

If adjusted, administrative data are a cost-effective instrument to monitor epilepsy frequency.

A critical appraisal of the literature helped in the preparation of an updated guideline for drug discontinuation in epilepsy in remission.

We have identified possible mechanisms associated with the severity of disease course which will help us to direct more effectively the pharmacological interventions to slow down at the very early stage, this devastating disease. In addition, these studies will help in the development of biomarkers able to predict the progression of the disease and to monitor the efficacy of experimental treatments.

We have seen that the faster disease course in SOD1 mutant mice depends on a earlier and greater accumulation of protein aggregates consequent to the reduced expression and function of chaperoness, as the alpha beta crystallin and cyclophilin A and a premature dysfunction of the proteasome system. An intervention on these system could therefore lead to a significant slowing of the disease.

Through the study of the role of TNFR2 we have demonstrated once again that the ALS is a multisystemic disorder and that in order obtain a therapeutic efficacy is important to consider not only the protection of the motor neurons but also to target other districts involved in the disease such as the muscles and the immune system.

We have brought to light, for the first time, the activation of MHCI complex in motor neurones of ALS models. Since this is a typical response of adaptive immunity, this suggests new perspectives to decipher the mechanisms of interaction between motor neurons and the immune system from which identify new therapeutic targets.

The human cord blood mononuclear cells injected into the cerebral ventricle significantly slow down the disease progression in two mouse models of motor neuron degeneration. Their effect...
is not due to cell replacement but is rather associated with the production and release of circulating protective factors which may act both at the central and/or peripheral level.

We have demonstrated the crucial involvement of some pro- and anti-inflammatory cytokines in seizures using experimental models of epilepsy in rodents, thus describing a new etiopathological mechanism which may be relevant for human epilepsy.

We have demonstrated that membrane-bound drug transport proteins are functionally activated by seizures and have a significant role in decreasing the brain concentrations of antiepileptic drugs in experimental models. Pharmacological intervention to block the activity of these proteins may contribute to reverse multidrug resistance in epilepsy.

The complement system is a relevant target in acute brain injury:

- Recombinant complement inhibitor (rhC1-INH) has a powerful neuroprotective action and a wide therapeutic window in brain ischemia/reperfusion injury
- Targeting mannose-binding lectin (MBL), an activator of the lectin complement pathway, leads to neuroprotection with a wide therapeutic window
- In subarachnoid hemorrhage (SAH) patients ficolin-3, an activator of the lectin complement pathway is associated to clinical and structural parameters of severity.

Microglia is associated to protective actions in the injured brain.

Mesenchymal stem cells drive protective microglia polarization in \textit{in vitro} and \textit{in vivo} injury.

Long term efficacy of human bone marrow mesenchymal stem cells in traumatized mice brain is not affected by immunosuppressive treatment.

TAAR1 modulates brain glutamate transmission and cognitive deficits induced by NMDA antagonists in the rat

Truncation of \textit{mecp2} gene models motor deficits of Rett syndrome and may cause alteration of brain glutamate metabolism

A single session of cocaine self-administration is sufficient to shape rat behaviour towards goal-directed behaviours and selectively up-regulate Arc expression in mPFC. This is the first evidence that the mPFC’s function is already profoundly influenced by the first voluntary cocaine exposure.

The use and the early phases of cocaine abstinence induce a finely tuned modulation of BDNF expression in the NAc and in the mPFC.

Short abstinence from contingent cocaine i.v. self-administration elevates $\alpha$CaMKII autophosphorilation in NAc and mPFC. The persistent enhancement in the mPFC of abstinent rats may represent a previously unappreciated contribution to initial incubation of cocaine-seeking.

Environmental stimuli associated to drug self-administration induce drug-seeking behaviour when presented to rodents after a long period of abstinence.
Bifeprunox, a partial agonist at DA D₂ and 5-HT₁A receptors, influences nicotine-seeking behaviour in response to drug-associated stimuli in rats.

GlyT1-inhibitors might offer a therapeutic opportunity for acute cue-controlled nicotine-seeking. The lack of persistent effects of the sub-chronic treatment associated with nicotine cues exposure suggests that short-term administration of GlyT1-inhibitor SSR504734 is not sufficient to promote extinction of nicotine-cue conditioned responding.

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Protezione del Consumatore della Commissione Europea (DG-SANCO), Bruxelles.
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Health, istituito dal Department of Mental Health della World Health Organization.
International Subcommittee della American Academy of Neurology
International Steering Committee dell’European Network on mental health promotion and mental disorder prevention (EMHPA).
International Subcommittee dell’American Academy of Neurology
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Working Group on Epilepsy della World Health Organization (WHO)

EVENT ORGANIZATION

11ª Giornata di studio sulla malattia di Alzheimer:
La contenzione fisica del paziente affetto da demenza
Apatia e demenze 16 March 2013, Ateneo Veneto, Venezia


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WebMedica, Grottaferrata (Roma).
World Health Organisation

**SCIENTIFIC PUBLICATIONS (2013)**

Agnoli L, Mainolfi P, Invernizzi R, Carli M
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LAY PRESS SELECTION (2013)

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RESEARCH ACTIVITIES

Labotatory of Biology of Neurodegenerative Disorders

Alzheimer's disease: genetic studies and clinical investigations
In collaboration with different neurological centers and the laboratory of Geriatric Neuropsychiatry it has been created a bank of blood samples for DNA of patients with Alzheimer's disease (AD), in familial (FAD) or sporadic form (SAD), and patients with vascular dementia (VD). In all subjects the diagnosis of dementia is performed according to the international guidelines. Since 2005 we started also the collection of blood samples from subjects with front-temporal dementia. The genetic studies are aimed to the identification of causal factors in FAD and risk factors in SAD. Mutations on genes encoding proteins involved in the physiopathology of AD were investigated. The pathogenic role of these mutations is under investigation using fibroblasts obtained from skin biopsy. Furthermore, we continued the screening of FAD samples for the genes encoding for presenilin 1 and 2 (PS-1 and PS-2) and APP, missense mutations in these three genes were associated with AD.

Alzheimer's disease: preclinical studies
The formation of β amyloid (Aβ) deposits in brain parenchyma and on the wall of cerebral blood vessels is an early event in AD and there are now numerous genetic, biochemical and neuropathological studies pointing to a causal role of Aβ in the pathogenesis of AD. Thus, prevention the formation of Aβ aggregates or their elimination once formed is a potential therapeutic approach to the disease. This aim is strongly persecuted with different strategies including the regulation of enzymes responsible of the synthesis and degradation of Aβ and the enzymes influencing the metabolism of amyloid precursor protein (APP). In the lab, we developed the idea to interfere directly with the Aβ deposits formation using anti-amyloidogenic drugs. The experimental studies have shown the potential therapeutic activity of these drugs in AD, and now they will be tested in a clinical setting.
Alzheimer’s disease: Translational studies
In the frame of the European Consortium IMI-PharmaCog have been set up several protocols for the MRI analysis in various transgenic mice models of Alzheimer’s disease (AD). The PharmaCog project focused on the optimization of the translational studies to facilitate the therapeutic approaches considering in experimental models and in the clinical studies the same parameters, behaviorally, biochemically and of imaging. In this contest it will be analyzed longitudinally in single, carrying human amyloid precursor protein mutated (APP) associated to AD, double carrying APP and mutated PS1 transgene, and triple transgenic mice carrying APP, PS2 and mutated tau transgene. We performed the MRI analysis in the same animals at 4, 8, 12, 18 and 24 months, the analysis has been structural, functional and spectrosocical. The strumental parameters (ROI, T2, DTI) have b een harmonized with the partners developing similar approaches in humans. The structural results confirm some common features in the three animal models: the progressively reduction with aging of volume of specific regions like hippocampus and striatum and a reduction of the entorhinal cortex thickness, while the olfactory bulbs are preserved.

The role of oligomers in the Alzheimer pathogenesis
Recent data have shown the essential role plays by oligomers, small and soluble aggregates of Aβ, in the Alzheimer pathogenesis and in particular in the cognitive decline associated to the disease. In collaboration with the Department of Biochemistry an Molecular Pharmacology we developed some in vivo models to analyze the neuronal dysfunction induced by Aβ 1–42 but not in monomeric or fibrillar species. The intracerebral application of these different forms confirmed that Aβ oligomers induced behavioral impairment while monomeric or fibrillar forms of Aβ did not affect the cognitive behavior.

Sirtuins and aging
The sirtuins are a family of conserved proteins with de-acetylation activity. In human the sirtuins are coded by 7 different genes and are localized in the citosol, within the nuclei and in the cellular mitochondria. SIRT-1, the better known sirtuin, is involved in the aging physiology and energetic metabolism, its activation induced beneficial effects in Alzheimer and Parkinson experimental models. We studied sirtuins from different points of view, genetic, cellular and behaviorally. The genetic studies are devoted to identify alterations associated to AD in Italian populations. During the screening of all sirtuin genes, we found several single nucleic polymorphisms that now are investigated in larger population (560 AD subjects). The cellular studies are focused on the role of SIRT-1 and SIRT-2 in the cell death mechanisms and oxidative stress in cellular models of AD. Since sirtuins have been involved in the energetic metabolism, and mental as well as physical exercise exert protective effect in AD, we are evaluating in AD animal models if sirtuins are able to mediate the beneficial effects of physical exercise and environmental stimulation.

Genetics of aging
In collaboration with Geriatric Neuropsychiatry Lab for the Monzino 80-plus study and with dr. Maurizio Gallucci from the ARGel Association in Treviso for Trelong study we collected a large number of blood samples from subjects over seventy. In these samples we are performing a genetic analysis to identify genetic profiles associate to the longevity and /or to the aging-associated pathologies with specific attention to the dementias. The aim is to cross the genotype/phenotype profile with pathologies and environmental aspects including style of life, diet and economical conditions to identify risks and protective factors. Initially the subjects were genotyped for ApoE, whom allele E4 is a well-known risk factor for Alzheimer’s disease and several other disorders and sirt-1 a gene codified for protein member of a enzymatic family
of sirtuins associated to the longevity in several experimental models. The results are interesting but before drawing any conclusion we need to consider the numerous other parameters collected in our database.

**Parkinson's Disease: genetic studies**

Parkinson’s disease (PD) is the second more diffuse neurodegenerative disorder with an unknown pathogenesis, however for PD several therapies are available and, although at the symptomatic level, their efficacies is well-established. In the etiological studies on PD the genetic component has been traditionally considered with scarce interest whereas the environmental causes were carefully evaluated. This orientation was based on the evidence that the exposure to several toxins can mimic the PD pathology. However the genetic studies in the last few years have completely changed the perspective with the identification of mutations on two genes, encoding for alpha-synuclein and parkin, associated to the juvenile forms of the disease. A mutation on alpha synuclein gene is an event extremely rare, only three mutations identified until now, the parkin mutations are numerous ether in puntiform or in deletion form. The mutations on alpha-synuclein gene are dominant while the parkin mutations are associated with PD in recessive form. We collected, in collaboration with several neurological centers, blood samples from PD subjects and the screening of the samples involved genes like alpha-synuclein, parkin, DJ-1 and other factors potentially involved in PD.

**Parkinson's disease: in vitro studies and in vivo studies**

The identification of the mutations associated to Parkinson’s disease (PD) gave a substantial contribute to understand the disease and allowed the development of cellular models to investigate the pathogenesis of the disease. In the past we showed the potential neurotoxic activity of alpha-sinuclein using the synthetic peptide homologous to the fibrilogenic fragment 61-95 (NAC) of the protein. Successively with help of dr. Negro at the Department of Biochemistry at the University of Padova we prepared cDNA vectors including the sequence of wild type and mutated alpha-synuclein Their transfection to the PC12 cells induced in specific conditions a cellular damage. More recently in collaboration with the University of Insubria we obtained the synthesis of synuclein wild type and mutated, together with the in vitro experiments where the primary cells were exposed to the synuclein, we have developed a in vivo model similar to that setting up with β amyloid oligomer. The small aggregates of α-synuclein are injected intraventricularly and the effect of cognitive decline has been evaluated, with different pharmacological interactions are investigated the similarities and the differences from the application of β amyloid.

**Laboratory of Cell Death and Neuroprotection**

**Synaptic Dysfunction**

Nowadays it is assumed that AD is a synapse-related pathology (synaptopathy) in which Aβ oligomers accumulate in the brain parenchyma and lead to synaptic dysfunction and loss, a phenomenon that precedes extensive amyloid deposition in the brain. However, the relationship between Aβ and synapses loss remains unclear. We set up a new in vitro model to study the cellular and molecular alterations that lead to AD synaptopathy. In this model we demonstrated that JNK plays a key role in the onset of AD synaptopathy. JNK in fact is activated in the postsynaptic compartment following Aβ oligomers exposure and it contributes to synaptic dysfunction acting on two main postsynaptic targets: caspase-3, which has been already described to be involved in AD synaptopathy and PSD-95. In particular JNK-mediated phosphorylation of PSD-95 promotes its removal from the PSD and consequently spine...
shrinkage and loss. This study can potentially be a breakthrough in the comprehension of AD pathogenesis and will help in developing effective and preventive therapeutic strategies in order to counteract or nullify the degenerative processes activated by Aβ.

By performing the first in vivo chronic-treatment with the specific cell-penetrating JNK inhibitor peptide D-JNKI1 we demonstrated that JNK regulates the main pathogenic mechanisms of AD and might hold promise as an innovative and therapeutic target against it. We obtained a complete rescue in TgCRND8 AD mouse model of both long-term potentiation and long-term recognition memory impairments in D-JNKI1 treated mice. The functional recovery shows a strong relationship with the JNK signaling pathway and reduction of Aβ oligomers, derived by APP cleavage, in our model. The treatment did not show any side-effect in vivo.

Studying the role of JNK in the mechanisms underlying synaptic plasticity, we observed that it is extensively expressed in the presynaptic compartment, where it controls the release of glutamate into the synaptic cleft. JNK inhibition through D-JNKI1 in fact is able to reduce the neurotransmitter release. In the presynaptic compartment JNK immunoprecipitates with syntaxin-2, a membrane protein participating in the exocytosis of presynaptic vesicles. JNK binding to syntaxin2 is inhibited by D-JNKI1, suggesting that this interaction is involved in the regulation of glutamate release. These results allow to better understand the mechanisms regulating synaptic plasticity and set the basis for the development of new molecules able to modulate the release of glutamate.

New anti-amyloidogenic strategies
APP gene was the first to have been found mutated in an inherited form of AD. Over the years missense mutations have been discovered in this gene and since they are mainly located in the vicinity of the exons encoding the peptide Aβ, are all able to influence APP processing and the consequent production of β-amyloid. We characterized a new APP gene mutation (A673V) recently discovered by Prof. Tagliavini’s group focusing our attention on its interaction with synaptic compartment, first one involved in the toxic events of AD. This mutation causes pathology only in homozygosity otherwise heterozygous subjects never develop pathology. For this purpose, we generated a new in vitro model and we compared the postsynaptic effects of WT and mutated Aβ peptides, confirming that the mutated one is more synaptotoxic. On the contrary the equimolar mix (WT and mut) showed no toxic effects on the biochemical composition of the postsynaptic density region (PSD) and on the dendritic spines number. The neuroprotective effects of Aβ mix (WT plus mut) lead us to generate a new cell-permeable peptide (D-TAT 1-6 A2V) based on APP mutated region. The peptide showed no toxicity in vitro, and if used in combination with WT Aβ prevented from receptors, PSD proteins and dendritic spines loss induced by Aβ. We confirmed this data also in a preliminary in vivo experiment on a murine model of AD. The treatment had no toxic effects on the animals and reverted amyloid-induced dendritic spines loss, increasing PSD marker levels at the postsynaptic compartments. These results are encouraging and set the basis for a promising strategy to prevent AD progression even in the early stages of synaptopathy.

New peptides modulating MKK7 activity
We proved in our lab that it’s possible to selectively block JNK pathological role, separating it from the physiological one, performed under cellular homeostasis conditions. JNK complete activation is induced by two upstream MKKs: MKK4 and MKK7; however only MKK7 is responsible for JNK activation after cellular stress conditions such as excitotoxicity, event at the base of different acute and chronic CNS pathologies. MKK7 in fact is activated by excitotoxic treatments such as NMDA, while MKK4 remains inactive. Through molecular modelling studies, we synthetized a selective MKK7 inhibitor designed on GADD45β’s interaction domain with the kinase. GADD45β is a NFkB-pathway member and it’s able to specifically
inhibit MKK7 activity with no interference with MKK4 activation. We have synthetized two new cell-permeable peptides (MKK7I): Gadd45β (69-86) that only contains the binding site region to MKK7, and Gadd45β (60-86) that represents the binding site and a second region, essential for MKK7 activation. We firstly verified the absence of intrinsic toxicity by administration of different dosages of peptides in primary cultures of cortical neurons. Afterwards we demonstrated that the peptides protect against neuronal death induced by excitotoxic stimuli (NMDA 100 μM) and hypoxic treatment. Our data clearly show that the addition of peptides at a concentration of 10 μM results in a strong inhibition of MKK7 phosphorylation that lasts till one hour from the toxic stimuli, without interfering with MKK4 phosphorylation. Therefore, the two designed peptides act specifically on MKK7 and present a neuroprotective effect against stress like NMDA-toxicity and anoxia. In addition we have verified in vitro findings in two animal model of permanent cerebral ischemia. Animals treated with MKK7I 1h before damage showed an encouraging reduction of the infarcted area, equal to 50%, when compared to controls treated with vehicle only, 24h after the injury. In order to identify the therapeutic window of post-ischemia intervention of MKK7I, we realized tissue samplings at different timings after the lesion, particularly focusing on the first 6 hours. We observed a decrease in JNK activation starting from 3 hours post lesion until 6 hours.

**Laboratory of Experimental Neurology**

**Role of inflammatory molecules in ictogenesis and epileptogenesis**

We are studying the role of IL-1beta and HMGB1 systems in the genesis and propagation of seizures and in the associated neurodegenerative phenomena. We have demonstrated that epileptic activity induces the synthesis of these pro-inflammatory molecules, danger signals and their specific receptors. In particular, IL-1beta and HMGB1 have proconvulsive actions while their receptor antagonists (IL-1Ra, Box-A, Toll-like receptors inhibitors) or IL-1beta synthesis inhibitors, have anticonvulsant activities. We are actively studying the role of these molecules in epilepsy models with the intent of promoting their clinical applications in drug-resistant epileptic patients. This possibility is encouraged by the clinical use of some of these molecules (e.g. anakinra, the IL-1R antagonist) in chronic inflammatory and autoimmune diseases in humans. We are studying pharmacological approaches to block IL-1beta- and HMGB1-signaling involved in the proconvulsive effects of these molecules.

**Role of Toll-like receptor signaling in seizures and neurological sequelae**

Infection and fever, which are concomitant with increased levels of pro-inflammatory molecules not only in the periphery but also in the brain, can be precipitating events of seizures; moreover, a causal link between CNS infection and epilepsy has been proposed. In the context of convergence of brain infection and the epileptic process, an obvious candidate is represented by the Toll-like receptor (TLRs) family. These receptors are pivotal for activation of innate immunity and inflammation following both infections or epileptogenic brain injuries. Moreover, we recently described that HMGB1 released from neurons and glia exposed to pro-convulsant stimuli lowers threshold to seizures by activating TLR4. The aims of the project is two-fold: (1) to characterize TLR2 and TLR3 inflammatory signaling in the brain of rodents exposed to infection-like challenges; (2) to investigate whether TLR2 and TLR3 signaling contribute to seizure threshold and cognitive dysfunctions. We propose to focus on novel targets, to develop new treatments for prevention of drug resistant epilepsy and associated comorbidities, since these are unmet clinical need.

**In vivo MRI to determine glia activation and blood-brain barrier damage**
This study is focused on \textit{in vivo} magnetic resonance imaging (MRI) and spectroscopy (MRS) techniques to evaluate the role of glia activation and blood-brain barrier damage in the epileptic process. Our intention is to explore whether these two phenomena can be used as biomarkers of epileptogenesis. This information may provide a clinically applicable method for predicting the development of spontaneous seizures in individual at risk, thus permitting to envisage preventive strategies.

\textbf{miRNA and inflammation: new opportunities for therapy in epilepsy associated pathologies}

Increasing evidence supports the critical role of microRNAs (miRNAs) in post-transcriptional gene regulation in several biological processes of the central nervous system. Specific miRNAs are considered to represent a new class of modulators of the inflammatory response. In particular, the miRNA-146a has been specifically associated with the regulation of the Toll-like and interleukin-1 receptors (TIRs) signaling, which represents a major pro-epileptogenic pathway activated in both experimental and human epilepsy. Interestingly, this miRNA has been shown to be upregulated in experimental models of epilepsy, as well as in human TLE. The overall goal of the project is to evaluate the role of miRNAs, with a special focus on miRNA-146a, in regulating inflammatory pathways and to study their role in ictogenesis and in epileptogenesis.

Identifying key regulators of the immune/inflammatory response for developing anticonvulsive/antiepileptogenic approaches

A key role of the brain immune response to pathogens or injuries is to activate homeostatic programmes in competent cells for tissue defense or repair. This task is achieved by inducing release of soluble inflammatory mediators acting as effector molecules on target cells. Resolution of inflammation is a highly coordinated and active process that is controlled by endogenous pro-resolving mediators and is instrumental to switch off inflammation after its onset. If this mechanism fails then inflammation might perpetuate resulting in varying degree of tissue injury or dysfunction. A crucial question is how microglia and astrocytes, or leukocytes, balance these tissue demands after injury, and how their behavior can be modified to ameliorate inflammation outcomes. Our hypothesis is that the brain immune response triggered by ictogenic or epileptogenic injuries is inefficiently controlled by pro-resolving endogenous molecules and their cognate receptors, thus resulting in chronic inflammation. Using experimental models of seizures and post-injury epilepsy, we will study the role of key pro-resolving molecules governing the post-injury inflammatory response. The final goal of the project is to demonstrate that incrementing the brain ability to activate efficiently pro-resolving mechanisms of inflammation represents a promising target for developing therapeutic strategies in epilepsy.

\textbf{Time-lapse single-cell Ca\textsuperscript{2+} imaging in cell cultures}

This project investigates whether astroglia-mediated inflammatory pathways can affect neuronal activity, by analyzing changes in intracellular Ca\textsuperscript{2+} neuronal signals. This is a well established read-out measure of cell activation following physiopathologic stimuli. Using time-lapse single-cell Ca\textsuperscript{2+} imaging in primary cultures of mouse hippocampal neurons, we study whether cell responses evoked by proinflammatory stimuli or activation of glutamate receptors are modified. In particular, the effects will be tested on the augmentation of the NMDA-mediated Ca\textsuperscript{2+} response provoked in neurons by cytokines and danger signals which are inflammatory mediators released by glial cells in diseased tissues.
Laboratory of Geriatric Neuropsychiatry

Population study on the prevalence of dementias in the older-old
Parallel to the progressive increase of individuals aged 80 years or older within the elderly population (65+), the number of demented patients of 80 years or older makes up an ever increasing fraction of the total population affected by dementia. As very often happens, the exclusion from studies of subjects in the oldest age classes tends to inevitably underestimate the total number of individuals affected by dementia present in the population. To fill this gap, a door-to-door population study on the prevalence, incidence, risk factors and evolution of dementias and age-associated cognitive deficits has been set up in an elderly population aged 80 years or older living in eight small towns of Varese Province. The survey was subsequently extended to all registered individuals aged 100 or older residing in the province of Varese. The study is funded by a grant from the Fondazione Italo Monzino, Milano.

Health and Anemia in the elderly population
A large survey in old residents of Biella (65 years or older) has been conducted in collaboration with the Local Health Authority of Biella (ASL 12) and with the Division of Hematology, University of Pavia and Fondazione IRCCS Policlinico S. Matteo, Pavia, to estimate the prevalence and incidence of anemia (mild, moderate and severe) in the elderly population and to investigate whether low hemoglobin concentration associated to alteration of CBC such as mean corpuscular volume, leukocytes and/or platelet cell counts could predict or were associated with myelodysplastic syndrome in the elderly. Prevalence of chronic kidney disease increases considerably with age but little is known about its clinical significance in the oldest old. We have investigated the association of all-cause mortality with a reduced glomerular filtration rate estimated using five commonly used equations [Cockcroft-Gault (C-G), Modification of Diet in Renal Disease (MDRD), MAYO Clinic quadratic equation, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), Berlin Initiative Study-1 (BIS-1)] in 700 subjects aged 85 and older taking part in the “Health and Anemia” Study.

Evaluating risk profiles in ambulatory and hospitalised elderly subjects
In collaboration with the Geriatric Division of the Ospedali Regionali of Lugano and Mendrisio, Switzerland, hospitalized and ambulatory patients are evaluated from a neuropsychological, functional and mobility point of view to estimate the impact of these factors on health-related outcomes and disease progression (Canton Ticino Study).

Longitudinal follow-up of individuals with mild cognitive impairment (MCI)
In collaboration with the Geriatric Unit of Ospedali Regionali of Lugano and Mendrisio, Switzerland, the follow-up study of all Mild Cognitive Impairment or Questionable Dementia (CDR 0.5) patients seen at the Memory Clinic of the Hospitals is continuing to estimate the rate of conversion to dementia and to evaluate the possible risk factors associated with conversion (Canton Ticino Study).

The Centenari a Trieste Project (CaT): Study of Cognitive, Biological and Social Features of the Population of Centenarians in Trieste and construction of a data-base
Centenarians agreeing to participate in the study will be interviewed about past and present lifestyles and will be evaluated for the presence of disability, depressive symptoms and, specifically, symptoms of cognitive impairment. The medical history and medication will be provided by general practitioners and by querying the Local Health Unit’s administrative databases. Sleep and heart rate variability will also be investigated. Finally, the consenting individuals will be picked up a few milliliters of venous blood to perform genetic determinations. The study will begin in early 2014.

A European Multicentre Double-Blind Placebo Controlled trial of Nilvadipine in Mild to Moderate Alzheimer’s disease (NILVAD Project - European Union FP7 Program)
In collaboration with the Trinity College Dublin and St. James’s Hospital Dublin together with other ten centres from eight European countries participating in the NILVAD Project. The study employs a randomized double-blind placebo controlled parallel design. The objectives of this study are to investigate the efficacy and safety of Nilvadipine (8 mg once a day) as a disease course modifying treatment for mild to moderate Alzheimer’s disease in a phase III double-blind placebo-controlled study. The primary efficacy outcome measures in this study is the change from baseline to week 78 in cognitive function, as assessed by the Alzheimer’s -Disease Assessment Scale (ADAS -Cog 12). A total of 500 subjects over age 50 years with mild to moderate Alzheimer’s disease (NINCDS-ADRDA criteria); 250 in the nilvadipine group and 250 in the placebo group. The total study duration will be 82 weeks. Patients will receive study medication for 78 weeks.

A randomized, double-blind study versus placebo for the evaluation of efficacy and tolerability of doxycycline administered by oral route in patients affected by Creutzfeldt-Jakob disease
The primary objective of this study was to evaluate the effects of doxycycline, compared with the effects of placebo, in increasing survival time of patients with Creutzfeldt-Jakob disease. The secondary objective was the evaluation of the effects of doxycycline treatment on the rate of disease progression as assessed by functional scales and neurological examination. Safety of treatment is assessed for all subjects.

Fatal Familial Insomnia (FFI): preventive treatment with doxycycline of at risk individuals
Department of Neuroscience, in collaboration with 3rd Department of Internal Medicine, Medicine Operative, Unit Oderzo – ASL 9 Treviso and with Fondazione IRCCS Istituto Neurologico “Carlo Besta” The objective of this study is to test whether the chronic administration of 100 mg of doxycycline can prevent (or postpone) the onset of FFI in members of a family carrying the genetic mutation of the prion protein. Survival of the treated individuals will be evaluated after 11 years.

Analyses of health data taken from linked administrative databases
Following the establishment of administrative databases to monitor medical expenditure reimbursed by the National Health Service, a new field is open to study health using indirect data coming from these sources. We are actively collaborating in analysing data on old subjects and patients with dementia related problems.
Quality of care of terminally ill oncological subjects
In 2000 we started a collaborative program with the hospice “via di Natale Franco Gallini” in Aviano (PN). The present aim of the collaborative research project is to investigate both the clinical and sociodemographic determinants associated with awareness of illness severity in a cohort of terminal cancer patients (n=1080) at the time of admission to the hospice, from 2001 to 2011

Laboratory of Inflammation and Nervous System Diseases

The complement system in stroke and traumatic brain injury experimental models
Previous studies of ours have indicated that the complement system may represent a novel target for reducing damage following acute brain injury. We have shown that C1-INH, an endogenous inhibitor of the complement system, protects against brain injury. Notably it has a wide therapeutic window, being effective also when administered up to 18 h after ischemia. Our data strongly suggest that this remarkable property of C1-INH is due to its ability to bind mannose-binding lectin (MBL), a key protein of the complement lectin pathway. Thus, we have recently focussed our attention on MBL as a novel target for brain injury. Our data show that MBL is deposited on the ischemic endothelium and we have hypothesized that this may represent a key pathogenetic event leading to brain damage. Based on these results, we have developed different strategies aimed at inhibiting MBL functions and all of them lead to neuroprotection with a wide time window of efficacy. In particular, we have identified a newly synthesized MBL-binding molecule (Polyman2) and we have demonstrated that it is effective in reducing neurological deficits and anatomical damage also when administered up to 24 h from the onset of brain ischemia in mice. Ongoing studies are aimed at understanding the pathogenetic mechanism by which MBL exert its toxic effect on the activated endothelium, elucidating this aspect provides an opportunity to identify new neuroprotective strategies. Another goal of this project is to evaluate the involvement of this protein in traumatic brain injury that we model in mice. Detailed analysis of the MBL gene in humans has revealed that a surprisingly high percentage of individuals (10-15% depending on the population considered) carries a genetic deficiency in MBL which leads to low circulating levels of MBL. In order to confirm the relevance of this protein also in humans, another part of this project is aimed at defining whether MBL deficiency in stroke patients limits the progression of the injury. More generally, the involvement of the complement lectin pathway in human neurological condition represents a major goal of our present and future investigations.

Stem cells as a therapeutic approach in stroke and traumatic brain injury
We have previously demonstrated a beneficial effect of neural stem cells after transient brain ischemia. Ethical issues involved in stem cell research and the limited availability of most adult stem cells outline the need to look for other cell populations. We have thus focussed on mesenchymal stem cells (MSC) obtained from cord blood (CB-MSC) or bone marrow (BM-MSC) that are available sources of progenitors with multilineage capacity and can represent ideal candidates for cell-based therapy after acute brain injury. We have assessed the effectiveness of CB-MSC (provided by Cell Factory, Ospedale Maggiore Policlinico, Milano) in reducing ischemic and traumatic brain injury (TBI) and investigated the
mechanisms triggered by their infusion in the injured brain. We have provided evidence that the beneficial role of stem cells is related to the ability to induce a switch from a “hostile” to an “instructive” status in the inflammatory cells present in the injured tissue including microglia/macrophage phenotypical switch, glial scar inhibition and activation of trophic events that promote healing and regeneration. More recently we have defined successful protocols of MSC infusion obtained from human donors (provided by the Laboratory for Cell Therapy “Stefano Verri, Ospedale San Gerardo, Monza) in the immunocompetent injured brain in mice. Since immunosuppression in acute brain injured patients could lead to deleterious infective complications and not be tolerated, these results represent a further step towards translation to clinical practice. Presently we are working on other crucial aspects that need to be fully clarified before proceeding safely to clinical application, namely: 1) the common/differential contribution to the therapeutic effect of MSC obtained from different sources (BM versus CB or amnion) has never been directly determined and needs to be addressed in order select the most effective cell population and to develop a successful therapeutic protocol; 2) there is evidence that MSC may interact with brain cells by direct cell-cell communication and/or by indirect secretion of factors and thereby promote functional and structural recovery, however which are the specific factors produced by MSC driving the beneficial effects and which is the cell type more prone to respond to MSC is still unknown; 3) assessment of the long term efficacy and safety issue of MSC.

In vivo real time imaging in ischemic mouse brain by two-photon microscopy

Ischemic stroke triggers local inflammation-related events, including blood brain barrier damage, leukocyte/monocyte recruitment and microglia activation, all contributing to ischemic damage progression after the acute event, thus being potential therapeutic targets. In vivo imaging of the brain at cellular resolution in 3D provides an ideal tool to get an insight into these dynamic events. We have recently established an original approach by means of two-photon microscopy (2-PM) that allows the visualisation and measurement of dynamic events taking place in the brain. Two-photon microscope benefits from high-energy electronic transition in a fluorescent molecule due to the cooperation of two low-energy photons, thus enabling imaging over long periods in living animals.

We have applied 2-PM to obtain highly detailed imaging and quantification of immune cell behavior within the ischemic territory. In particular, we have focused on the tracking of infiltrated lymphocytes (in collaboration with the Centre For Biophotonics at the Strathclyde University of Glasgow) and of activated microglia. As regards the analysis of lymphocytes, we collected data as number of infiltrated lymphocytes, their track velocity, displacement rate and meandering index thus providing a comprehensive description of lymphocyte behaviour in the brain. We found that, after ischemia, lymphocytes split into two different patterns of motility behaviour and move along the perivascular space prior to brain tissue invasion. Microglia were analyzed by assessing their motility (displacement rate) and morphological features (Sholl analysis). We found that, following focal ischemia, microglia were stationary, but highly dynamic in modifying their shape, being able to develop their ameboid morphology within 24h after ischemia. In mice deficient for the receptor of fractalkine, a chemokine involved in the control of microglia activation, ameboid transformation was prevented with significant reduction in ischemic volume.

Ongoing studies are aimed at elucidating the dynamism of other immune cells associated with the evolution of ischemic damage over time. Thus our studies will provide the basis of a rationale manipulation of the immune response for therapeutic purpose in brain ischemia.

Temporal pattern of expression and colocalization of microglia/macrophage phenotype markers following acute brain injury
Microglia, the major cellular contributors to post-injury inflammation, have the potential to act as markers of disease onset and progression and to contribute to neurological outcome of brain trauma and stroke. After acute injury, these resident cells are rapidly activated and undergo dramatic morphological and phenotypic changes. This intrinsic response is associated to recruitment of blood-born macrophages which migrate into the injured brain parenchyma. Activated microglia and recruited macrophages (M/M), can affect neuronal function and promote neurotoxicity through the release of several harmful components such as inflammatory cytokines, proteases and reactive oxygen and nitrogen species. On the other hand they also possess protective qualities and promote neurogenesis and lesion repair, an action that we have previously documented. These different activation states are characterized by a specific pattern of phenotypic markers, whose expression depends on the temporal evolution of the brain lesion. Our ongoing studies aim at getting insight on previously unexplored aspects of M/M phenotype changes induced by acute brain injury, namely, the presence of specific phenotype markers, their temporal expression, whether or not there are concomitantly expressed by the same subpopulation, whether they are expressed at distinct phases or locations in relation to the lesion.

**Laboratory of Molecular Neurobiology**

**Study of the mechanisms governing the pathogenesis and the course of the Amyotrophic Lateral Sclerosis**

Comparative analysis of two murine models of familial ALS with phenotypic differences of disease for the identification of prognostic markers and therapeutic targets.

We continue, the comparative study between the two strains of mice bearing the same number of copies of the transgene for the SOD1 with human mutation G93A, which show a phenotype of ALS very different from each other by age of onset and duration of disease. After we described in detail the profile of gene expression in motoneurons of the two murine models of ALS at the onset of symptoms (data published in Nardo et al. Brain 136: 3305-32), our attention focused on the study of the mechanisms of protein quality control. In fact the comparison between the two models showed a different accumulation of insoluble mutant SOD1 and in general of aggregated proteins in the spinal cord of the two mice. We therefore investigated, in both models, the main physiological mechanisms of the degradation of proteins such as:

1) The chaperones important for the "refolding" of mutant proteins and their processing toward the "pathways" of degradation; 2) the systems for protein degradation such is the proteasome and the autophagy. We have observed that some chaperones as the alpha-beta-crystalline and the ciclophilin A are more expressed in ALS mice with slow as compared to fast disease progression, the first already at the basal state while the second increases significantly during the progression of the disease only in mice with slow disease course. In addition, the levels of certain subunits of the proteasome, the main system of protein degradation decreases more precociously in mice with rapid disease progression compared to those in whom the disease progresses more slowly.

These data support the hypothesis that a malfunction of the mechanisms of protein degradation is at the base of a rapid worsening of the disease. The modulation of these systems could then lead to a slowing of the disease. These results are described in a manuscript currently under revision. This project funded by MNDA U. K. and EUROMOTOR FP7 program is based on the collaboration with the Sheffield Insitute of Translational Neuroscience (SITraN) in Sheffield, UK.
Role of neuroinflammation and neuroimmunity in the pathogenesis and progression of ALS.

Our recent studies and other evidence indicate that the involvement of the immune system in ALS is no longer a secondary event following the progressive degeneration of motor neurons but rather it can be a causative phenomenon in governing the development and in particular the severity of the ALS. Our interest in this regard is aimed to the study of the following mechanisms:

*Studies in vitro and in vivo on the role of TNFalpha pathway in the pathogenesis of ALS.*

Several lines of evidence have demonstrated the involvement of neuroinflammation in the development and progression of amyotrophic lateral sclerosis (ALS). In particular, the cytokine TNFalpha has often been referred to as one of the main mediators of this phenomenon. Our previous studies showed an increased expression of TNFR1 and 2 receptors in the motor neurons of SOD1-G93A mice already at an early state of the disease (Veglianese et al. 2009). To further investigate the role of the activation of these pathways on the death of motor neurons, we performed in vitro studies using co-cultures of neurons and astrocytes taken from the spinal cord of SOD1-G93A transgenic mice. The use of this cell model showed that the functional block or ablation of the TNFR2 receptor, on both astrocytes or neurons, prevents the death of motor neurons. In agreement with these findings, we observed partial protection of motor neurons and neuromuscular junctions in SOD1-G93A mice lacking the receptor TNFR2 (SOD1-G93A/TNFR2-/-). However, this apparent protective phenomenon was not enough to significantly improve the symptoms or prolong the survival of these animals (manuscript in preparation). Recent studies have shown that TNFR2 is essential for the stabilization of regulatory T cells (Treg). It was also seen that these cells play a protective role important in ALS particularly on the disease progression. Therefore the altered stability of Treg given by the lack of TNFR2 could have masked the protective effect on motoneuron observed in mice SOD1-G93A/TNFR2-/-, an aspect that we are now investigating. This study emphasized once again that the ALS is a neurodegenerative multisystemic disorder. Then, therapeutic treatments should be addressed at different levels in order to obtain a real efficacy in halting the disease. Our next experiments are pointing in this direction.

This study was supported by the Regione Lombardia (Project Nepente) and is now made collaboration with the Department of Immunology of the Humanitas Foundation for the research of Milan

*Pathogenic role and possible clinical use of the axis CCL2/CCR2 in the regulation of immune responses in ALS.*

This translational research project aims to understand the role of the axis CCL2/CCR2 in the induction and maintenance of the immune response in ALS. The project is based on the assumption that an increase of the early expression of CCL2/MCP-1 observed in the spinal cord of the mice and presumably from the ALS patients, can attract monocytes and lymphocytes from the blood able to exert a potential protective effect motoneuron damaged. We therefore intend to examine whether the axis CCL2/CCR2 could impact on ALS pathogenesis at three distinct levels: 1) via a direct effects of on the mobilization of different monocytes and lymphocytes subsets in the bloodstream of both animal models and patients with ALS; 2) via a direct impact in the control of macrophage/microglia and T lymphocytes (Th2) polarization which determine the balance between cytotoxic (type 1) and cytoprotective (type 2) activation; 3) via direct effects on the recruitment of monocytes and T
lymphocyte subsets in the CNS and peripheral nerves and their possible protective activity on motor neurons ad axons. So far we have confirmed that in the spinal cord of SOD1 mutant mice there is an upregulation of CCL2 which is not accompanied by an increase of CCR2 suggesting a reduced recruitment of CCR2 positive cells in the damaged area. Accordingly, reduced levels of CCR2 were found in the monocytes/macrophages of SOD1G93A mice as well as in the monocytes of ALS patients with the most severe disease. Therefore, we suggest that in ALS the downregulation of CCR2 in monocytes/macrophages reduce their recruitment to damaged areas. What is the mechanism involved and how this may affect the course of pathology in mice is still ongoing.

This project supported by the Italian Agency for the research on ALS (ARISLA) is based on collaboration between our group, the Department of Immunology of Fondazione Humanitas per la Ricerca of Milano and the Fondazione Salvatore Maugeri IRCCS, Istituto Scientifico di Milano.

Role of The Major Histocompatibility Complex I (MHC-I) In Amyotrophic Lateral Sclerosis

From our recent studies have shown for the first time that in motoneurons of the SOD1G93A mice, since the symptoms onset, there is a significant increase in the expression of the complex of Major Histocompatibility Complex I (MHC-I) and the beta 2 microglobulin(b2m), a typical mechanism of adaptive immunity that promotes the presentation of antigens on the membrane for recognition by cytotoxic T lymphocytes, CD8. In particular, we observed that the MHCI and the b2m molecules increase considerably in nerves and in neuromuscular junctions in mice with a slower progression of disease suggesting a possible protective action of this system.

In order to understand the role that this complex plays in pathogenetic mechanisms as well as on the progression of the disease we have crossed the mice SOD1G93A with mice lacking b2m and therefore lacking of the adaptive immune response. The experiments are still in progress.

This project is funded by the Foundation Thierry Latran for the Research on ALS from France and is made in collaboration with the Department of neuroscience of the Karolinska Institute in Stockholm, Sweden.

Studies aimed to identify biomarkers for the diagnosis and progression of the ALS

Following the identification of a panel of protein markers that can discriminate with high significance and specificity patients with the ALS from control patients with other diseases though the collaboration with the Translational Proteomics Laboratory of the Department of Biochemistry, directed by Dr. Valentina Bonetto we have also contributed to validate some of these proteins in the peripheral blood cells and in the spinal cord of SOD1G93A transgenic rats. This study allowed to identify some proteins non only as easy detectable biomarkers for the diagnosis of ALS but also as important factor relevant to the pathogenesis of the disease. They are going to be examined in depth.

Laboratory of Experimental Psychopharmacology

Drug Abuse: Neural basis of drug self-administration

To separate the direct pharmacological effects of cocaine from those associated with active drug self-administration we employed a yoked control-operant paradigm and investigated the expression of well established markers of the rapid action of cocaine, i.e. the inducible early genes, such as Activity-Regulated Cytoskeletal-associated protein (Arc), and trophic factors, such as Brain Derived Neurotrophic Factor (BDNF), in rats after a single intravenous (i.v.) cocaine self-administration session. Animals self-administering cocaine (SA) did more active
lever-presses than yoked-cocaine (YC) and yoked-vehicle (YV) animals. This goal-oriented behaviour was accompanied by a selective increase in Arc mRNA levels in the medial prefrontal cortex (mPFC). These findings demonstrate that a single session of cocaine i.v. self-administration is sufficient to shape rat behaviour towards goal-directed behaviours and selectively up-regulate Arc expression in mPFC (of SA animals), providing the first evidence that the mPFC’s function is already profoundly influenced by the first voluntary cocaine exposure. Ongoing studies are evaluating whether this effect is peculiar to cocaine or common to other drugs of abuse.

BDNF dynamic changes were investigated in the nucleus accumbens (NAc) and mPFC during use and the early phases of cocaine abstinence after chronic exposure by employing a “yoked control-operant paradigm”. The effect on BDNF was region-specific and dependent on the withdrawal time. In the NAc, BDNF protein levels increased immediately after the last self-administration session, with a larger increase in passively cocaine-exposed rats. In the mPFC, BDNF expression was elevated 24 hours after the last self-administration session, independently of how the drug was encountered. No changes were found in NAc and mPFC 7 days after the last self-administration session. Analysis of transcript levels in the mPFC indicated that action on exon I might contribute to BDNF’s cortical induction.

Increases in alpha calcium/calmodulin-dependent protein kinase type II (αCaMKII) activity in the nucleus accumbens shell has been proposed as a core component in the motivation to self-administer cocaine and in priming-induced drug-seeking. Since cocaine withdrawal promotes drug-seeking, we hypothesized that abstinence from cocaine self-administration should enhance αCaMKII as well. Short-term abstinence from contingent, but not non-contingent, cocaine i.v. self-administration elevates αCaMKII autophosphorylation, but not the kinase expression, in a dynamic, time- and brain region-dependent manner. Increased αCaMKII autophosphorylation in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC), but not dorsolateral striatum (dlS), was found 24 h, but not immediately, after the last cocaine self-administration session. Notably, in the mPFC, but not NAc and dlS, αCaMKII autophosphorylation was still enhanced 7 d later.

Neural basis of “drug craving” and “relapse” in the drug abuse assumption

Drug craving, defined as the desire to experience the effect(s) of a previously experienced psychoactive substance is a cardinal feature of drug addiction and is clinically significant because of its potential link to relapse. To provide useful indications to the development of novel therapeutic approaches to prevent the use and abuse and the relapse of drug assumption following the outcome of craving, we elaborated experimental models of self-administration and relapse induced by cocaine, nicotine and alcohol-associated cues, after a period of abstinence. Ongoing studies are evaluating the role of several neurochemical mechanisms potentially involved in the drug-seeking behaviour.

Search for pharmacological agents modulating drug craving and relapse

Environmental stimuli associated with the intake of psychotropic substances of abuse may have the ability to induce the craving that often preludes to relapse in formally detoxified patients. Studying nicotine in an experimental model of extinction-reinstatement induced by the presentation of environmental stimuli associated with self-administration of psychotropic substance of abuse, it was found that bifeprunox, a high-affinity partial agonist of dopamine (DA) D2 receptors and serotonin1A (5-HT1A) receptors, preferentially reduced nicotine-seeking behaviour in response to drug-associated stimuli in rats after a long period of abstinence. Pharmacological stimulation of N-methyl-D-aspartate receptors (NMDAR) could enhance the outcome of cue-exposure therapy for smoking cessation. NMDAR stimulation can be achieved by increasing pharmacologically the synaptic levels of glycine, a necessary co-agonist. Here, we
evaluate the effects of SSR504734, a selective inhibitor of glycine type I transporter (GlyT1) in an extinction-reinstatement procedure inducing robust and lasting nicotine-seeking behavior in rats. Acute pre-treatment with SSR504734 reduced nicotine-seeking but not sucrose-seeking behavior without influencing rats' locomotor activity. Sub-chronic treatment during daily exposure to nicotine-conditioned cue reduced nicotine-seeking; however, this effect was transient, with return to responding at 72 hours. Full recovery of responding was observed after 1 month suggesting that SSR504734 sub-acute treatment did not engage the long-term plasticity mechanisms probably involved in nicotine-seeking.

Laboratory of Epidemiology and Social Psychiatry

Randomised controlled trial of the Italian Group for the Study of the Second Generation Antipsychotics – GiSAS
The study aims at evaluating efficacy and safety of three antipsychotic drugs - aripiprazole, olanzapine and haloperidol – by a pragmatic design and involving a large sample of patients with schizophrenia treated in community psychiatric services across Italy with a 12 months follow-up.

Monitoring of self-harm and suicide attempts
Data collection is active in all Emergencies of the Province of Trento, covering a total of 530,000 inhabitants. Between July 2009 and November 2013 a total of 596 events were registered.

Regional drug prescription database
Lithium utilization from 2000 to 2010 was analysed. Dispensing data of all community-dwelling residents, representing about 30% of the whole Lombardy population, were drawn from the regional administrative database.

Survey and ecological study of drug abuse in the area of Como
Waste waters can be analysed in order to quantify use of specific drugs in the population served by a specific waste water area. In order to attribute the detected consumption to specific population groups, a general population survey and a survey in a randomised sample of schools were conducted in the area of Como. Besides waste waters analyses consumption was investigated in drug addiction services, in the general population and in school population.

The Health of the Nation Outcome Scales – Children and Adolescents, HoNOSCA
The HoNOSCA is evaluated for its reliability: inter-rater reproducibility was conducted using 35 trained psychologists and psychiatrists on 8 vignettes and the validation study is starting in 9 services.

Manual of family therapy for families with adoptive children showing problems
The phases of the therapy were described and operationalized. Validation is conducted by a group of 20 psychologists who identify the phases in the videos of real therapies.
Prion's disease

Prion diseases, also known as transmissible spongiform encephalopathies, are progressive and invariably fatal degenerative disorders of the central nervous system that affect humans and other animals. Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) syndrome, and fatal familial insomnia (FFI) are the most common forms in humans; scrapie of the goat and sheep, bovine spongiform encephalopathy (“mad cow disease”), and chronic wasting disease of deer and elk are the best-known examples of prion zoonoses. These diseases result from the conformational change of a cellular protein of unclear function (denominated prion protein, PrP) into a self-propagating pathogenic isoform that accumulates in the brain of the patients and causes neuronal dysfunction and degeneration through an unknown mechanism. Three different manifestations of prion diseases are recognized: sporadic, infectious and genetic. Genetic prion diseases display autosomal dominant inheritance and are linked to insertional and point mutations in the PrP gene, on chromosome 20. These mutations are presumed to favor the conformational conversion of PrP into a pathogenic isoform. Interestingly, different mutations are associated with different types of prion disease (CJD, GSS or FFI). The research activity in the laboratory of Prion Neurobiology is focused on two main questions: 1) What causes neuronal dysfunction in inherited prion diseases? 2) How do different PrP mutations cause different diseases? We have developed a research program to tackle these questions, using transfected cells, transgenic mice and primary neuronal cultures for complementary exploration of responses to mutant prion proteins. These experimental models are being analyzed with a wide range of molecular and cell biology techniques, as well as protein chemistry and proteomics. The major achievements of the laboratory are the development of the first transgenic mouse model of Creutzfeldt-Jakob disease that recapitulates the cognitive, motor and neurophysiological abnormalities of the human disorder (Dossena et al., *Neuron*, 2008), and the discovery of the molecular mechanism by which mutant PrP induces neuronal dysfunction (Senatore et al., *Neuron*, 2012).

Molecular mechanisms of synaptic dysfunction in genetic prion diseases

How mutant PrP leads to neurological dysfunction in genetic prion diseases is unknown. Tg(PG14) mice synthesize a misfolded mutant PrP which is partially retained in the neuronal endoplasmic reticulum (ER). As these mice age, they develop ataxia and massive degeneration of cerebellar granule neurons. We found that motor behavioral deficits in Tg(PG14) mice emerge before neurodegeneration and are associated with defective glutamate exocytosis from granule neurons due to impaired calcium dynamics. We then discovered that PrP interacts with the voltage-gated calcium channel $\alpha_2\delta-1$ subunit which promotes the anterograde trafficking of the channel. Owing to ER retention of mutant PrP, $\alpha_2\delta-1$ accumulates intracellularly, impairing delivery of the channel complex to the cell surface. Thus mutant PrP disrupts cerebellar glutamatergic neurotransmission by reducing the number of functional channels in cerebellar granule neurons. These results link intracellular PrP retention to synaptic dysfunction, indicating new modalities of neurotoxicity and potential therapeutic strategies.

Mechanism of toxicity of deleted form of prion protein

Insight into the normal function of PrP, and how it can be subverted to produce neurotoxic effects, is provided by PrP molecules carrying deletions encompassing the conserved central region. The most neurotoxic of these mutants carries a short deletion in the central core of the molecule (called $\Delta$CR). This mutant produces a spontaneous neurodegenerative illness when expressed in transgenic mice, and this phenotype can be dose-dependently suppressed by co-expression of wild-type PrP. Whether the toxic activity of $\Delta$CR PrP and the protective activity or wild-type PrP are
cell-autonomous, or can be exerted on neighboring cells, is unknown. To investigate this question, we have utilized co-cultures of differentiated neural stem cells derived from mice expressing ∆CR or wild-type PrP. Using this system, we found that while ∆CR-dependent toxicity is cell-autonomous, the rescuing activity of wild-type PrP can be exerted \textit{in trans} from nearby cells. These results provided important insights into how ∆CR PrP subverts a normal physiological function of PrP, and the cellular mechanisms underlying the rescuing process.

**A new method for detecting toxic amyloid-β oligomers involved in Alzheimer's disease**

Soluble oligomers of the amyloid-β peptide play a key role in the pathogenesis of Alzheimer’s disease, but their elusive nature makes their detection challenging. We have developed a new immunoassay based on surface plasmon resonance (SPR) that specifically recognizes biologically active amyloid-β oligomers. This assay allows specific recognition of oligomeric intermediates, discriminating them from monomers and higher order aggregates. The species recognized by SPR generate ionic currents in artificial lipid bilayers and inhibit the physiological pharyngeal contractions in the nematode \textit{Caenorhabditis elegans}, a new method for testing the toxic potential of amyloid-β oligomers. With these assays we found that the formation of toxic oligomers is inhibited by epigallocatechin gallate and increased by a mutation linked to early onset dementia. The SPR-based immunoassay provides new opportunities for detection of toxic amyloid-β oligomers in biological samples and could be adapted to study misfolding proteins in other neurodegenerative disorders.

**Laboratory of Neurochemistry and Behavior**

**Trace amine-associated receptor1 as a potential target to counteract cognitive deficits**

The role of trace amine-associated receptor1 (TAAR1) in the central nervous system has been investigated in the last few years after suitable tools, such as selective TAAR1 agonists and antagonists and TAAR1 knockout and transgenic animal, become available. Most studies have been focused on monoaminergic neurotransmission and little is known on their potential role in counteracting cognitive deficits caused by dysfunctional glutamatergic transmission. We have recently investigated the effect of molecules acting on a variety of neurotransmitter receptors for their ability to counteract attention deficit caused by antagonists of the glutamatergic NMDA receptor. These studies were done in rats and mice using the 5-choice serial reaction time task, a behavioral task analogous to the “continuous performance test used in schizophrenic patients to assess their attention and vigilance. Using this approach, we found that attention deficits caused by NMDA receptor blockade are associated to excessive release of glutamate from nerve endings of the rat and mouse prefrontal cortex. Preliminary results show that the overexpression of TAAR1 suppresses the release of glutamate induced by phencyclidine, a NMDA receptor antagonist, used to model cognitive symptoms of schizophrenia in rodents. Based on these findings, we hypothesized that the stimulation of TAAR1 receptors may improve attention deficits induced by NMDA receptor antagonists. Studies are ongoing to evaluate this hypothesis.

**Experimental model of Rett syndrome**

Rett syndrome is a X-linked genetic disorder predominantly caused by the loss-of-function of the \textit{mecp2} gene. Females are predominantly affected. After a normal development until 6-18 months, a regression phase begins and patients start to show a spectrum of abnormalities including neurological symptoms such as ataxia, aphasia and apraxia, abnormal motor functions irregular breath, disruption of the sleep/wake pattern, autistic symptoms, repetitive hand
movements (hands-wrangling), convulsions back deformities and feeding abnormalities. Experimental models are fundamental for investigating the biological bases of the disease and are necessary for the development of potential therapies. With this aim and the support of the Italian Rett Syndrome Association (AIRETT), we characterize the behaviour of mecp2<sup>308/-</sup> homozygous female mice, which carry a truncated gene. Differently from conventional knockout mice that survive for few weeks, gene truncation is associated with a milder phenotype and prolonged survival (>10 months), which allows the investigation of motor and cognitive functions and behavior at different ages. Mecp2<sup>308/-</sup> mice showed early onset motor deficits (motor performance and coordination), tremors, clasping and kiphosis worsening over time. Cognitive performance as well as emotional and social behavior were apparently not affected. We now planned to extend these observations to mice carrying different type of mutations of the mecp2 gene to confirm our findings and investigate the underlying mechanism, which based on preliminary results may be linked to an alteration of brain glutamante metabolism.

**Laboratory of Neurological Disorders**

**Epidemiological studies on amyotrophic lateral sclerosis (ALS)**

Included are studies on the incidence, risk factors and mortality of ALS. The data are obtained from a regional registry of the disease activated in 1998 and including all patients with newly diagnosed ALS identified in the Lombardy region. Using similar study protocols, the same data are collected in three additional regional registries (from Piemonte, Liguria and Puglia) included in a network with the Lombardy registry. Information obtained from patients enrolled in the Lombardy registry and from cases examined by members of the Italian ALS Study Group has been used to assess the validity and reliability of diagnostic criteria for ALS and selected disability scales. Based on the data recorded, the annual incidence of ALS is comparable to that obtained in other Western countries where ALS registries have been activated, and is among the highest ever published (1.9 per 100,000). Mortality of ALS has been found to be comparable to that of studies from similar populations studied with the same protocol. The study on the validation of the current diagnostic criteria for ALS (the El Escorial criteria) showed that to be considered valid and reliable, the criteria should be used after proper training of the investigators.

In October 2004, the Laboratory of Neurological Disorders has started a European collaborative group for the ALS registries (EURALS) with the intent to create a common database (completed in the year 2005) with the participation of the existing regional and national disease registries. With the collaboration of the UK and Irish groups participating in the EURALS collaboration, a scientific report has been published on a meta-analysis of the incidence of ALS, performed by pooling data from the 1998-99 cohorts of patients enrolled in the population-based registries. Two studies have been recently concluded: 1. A case-control study on trauma and risk of ALS (in collaboration with the Italian registries); 2. A survey of the prevalence of cognitive impairment and extrapyramidal signs in patients with newly diagnosed ALS (Italian registries); 3. A study on the correlation between ALS and coffee intake; 4. A comparative study of the genotype and phenotype of early onset and late onset ALS; 5. A case-control study of sport, physical activity, and trauma and risk of ALS (in collaboration with partners of the EURALS group); 6. A study on the long-term survival of ALS. The following investigations are still in progress: 1- A study in a population-based incident cohort 1998-2002, aimed to verify the correctness of the diagnosis during follow-up; 2. A study comparing cognitive impairment and extrapyramidal signs in a sample of ALS patients and in a matched control population in Lombardy; 3. An observational study to identify environmental and and genetic risk factors in
some European populations. 4. A survey on dietary factors in patients with ALS and healthy controls to investigate the effects of alimentary habits on the disease risk.

**Therapeutic trials in neurological disorders**

During the year 2010 seven therapeutic trials sponsored by the Italian Drug Agency (AIFA) and a therapeutic trial sponsored by the Italian Ministry of Health were ongoing. Included are: 1. A randomized double-blind parallel-group placebo-controlled trial on the efficacy and tolerability of L-acetylcarnitine in ALS; 2. A randomized open-label parallel-group trial comparing Erythropoietin to Methyl-prednisolone in patients with acute spinal cord injury; 3. A randomized double-blind parallel-group placebo-controlled trial on the efficacy and safety of valproate in medication-overuse headache; 4. A randomized open-label trial of the efficacy of a comprehensive rehabilitation program for the prevention of falls in Parkinson’s disease; 5. A randomized open-label trial on the efficacy of an active monitoring of the adverse effects of antiepileptic drugs and of relevant drug interactions; 6. A randomized open-label trial on the efficacy of an educational program for physicians working in nursing homes. 7. A multicenter, randomized, double-blind, placebo controlled, parallel-group trial of intravenous immunoglobulin vs. methylprednisolone in patients with chronic inflammatory demyelinating polyradiculoneuropathy. The first trial aims at finding a potentially effective drug in a clinical condition for which there is only one product (Riluzole) with at best modest efficacy on survival. L-acetylcarnitine has been found to improve survival in experimental models of motor neuron disease. The second trial intends to verify the efficacy of erythropoietin, a drug shown to mitigate the effects of traumatic spinal shock and accelerate recovery in experimental animals. The drug chosen for comparison (methylprednisolone at high doses) has been selected for being the present gold standard in clinical practice. The third trial aims at verifying whether valproate (a drug commonly used for the prophylaxis of migraine) abates symptoms occurring in drug-overuse headache, a common and frequently invalidating variety of chronic idiopathic headache. The fourth trial aims at assessing whether a comprehensive rehabilitation program compared to usual care is followed by a reduction in the incidence of falls in patients with Parkinson’s disease at risk of falls. The fifth trial aims at verifying the added value of an active monitoring of adverse drug interactions compared to usual care in patients receiving antiepileptic drugs associated to other compounds. The sixth trial aims to verify the added value of a web-based educational program in reducing the number of inappropriate prescriptions compared to usual care. The seventh trial aims at evaluating the comparative efficacy and tolerance of IVIg or corticosteroids over a 6 month period, which remains unclear.

The laboratory of neurological disorders is the coordinator of the first trial and a partner in the other trials, where the main tasks include protocol and CRF preparation, statistical analysis, and preparation of the final scientific report.

**Public knowledge and attitudes towards epilepsy**

Two national population-based surveys have been conducted to assess the knowledge and attitudes of the Italian population towards epilepsy. The first study was a telephone interview of 819 women and 737 men aged 18 or older to verify the basic knowledge of the frequency, causes and characteristics of the disease and their attitudes towards the affected individuals. The answers were compared to those of a previous interview performed 25 years before. The interviewees showed satisfactory basic knowledge, with few exception, and an overall improvement in the acquired notions and the attitudes when compared to the responders in the antecedent survey. However, about half of them still considered epilepsy a psychiatric disorder and a source of important limitations in everyday life. Knowledge and attitudes varied with age, gender and education. A second telephone survey involved 600 primary and secondary school teachers. As with the previous interview, respondents showed a satisfactory basic knowledge but some negative attitudes towards epilepsy and several of them declared being unable to
manage an epileptic seizure. A third survey was conducted on 582 Italian elementary school teachers. All interviewees were aware of the existence of epilepsy and most had direct experience with the disease. Answers about frequency, causes, outcome and response to treatments were variable and independent on age, residency and years of experience. Teachers had positive attitudes towards epilepsy, except for driving and sports. Epilepsy and its treatment were considered a source of learning disability and social disadvantages. Several teachers declared being unable to help a seizing child.

**Barriers toward epilepsy surgery**

Epilepsy surgery is a valuable therapeutic option in patients who do not respond to the available drugs. Knowledge and attitudes toward epilepsy surgery have been tested through a questionnaire survey in a sample of 183 neurologists and child neurologists in Italy and then to patients (adults and adolescents) and their relatives. The responses to the first investigation (only neurologists) were compared to those of a group of epilepsy experts. The study showed a significant heterogeneity of responses, two thirds of them non-aligned to those of epilepsy experts who were largely in favor of surgery. The only variables associated with negative attitudes were the small number of surgical candidates among their patients and the region of specialty attainment. The second survey was conducted in 228 adults patients with epilepsy in tertiary referral centers in Lombard. The responses showed that patients, even those who were possible candidates for surgery, had received insufficient information and were therefore unwilling to accept the treatment. Their opinion changed when detailed information on the risks and benefits of surgery was given.

**Prevalence and incidence of epilepsy in northern Italy**

The study aim was to calculate the prevalence and incidence of epilepsy in a well-defined area of Lombard, using administrative data for the period 2000-2008 provided by the regional database. Included were patients fulfilling the ICD 9 code for epilepsy and seizures and/or the disability exemption code for epilepsy, the presence of EEG, and antiepileptic drugs prescriptions in variable combinations. The validity of the diagnostic criteria was assessed examining a sample of patients with epilepsy through their caring physicians. The best and most conservative algorithm included EEG and selected treatment schedules (sensitivity 85.9%; specificity 99.8%; positive and negative predictive values 64.2% and 99.9%). Based on these values, data obtained from administrative records were adjusted to provide prevalence ratios and incidence rates of respectively 5.95 per 1,000 and 46.68 per 100,000 per year. These data are comparable to those of accurate epidemiological surveys done in industrialized countries.

**Guidelines for the treatment of epilepsy**

The Italian League Against Epilepsy has issued evidence-based guidelines to help practicing physicians in their decision to stop or withhold antiepileptic drugs (AEDs) in patients achieving a prolonged period of seizure freedom. 6 neurologists and 2 child neurologists examined the literature, assessing the quality of the reports, and made the following recommendations: (1) antiepileptic treatment might be discontinued after a minimum period of 2 years of seizure freedom; (2) in children, AED discontinuation could be considered after less than two seizure-free years; (3) factors, such as abnormal EEG at the time of treatment discontinuation, a documented etiology of seizures, partial seizures, or an older age at disease onset, enhance the risk of relapse; however, patients should not be encouraged to withhold treatment unless a combination of two or more of these factors is present; (4) female sex, family history of epilepsy, history of febrile seizures, disease length/severity, and number and type of drugs taken should not influence the decision to stop treatment; (5) epilepsy syndrome should be always included in the decision process; (6) slow (at least 6 months) AED discontinuation should be encouraged; in any case the duration of the tapering period should be tailored to the patient's
needs and preference; and (7) patient discontinuing treatment should be followed for no <2 years. As a general habit, the decision to stop treatment should be discussed and shared with each patient, taking into account social and personal complications of a seizure relapse and the medical complications of chronic AED treatment.

Laboratory of Quality Assessment of Geriatric Services

PHARMACOEPIDEMIOLOGICAL STUDY USING ADMINISTRATIVE DATABASE

Utilisation, integration and implementation of administrative database for the assessment of prescribing appropriateness and in-hospital and community therapeutic continuity.

The availability of computerized system for the management and care of community-dwelling and in-hospital patients represents an opportunity for developing and implementing new strategy in the field of the evaluation, monitoring and implementation for the appropriateness of drug prescription and the continuity of care. A collaborative study has been set up with the Health Directorate of Lombardy Region, the Bergamo Local Health Unit and the hospital “Azienda Ospedali Riuniti” of Bergamo with the goal to test in some critical prescribing fields the effectiveness of multidisciplinary integrated interventions and educational events in improving the prescribing practice and to implement the utilisation of generic drugs.

In the Working Group on the appropriateness prescribing in the elderly an interactive database has been done for the evaluation of prescription drugs with the aims of:
- identifying of drug interactions
- identifying of inappropriate prescribing
- Analysing the anticholinergic effects
- assessing the appropriate dosage in case of impaired renal function.

Drug interactions in elderly patients

In a collaborative study with Lombardy Region we analysed all prescriptions dispensed from January 1, 2003 to December 31, 2003 to individuals aged 65 or more registered under the Local Health Authority of Lecco, a northern Italian province with a population of almost 330,000 persons. Elderly who received at least two co-administered prescriptions were selected to assess the presence of DDIs. 9115 elderly (16%) were exposed to potentially severe drug-drug interactions and 61% were women. A total of 13,520 severe drug interactions were recognized, mainly involving cardiovascular drugs (56.8% of the cases). The prevalence of potentially severe DDI increased at rising of the patient’s age and of the number of chronic drugs prescribed. At univariate and multivariate analysis age and number of chronic drugs were associated with an increasing risk of DDIs. Elderly constitute a population at high risk of DDIs. Since physicians still have some difficulty in managing this topic, it is essential to provide them with adequate information on which factors raise the risk of DDIs.

Prevalence and appropriateness of antidepressant use in elderly

The objectives of the present study were to investigate the prevalence and the appropriateness of antidepressant (AD) use in the elderly population living in three Local Health Unit of Lombardy Region by using a population-based prescription dataset. Changes in the patterns of antidepressant prescribing from 2000 to 2007 were investigated and put into relation with the rates of depressive disorders in Lombardy. The 1-year prevalence of “AD use” increased dramatically from 2000 to 2007. The greatest shift occurred between 2000 and 2003 when the
global prescription almost doubled increasing from 5.5% to 9.9%. The most pronounced increase was seen in females who in 2007 reached a 1-year prevalence of AD use of 13.8%. The prescription of TCAs and other ADs remained stable across the years, thus the observed changes were mainly attributable to SSRIs. The SSRIs accounted for 44.8% of “AD use” in 2000 and rose to 75.7% in 2007. The most prescribed antidepressant was citalopram: its 1-year prevalence increased about sixfold and, in 2007, peaked at 3.3%. Citalopram was followed by two SSRIs: paroxetine (2.2%) and sertraline (1.9%).

The declining use of reboxetine in years 2000-2006
To compare the use of reboxetine with that of fluoxetine and paroxetine in a large population sample of subject living in Lombardy Region, and to compare reboxetine prescribing trends with those of mirtazapine. As expected, the use of paroxetine and fluoxetine peaked in 2002 and then decreased. The prescription rates of mirtazapine gradually increased all through the study period: from 0.07% in 2000 to 0.13% in 2006. On the contrary, the prescription rates of reboxetine showed a different trend and progressively decreased from 0.20 in 2000 to 0.04 in 2006. The annual rates of the prolonged use of paroxetine and fluoxetine significantly increased over time: from 58% in 2000 to 63% in 2006 (p<0.001) and were characterised by a highly significant heterogeneity (p<0.001). Also reboxetine prolonged use showed a statistically significant growth: from 33% in 2000 to 52% in 2006 (p<0.001). It increased by 4% per year with no significant heterogeneity. The overall proportion of prolonged use, however, was significantly lower for reboxetine (42%) than for paroxetine (57%; OR: 0.55, 95% IC: 0.53-0.57, p<0.001) and fluoxetine (58%; OR: 0.53, 95% IC: 0.51-0.55, p<0.001).

Across the study period the annual rates of persistence ranged 21-27% for reboxetine, 28%-43% for paroxetine and 30%-46% for fluoxetine. There was a certain fluctuation in annual rates and no significant time trends were evident. The overall proportion of persistence was significantly lower for reboxetine (23%) than for paroxetine (34%; OR: 1.67, 95% IC: 1.56-1.79, p<0.001) and fluoxetine (36%; OR:1.89, 95% IC: 1.76-2.03).

Changes in co-prescribing warfarin and potentially interacting drugs and risk of major bleeding in community-dwelling elderly people.
To analyze the rate and trend of co-prescribing warfarin and potentially interacting drugs (PIDs) and the risk of hospitalization for major bleeding in community-dwelling elderly people, a cohort of community-dwelling elderly people (aged 65 years or more) who received at least one prescription for warfarin during the period 2001-2007 was drawn from Lombardy Region administrative database (northern Italy) was analysed. Age, local health unit (LHU) of residence, number of drugs and co-prescribed PIDs were predictors of hospitalization for hemorrhage, but the risk decreased during the study period (OR 0.94; 95% CI, 0.89-0.99). Compared with prescribing warfarin alone, coprescribing antibacterial drugs, calcium antagonists, allopurinol, omeprazole and ranitidine increased the risk of hospitalization for major bleeds. Over time, the rate of users warfarin of alone increased, and the percentage of those co-prescribed of PIDs fell slightly ($\chi^2$ trend: 3.74; p<0.001). No differences were found in the interaction between the co-prescription of warfarin with PID and years of prescription.

Antipsychotics prescription and cerebrovascular events in Italian older persons
The primary aim of this study was to evaluate the association between any antipsychotic prescription and cerebrovascular events (CVEs) in Italian elderly; second, to compare the effect of typical and atypical antipsychotics on CVEs; and, third, to investigate the effect of antipsychotics on CVEs in the subgroup of persons co-prescribed with acetylcholinesterase inhibitors (AChEIs). Administrative claims from community-dwelling people aged 65 to 94 years living in Northern Italy were analysed using a retrospective case-control design, from
2003 to 2005. 3855 cases of CVEs were identified and matched with 15420 controls. When antipsychotics were categorized according to number of boxes prescribed during the observational period, being prescribed with at least 19 boxes of typical antipsychotics was significantly associated with CVEs (OR=2.4; 95%CI=1.08-5.5). An interaction was found between any antipsychotic and AChEI co-prescription on CVEs (OR=0.46; 95%CI=0.23-0.92).

In conclusions, only typical antipsychotics were associated with an increased odd of CVEs but the association was duration-dependent. Persons prescribed simultaneously with AChEI and antipsychotics may be at a lower risk of CVEs.

New prescriptions of spironolactone associated with angiotensin-converting-enzyme inhibitors and/or angiotensin receptor blockers and their laboratory monitoring from 2001 to 2008.

The aim of the study was to analyse, in older community-dwelling people living in Italy’s Lombardy region, 8-year trends in new users of spironolactone co-prescribed with angiotensin-converting-enzyme inhibitors (ACE-Is) and/or angiotensin receptor blockers (ARBs); blood test monitoring; and independent predictors of appropriate blood test monitoring. Only new users of spironolactone co-prescribed with ARBs increased from 2001 to 2008 (P<0.001). In the 6 months before starting the co-prescriptions 96 to 100% of patients measured serum creatinine (mean 99.3%), sodium (97.3%) and potassium (98.6%). Within 3 months of starting the co-prescriptions 96 to 99% of patients measured serum sodium (mean 97.3%) and potassium (98.6%), but on average only 48% of them (range 43 to 53%) measured serum creatinine. Our results support the need for greater awareness within the medical community of the potential renal toxicity of the association of spironolactone with ACE-Is and/or ARBs. Adequate short-term monitoring of serum creatinine in all older community-dwelling people who receive such co-prescription is necessary in order to ensure safe usage of these medications.

Adverse drug reactions caused by drug-drug interactions in elderly outpatients

Although the prevalence of drug-drug interactions (DDIs) in elderly outpatients is high, many potential DDIs do not have any actual clinical effect, and data on the occurrence of DDI-related adverse drug reactions (ADRs) in elderly outpatients are scarce. This prospective cohort study was aimed to determine the incidence and characteristics of DDI-related ADRs among elderly outpatients (aged ≥60 years) as well as the factors associated with these reactions. The incidence of DDI-related ADRs was 6%. Warfarin was the most commonly involved drug (37% cases), followed by acetylsalicylic acid (17%), digoxin (17%), and spironolactone (17%). Gastrointestinal bleeding occurred in 37% of the DDI-related ADR cases, followed by hyperkalemia (17%) and myopathy (13%). The multiple logistic regression showed that age ≥80 years, a Charlson comorbidity index ≥4, consumption of five or more drugs, and the use of warfarin were associated with the occurrence of DDI-related ADRs. With regard to severity, approximately 37% of the DDI-related ADRs detected in our cohort necessitated hospital admission. All DDI-related ADRs could have been avoided (87% were ameliorable and 13% were preventable). The incidence of ADRs not related to DDIs was 10% (n = 44). The incidence of DDI-related ADRs in elderly outpatients is high; most events presented important clinical consequences and were preventable or ameliorable.

DRUG UTILIZATION STUDIES

Drug utilisation in elderly patients

In a collaborative study with the Health Directorate of the Lombardy Region, a drug utilization study aimed to investigate the overall drug prescription rate, the prescribing pattern of chronic therapies and polypharmacy in relation to gender and different age groups of community-
dwelling elderly people has been set up. All prescription for elderly aged 65 years or older (n=1767 239), reimbursed by the National Health Service (NHS) and dispensed by retail pharmacies of the 15 local health units (LHU) in the Lombardy Region between 1 January and 31 December 2005 were analyzed. During the year of the study, 1555142 elderly (88% of the elderly population) received at least one drug prescription (89% women and 87% men). The overall prescription prevalence rate was slightly higher in women than in men (OR 1.20; 95% CI: 1.19-1.21), and increased up to 75 years of age in both sexes, reaching a plateau which persisted until 85 years. Each treated elderly received an average of 5 drugs (active substances) (median 4, interquartile range 2-7), without any difference between genders; 76% of the elderly (76% women and 75% men) received at least one chronic drug, 46% were exposed to polypharmacy (46% women and 45% men) and 20% to chronic polypharmacy (18% women and 22% men). Age and LHU of residence were predictors for chronic polypharmacy exposure and at multivariate analysis, elderly in age groups of 75-79, 80-84 and 85-89 years had the highest risk to be exposed to chronic polypharmacy (OR 2.25; 95%CI: 2.23-2.27, OR 2.68; 95%CI: 2.65-2.71, and OR 2.84; 95%CI: 2.79-2.89 respectively).

**Antipsychotic use in a sample of Italian Alzheimer Special Care Units**

An observational prospective study was set up to evaluate the frequency of antipsychotic use and their association with BPSD in institutionalised patients with dementia in northern Italian Alzheimer’s special care units (ASCU). Sixty percent of 319 patients were taking at least one antipsychotic, 49% typical and 51% atypical. Forty five percent were exposed to one antipsychotic, 14% two and 1% three. Risperidone was the most frequently prescribed antipsychotic followed by promazine, olanzapine and haloperidol. In 40% of the cases, another hypnotic or sedative drug was simultaneously administered. Antipsychotics were significantly associated with female sex, older age and higher NPI score, but did not significantly influence mortality, hospitalisation, falls or use of physical restraint at follow-up.

**Antipsychotic use and mortality in the “very old” with dementia: the Monzino 80-plus study**

To investigate the association between the use of antipsychotic drugs and the risk of death in the very old general population affected by dementia we analysed the data of The Monzino 80-plus study. This is an ongoing, prospective population-based study of all eighty years or older residents in eight municipalities of Varese province, Italy. The diagnosis of dementia was based on DSM-IV criteria. Information on drug use at baseline was obtained from participants and/or caregivers. At baseline, 33.6% of the elderly participants (n1/44618) were found to be affected by dementia. The use of antipsychotics was much more common among demented than non demented elderly (19.1% vs 2.3%, p<0.0001). Frequency of antipsychotic drug use was similar among demented persons living at home and those institutionalized (18.8% vs 20.0%, p1/40.73). After a follow-up period of 1, 2, 3, or 4 years, no significant differences (p>0.33) in death rate were found between demented elderly taking antipsychotics (29.7%, 48.3%, 61.9%, 64.4%) and those not taking antipsychotics (34.4%, 49.0%, 60.6%, 67.2%). The difference remained not significantly different also when potential confounders (age, sex education, smoking history, BMI, stroke, diabetes, hypertension, myocardial infarction, heart failure, COPD) were entered into a logistic regression model (p 1/4 0.46).

**Pattern of Cholinesterase Inhibitors Use in Alzheimer’s disease: Results of the EPIFARM-Elderly Project**

This study was aimed to examine the trend of cholinesterase inhibitors (ChEIs) use during 2002-2007 and to estimate the rate of Alzheimer’s disease (AD) patients treated with ChEIs. Individuals aged 65 years or older who received at least one prescription of ChEIs between January 1, 2002 and December 31, 2007 were included in the study. ChEIs utilization was
estimated using prescription data of drugs reimbursed by the Italian National Health Service between January 1, 2004 and December 31, 2007 in three provinces of the Lombardy region (Milan, Lecco and Brescia), Italy. The rate of elderly who received at least one prescription of ChEIs increased from 0.5% in 2002 to 0.7% in 2004 and then remained unchanged until to 2007. The percentage of mild to moderate AD cases taking ChEIs was rather low (19-20%), and fairly stable overtime in the less treated oldest age groups (80+), while decreased in the youngest (65-79 years). In incident AD cases, the percentage of newly treated patients decreased overtime in the overall group (from 11.7% in 2004 to 8.0% in 2007) as well as in each age class. In the cohort of incident AD cases who started the treatment during 2004, nearly 40% were also in treatment three years later.

Co-prescription of antipsychotics in patients treated with cholinesterase inhibitors: the EPIFARM-Elderly Project

The objective of the study was to assess co-prescribing of antipsychotics in elderly taking cholinesterase inhibitors (ChEIs) from 2002 to 2008 and the changes subsequent to two main official warnings issued by the Italian Medicines Agency to restrict their use. Elderly patients aged 65-94 years who received at least one prescription of ChEIs between 1 January 2002 and 31 December 2008 were selected. Co-prescribing of atypical antipsychotics in patients exposed to ChEIs declined from 21.0% in 2002 to 14.6% in 2008 (OR 0.92; 95% CI: 0.90, 0.94; p<0.001), while the prescribing prevalence of typicals slightly increased (OR 1.08; 95% CI: 1.03, 1.13; p=0.001). In relation to the two warnings, the prevalence of patients who received a co-prescription of antipsychotics was significantly lower in 2005 than 2004 (23.1% vs. 28.0%; OR 0.79; 95% CI: 0.73-0.86; p<0.001) and in 2007 than 2006 (19.4% vs. 23.0%; OR 0.79; 95% CI: 0.73-0.86; p<0.001). After the first safety warning the prevalence of prescriptions for risperidone and olanzapine dropped significantly, and there was a significant increase for quetiapine. Haloperidol prescriptions increased, especially after the second warning. Despite regulatory warnings issued to discourage the use of antipsychotics, they are still frequently prescribed to patients taking ChEIs. Awaiting further studies to clarify their therapeutic role, physicians should prescribe antipsychotics very cautiously and only after careful risk-benefit assessment.

Within region differences in outpatient antibiotic prescription

To assessed antibiotic patterns of use and geographical distribution of prevalence and consumption by age in 15 Local Health Units (LHUs) of Italy’s Lombardy region, we retrospectively analysed administrative claims for the community-dwelling population in 2005. A total of 3,120,851 people (34% of the population) received at least one antibiotic drug prescription. The highest prescription prevalence was observed in the 0-17 and 80 or more year age ranges (41.6% and 41.9%, respectively). Large differences were found in prevalence rates between different LHUs (ranging from 28.7% in Milan to 39.4% in Brescia) and in DIDs (ranging from 12.2 DID in Sondrio to 19.8 DID in Brescia). The age and residence of the population were the main determinants of drug exposure. In particular, patients aged <18 years (OR=1.73; 95% CI 1.73, 1.74), aged 65 or older (OR=1.64; 95% CI 1.63, 1.65), and those that live in Brescia (OR 1.66, 95% CI 1.65, 1.66) had a statistically significant higher risk of antibiotic drug exposure. A careful monitoring should be carried out to reduce antibiotic resistance and improve the rational use of drugs.

Geographical differences in the prevalence of chronic polypharmacy in elderly people

The aim of the study was to compare the geographical differences in the prevalence of chronic polypharmacy in community-dwelling elderly people over eleven years. This study analyzed nearly two million patients aged 65-94 years recorded in the Drug Administrative Database of
the Lombardy Region (Northern Italy) from 2000 to 2010. Chronic polypharmacy was defined as taking five or more drugs in one month for at least six months (consecutive or not) in a year. Our results showed clusters of high and low prevalence rates of chronic polypharmacy and they were not influenced by age. Chronic polypharmacy was just weakly correlated with hospital admission (2000: $\rho=0.08, \ p=0.0032$; 2005: $\rho=0.11, \ p<0.0001$; 2010: $\rho=0.18, \ p<0.0001$), but not with mortality. In conclusions, there were geographical differences in the prevalence of elderly people with chronic polypharmacy, only partly explained by health indicators. These findings highlight the need for targeted efforts on prescription practice to reduce polypharmacy.

Validation of healthcare administrative data for the diagnosis of epilepsy. Administrative databases have become an important tool to monitor diseases. Patients with epilepsy could be traced using disease-specific codes and prescriptions, but formal validation is required to obtain an accurate case definition. The aim of the study was to correlate administrative data on epilepsy with an independent source of patients with epilepsy in a district of Lombardy, Northern Italy, from 2000 to 2008. Data of nearly 320 600 inhabitants in the district of Lecco collected from the Drug Administrative Database of the Lombardy Region were analysed. Among them were included patients who fulfilled the International Classification of Diseases 9 (ICD-9) codes and/or the disease-specific exemption code for epilepsy and those who had at least one EEG record and took antiepileptic drugs (AEDs) as monotherapy or in variable combinations. To ascertain epilepsy cases, 11 general practitioners (GPs) with 15 728 affiliates were contacted. Multiple versions of the diagnostic algorithm were developed using different logistic regression models and all combinations of the four independent variables. Among the GP affiliates, 71 (4.5/1000) had a gold standard diagnosis of epilepsy. The best and most conservative algorithm included EEG and selected treatment schedules and identified 61/71 patients with epilepsy (sensitivity 85.9%, CI 76.0% to 92.2%) and 15 623/15 657 patients without epilepsy (specificity 99.8%, CI 99.7% to 99.8%). The positive and negative predictive values were 64.2% and 99.9%. Sensitivity (86.7%) and the positive predictive value (68.4%) increased only slightly when patients with single seizures were included. A diagnostic algorithm including EEG and selected treatment schedules is only moderately sensitive for the detection of epilepsy and seizures. These findings apply only to the Northern Italian scenario.

STUDY FOR THE IMPROVEMENT OF THE APPROPRIATENESS OF DRUG PRESCRIPTION

Rationalization of drug prescribing in patients resident in the Bergamo Local Health Authority
In a study aimed to improve the quality of drug prescribing of general practitioners (GPs) in selected therapeutic areas (non-steroidal anti-inflammatory drugs, proton pump inhibitors, antibiotics, and antihypertensive agents, conducted among 160 GPs of the Bergamo Local Health Authority, we found a reduction of inappropriate prescribing of nearly 3% in all the indicators of drug utilization and cost analyzed.

Medication non-adherence among elderly patients newly discharged and receiving polypharmacy
Poor adherence may have a major impact on clinical outcome, contributing to substantial worsening of disease, increased health care costs and even death. With increasing numbers of medications low adherence is a growing concern, seriously undermining the benefits of current medical care. Little is known about medication adherence among elderly recently discharged with high comorbidity, living at home and requiring complex medication regimens. To describe the adherence to drug prescriptions in a cohort of elderly patients receiving polypharmacy and discharged from an Italian internal medicine ward, we conducted an observational study asking
patients or caregivers for information about adherence at two follow-up times. A sample of elderly patients (65 years or older) discharged from an internal medicine ward in Italy throughout 2012 were enrolled. They were followed for three months after discharge with a structured telephone interview to collect information on drug regimens and medication adherence 15 days (first follow-up) and three months (second follow-up) after discharge. Demographic variables including age, sex, marital status and caregiver were collected. Among 100 patients recruited information on medication adherence was available for respectively 89 and 79 patients at first and second follow-up. Non-adherence was reported for 49 patients (55.1%) at the first follow-up and for 55 (69.6%) three months from discharge. Voluntary withdrawal of a drug and change of dosage without medical consultation were the main reasons for non-adherence at both follow-ups. The number of drugs prescribed at discharge was related to medication non-adherence at both follow-up interviews. No association was found between age and non-adherence. Only 25 patients (28.1%) at the first follow-up and 20 (25.3%) at the second understood the reasons for their medications. Low medication adherence is a real, complex problem for older patients receiving polypharmacy. We found that the increasing number of drugs prescribed at hospital discharge is correlated to non-adherence and a high percentage of patients did not understand the purpose of their medications. Simplification of drug regimens and reduction of pill burdens as well as better explanations of the reason for the medications should be targets for intervention.

Effect of an integrated e-learning intervention, focused on “Comprehensive Geriatric Assessment” to improve the quality of drug prescribing in hospitalized elderly patients. The ELICADHE-AIFA Project

Health care systems are increasingly challenged by the complex management of geriatric patients with multiple chronic conditions that receive multiple drugs. The traditional clinical assessment does not provide thorough information on all the needs of these patients. Comprehensive Geriatric Assessment (CGA) provides such information, offers a more specific and sensible care plan for each patient, and may improve the quality of prescribing. With the aim to evaluate whether an integrated e-learning program of medical education, focused on teaching and implementing CGA added to geriatric pharmacological notions (GPNs) (intervention) is superior to delivering only GPNs (control) in reducing the prescription of potentially inappropriate drugs (PID) or potential drug-drug interactions (PDDI) in hospitalized elderly, a cluster randomized single-blind controlled study was set up in a sample of elderly patients (aged 75 years or more) consecutively admitted to 20 geriatric and internal medicine hospital wards, and randomized to study intervention or control group. Secondary aims are to assess the clinical impact of the integrated e-learning intervention on the length of hospitalization, in-hospital and overall mortality, re-hospitalization, institutionalization and persistence of the effect of improving quality of drug prescribing during a follow-up of 12 months.

The results of this project should help to provide National Health Service with indications on the clinical impact of a e-learning intervention in improving pharmacological use in hospitalized elderly patients.

The results of the pilot study indicate that 26% of patients in the intervention group and 18% in the control group were treated on admission with at least one inappropriate medication according to the Beers criteria. These percentages drop respectively to 21% and 16% at discharge. 56% of patients in the intervention group and 77% in the control group were taking medications at admission with the risk of potential interactions (12% and 15% of patients respectively were at the risk of drug interactions whose clinical significance was considered as a major). Regarding the use of inappropriate drugs or duplicate emerges a reduction in both groups, while in relation to drug interactions, there is a drop for those classified with greater clinical relevance.
STUDY ON MULTIMORBIDITY AND POLYPHARMACY IN HOSPITALIZED ELDERLY PATIENTS

The REPOSI Study

The Registro POliteria SImi (REPOSI) study is a collaborative effort between the Italian Society of Internal Medicine (SIMI-Società Italiana Medicina Interna) and the Mario Negri Institute for Pharmacological Research. It was designed with the purpose to set up a network of internal medicine and geriatric wards in order to investigate patients aged 65 years or older affected by multiple diseases and prescribed with polypharmacy. Participation to the network was on a voluntary basis. During a period of four weeks, three months apart each from the other, the 38 wards involved in the study, recruited 1332 elderly patients (aged 65 years or older). The main results from the analyses of this cohort of hospitalized elderly patients are the following:

1. at hospital admission 52% of patients taken five or more different drugs (polypharmacy) and were in the ward for a mean of 11 days.
2. The comparison discharge-admission showed an increasing rate of patient with polypharmarcy (+13%) and with multiple disease (+16%).
3. No difference emerged in terms of in-hospital mortality between patients with polypharmacy and the other ones.
4. At multivariate analysis the in-hospital mortality and hospital stay were positively associated with age, adverse clinical events, and comorbidity (Charlson Index).

Furthermore, with aim of recognizing clusters of diseases among the hospitalized elderly, and of identifying groups of patients at risk of in-hospital death and adverse clinical events according to disease clustering, a regression analysis was done. Patients affected by the clusters including heart failure (HF) and either chronic renal failure (CRF), or chronic obstructive pulmonary disease had a significant association with in-hospital death (OR=4.2;95%CI=1.6-11.4; OR=2.9;95%CI=1.1-8.1, respectively), as well as patients affected by CRF and anaemia (OR=6.0;95%CI=2.3-16.2). The cluster including HF and CRF was also associated with adverse clinical events (OR=3.5;95%CI=1.5-7.7). The effect of both HF and CRF and CRF and anaemia on in-hospital death was additive.

Other analyses:

- Another analysis was done with the aims to evaluate the rate of prescriptions of drugs for peptic ulcer or gastro-oesophageal reflux disease (GERD) in elderly patients at admission and discharge in a sample of internal medicine wards, and to analyze their appropriateness of use in relation to the evidence-based indications. The appropriateness of drug prescriptions for peptic ulcer and GERD were retrospectively evaluated taking into account the presence of conditions requiring their use or the use of gastro-toxic drugs combination. Among 1155 patients eligible for the analyses, elderly treated with drugs for the treatment of GERD or peptic ulcer were 466 (40.3%) at hospital admission and 647 (56.0%) at discharge. 65.2% of patients receiving a drug for peptic ulcer or GERD at admission and 64.1% at discharge were inappropriately treated. Among patients inappropriately treated the number of other drugs prescribed was associated with an increased use of drugs for peptic ulcer or GERD, also after adjustment for age, sex and number of diagnoses at admission (OR 95%CI=1.25 (1.18-1.34), $p=.0001$) or discharge (OR 95%CI= 1.11 (1.05-1.18), $p=0.0003$).

- With the aim to evaluate the association between the presence of bacterial or community-acquired pneumonia (CAP) and the use of drugs inhibiting gastric acid secretion such as proton pump inhibitors (PPIs) and antagonists H2-receptor (anti-H2) was conducted logistic regression analysis. A statistically significant association between the presence of bacterial infection and use of PPI was found. This association was greater in elderly receiving the drug for more than 14 days and even after adjusting results for age, sex and comorbidity.

- To evaluate the adherence to current guidelines on cardio-embolic prophylaxis in elderly
(≥65 years old) patients admitted with an established diagnosis of AFF to the Italian internal medicine wards participating in REPOSI registry project, we retrospectively analyzed registry data collected from January to December 2008. At admission, CHADS2 score was ≥ 2 in 68.4% of patients, at discharge in 75.9%. Among patients with AFF 26.5% at admission and 32.8% at discharge were not on antithrombotic therapy, and 43.7% at admission and 40.9% at discharge were not taking an appropriate therapy according to the CHADS2 score. Among elderly patients admitted with a diagnosis of AFF to internal medicine wards, an appropriate antithrombotic prophylaxis was taken by less than 50%, with an underuse of VKAs prescription independently of the level of cardio-embolic risk. Hospitalization did not improve the adherence to guidelines.

- To explore the association of dementia with in-hospital death in acutely ill medical patients, we analyzed data on 1332 in-patients aged 65 years or older enrolled in the REPOSI study. After multiadjustment, the diagnosis of dementia was associated with in-hospital death (OR = 2.1; 95% CI = 1.0 - 4.5). Having dementia and at least one adverse clinical event during hospitalization showed an additive effect on in-hospital mortality (OR = 20.7; 95% CI = 6.9 – 61.9). Acutely ill elderly patients affected by dementia are more likely to die shortly after hospital admission. Having dementia and adverse clinical events during hospital stay increases the risk of death.

- We assessed which clusters of diseases are associated with polypharmacy in acute-care elderly inpatients. Among clusters of diseases, the highest mean number of drugs (N=8) was found in patients affected by heart failure (HF) plus chronic obstructive pulmonary disease (COPD), HF plus chronic renal failure (CRF), COPD plus coronary heart disease (CHD), diabetes mellitus plus CRF, and diabetes mellitus plus CHD plus cerebrovascular disease (CVD). The strongest association between clusters of diseases and polypharmacy was found for diabetes mellitus plus CHD plus CVD, diabetes plus CHD, and HF plus atrial fibrillation (AF).

- We evaluate the prevalence of antidepressant prescription and related factors in elderly inpatients, as well as the consistency between prescription of antidepressants and specific diagnoses requiring these medications. The number of patients treated with antidepressant medication at hospital admission was 115 (9.9%) and at discharge 119 (10.3%). In a multivariate analysis, a higher number of drugs (OR = 1.2; 95% CI = 1.1–1.3), use of anxiolytic drugs (OR = 2.1; 95% CI = 1.2–3.6 and OR = 3.8; 95% CI = 2.1–6.8), and a diagnosis of dementia (OR = 6.1; 95% CI = 3.1–11.8 and OR = 5.8; 95% CI = 3.3–10.3, respectively, at admission and discharge) were independently associated with antidepressant prescription. A specific diagnosis requiring the use of antidepressants was present only in 66 (57.4%) patients at admission and 76 (66.1%) at discharge.

- We evaluated which factors were associated with a risk of hospital readmission within 3 months after discharge of a sample of elderly patients admitted to internal medicine and geriatric wards. We found that nineteen percent of patients were re-admitted at least once within 3 month after discharge. Multivariate logistic regression analysis showed that only AEs during hospitalization, previous hospital admission, and vascular and liver diseases were significantly associated with likelihood of readmission. In conclusions, the results demonstrate the need for increased medical attention towards elderly patients discharged from hospital with characteristics such as AEs during the hospitalization, previous admission, vascular and liver diseases.

- We evaluated the association between anticholinergic burden and both cognitive and functional status, according to the hypothesis that the cumulative anticholinergic burden, measured at Anticholinergic Cognitive Burden (ACB) scale and Anticholinergic Risk Scale (ARS), increases the risk of cognitive decline and impairs the activities of daily living in elderly patients admitted to internal medicine and geriatric wards. The mean SBT score for patients treated with anticholinergic drugs was higher than in patients receiving no
anticholinergic medications as also indicated by the ACB scale, even after adjustment for age, sex, education, stroke and TIA (9.2; 95% CI, 8.6-9.9 vs 8.5; 95% CI, 7.8-9.2; p=0.05). There was a dose-response relation between total ACB score and cognitive impairment. Patients identified by the ARS had more severe cognitive and physical impairment, and the dose-response relation was clear for ability in activities of daily living. No correlation was found with length of hospital stay.

- We evaluated the prevalence of patients exposed to potentially severe drug-drug interactions (DDIs) at hospital admission and discharge and to evaluate the related risk of in-hospital mortality and of adverse clinical events, re-hospitalization and all-cause mortality at 3 months follow-up. Multivariate analysis found a significant association with an increased risk of mortality at 3 months in patients exposed to at least 2 potentially severe DDIs (OR=2.62; 95% CI, 1.00-6.68; p=0.05). The cause of adverse clinical events was potentially related to severe DDIs in 2 patients who died during hospitalization, in 5 patients re-hospitalized and in one who died at 3 months follow-up after discharge. Hospitalization was associated to an increase in potentially severe DDIs. Careful monitoring for potentially severe DDIs, especially for those created at discharge or recently generated, is important to minimize the risk of associated harm.

- We evaluated the prevalence of PIMs comparing the 2003 and 2012 versions of Beers criteria and the related risk of adverse clinical events, re-hospitalization and all-cause mortality at three month follow-up. To also examine the association between anticholinergic burden according to the Anticholinergic Cognitive Burden (ACB) scale and the risk of adverse outcomes. Prevalence of patients receiving almost one PIM according to the Beers criteria were 20.1% and 23.5% with the 2003 and 2012 version, respectively. Prescription of PIMs according to those criteria was not associated with an increased risk of adverse clinical events, re-hospitalization and all-cause mortality at three month follow-up in both univariate and multivariate analysis, after adjusting for age, sex and CIRS comorbidity index. On the other hand, anticholinergic drugs assessed according to the ACB scale was associated with an increased risk of re-hospitalization in both univariate (OR=1.73, 95%CI, 1.16-2.56, p=0.006) and multivariate models (OR=1.67 95%CI, 1.12-2.53, p=0.01).

- Scores for cardio-embolic and bleeding risk in patients with atrial fibrillation are described in the literature. However, it is not clear how they co-classify elderly patients with multimorbidity, nor whether and how they affect the physician’s decision on thromboprophylaxis. Four scores for cardio-embolic and bleeding risks were retrospectively calculated for ≥65 year old patients with atrial fibrillation enrolled in the REPOSI registry. At admission, among 543 patients the median scores (range) were: CHADS2 2 (0-6), CHA2DS2-VASc 4 (1-9), HEMORR2HAGES 3 (0-7), HAS-BLED 2 (1-6). Most of the patients were at high cardio-embolic/high-intermediate bleeding risk (70.5% combining CHADS2 and HEMORR2HAGES, 98.3% combining CHA2DS2-VASc and HAS-BLED). 50-60% of patients were classified in a cardio-embolic risk category higher than the bleeding risk category. In univariate and multivariable analyses, a higher bleeding score was negatively associated with warfarin prescription, and positively associated with aspirin prescription. The cardio-embolic scores were associated with the therapeutic choice only after adjusting for bleeding score or age.

- We assessed the prognostic value of glomerular filtration rate (eGFR) on in-hospital mortality, hospital re-admission and death within 3 months, in a sample of elderly patients (n = 1,363) admitted to 66 internal medicine and geriatric wards of REPOSI Registry. Based on eGFR, calculated by the new Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, subjects at hospital admission were classified into three groups: group 1 with normal eGFR (≥60 ml/min/1.73 m2, reference group), group 2 with moderately reduced eGFR (30-59 ml/min/1.73 m2) and group 3 with severely reduced eGFR (<30 ml/min/1.73 m2). Patients with the lowest eGFR (group 3) on admission were more likely to be older, to have a greater cognitive and functional impairment and a high rate of
comorbidities. Multivariable logistic regression analysis showed that severely reduced eGFR at the time of admission was associated with in-hospital mortality (OR 3.00; 95% CI 1.20–7.39, p = 0.0230), but not with re-hospitalization (OR 0.97; 95% CI 0.54–1.76, p = 0.9156) or mortality at 3 months after discharge (OR 1.93; 95% CI 0.92–4.04, p = 0.1582).

On the contrary, an increased risk (OR 2.60; 95% CI 1.13–5.98, p = 0.0813) to die within 3 months after discharge was associated with decreased eGFR measured at the time of discharge. Our study demonstrates that severely reduced eGFRs in elderly patients admitted to hospital are strong predictors of the risk of dying during hospitalization, and that this measurement at the time of discharge helps to predict early death after hospitalization.

- We examined data of REPOSI, a cross-sectional prospective study held in 70 Italian internal medicine and geriatric wards, and evaluated the appropriate use of allopurinol according to the presence of gout or antineoplastic-induced hyperuricemia. In order to find predictor of inappropriate prescription of allopurinol we compared the characteristic of inappropriately treated patients with those appropriately not treated. The xanthine oxidase inhibitor allopurinol is estimated to be the treatment of choice for gout, but it’s often inappropriately used in asymptomatic hyperuricaemia, even if it has a great potential for rare but severe skin and systemic life-threatening side effects. In addition little evidences support a role of allopurinol in the prevention of cardiovascular diseases. To examine the appropriateness of allopurinol prescription in the treatment of gout in a sample of elderly patients (65 years old or older) at hospital admission and discharge and to evaluate the predictors of inappropriate prescription at hospital discharge. Among 2,712 patients eligible for the analysis, 303 (11.2%) were treated with allopurinol at hospital admission and 292 (12.6%) among 2,314 patients discharged. Only 16 (5.3%) of patients receiving allopurinol at admission and 22 (7.5%) at discharge were appropriately treated. Among these, asymptomatic hyperuricemia, polytherapy, chronic renal failure, diabetes, ischemic cardiomyopathy, BPCO and atrial fibrillation was significantly associated with greater use of allopurinol. Prevalence of inappropriate prescription of allopurinol remained almost the same at admission and discharge. Inappropriate use of this drugs is principally related to asymptomatic hyperuricemia. Careful assessment of clinical conditions and stricter adherence to evidence-based guidelines are essential for a rational use.

ASSESSMENT OF QUALITY OF SERVICES FOR ELEDELRY PEOPLE

Quality assessment of services on dementia
A sample of Lombardy Region Alzheimer Special Care Units (ASCU) was compared with traditional nursing homes to assess their effects on main clinical outcome in a sample of 450 residents followed for 18 months. Patients admitted at ASCU had a lower risk of hospitalisation, use of physical restraints, and a higher probability of withdrawing antipsychotics than patients admitted to NH. No difference was reported on overall mortality and falls.

Census and quality assessment of the Lombardy Region Alzheimer Evaluation Unit (AEU)
A collaborative study with the Italian Alzheimer Association (Federazione Alzheimer Italia) was organised with the aim to assess the quality of Lombardy Region AEU. After a census of the 81 AEU active in the Lombardy Region, a random sample of 18 AEU was selected for the quality evaluation by specific indicators that covered all the three axes of quality (structure, process and outcome). The overall quantitative score for each of the three axes was nearly 50% of the available score. The comparison of the 18 AEU sowed some differences in all the three quality axes, in particular the process axis. The results of the study highlight the need to improve the standard of these services in order to better meet the needs of families and patients with Alzheimer Disease.
Caregivers’ perceptions of the therapeutic benefits of cholinesterase inhibitors
The aim of the study was to collect opinions, perceptions, and expectations on the therapeutic benefits of ChEIs and the impact on the care of the patient in a large sample of caregivers. This used an ad-hoc online questionnaire that was accessible for nearly four months on the Federazione Alzheimer Italia website and had three sections: 1) information on the patient with dementia; 2) information on the caregiver’s perception of the therapeutic benefits of ChEIs; 3) information on caregivers. During the access time, 439 questionnaires were filled, and 369 were validated for inclusion in the analysis; of these, 329 also had information on caregivers. Caregivers’ beliefs about the effectiveness of dementia treatment, their expectations and changes in their lives were clear.

Computerized prescription support system (intercheck®)
Computerized Prescription Support Systems (CPSSs) are programs or software developed to highlight inappropriate prescribing and minimize the occurrence of adverse drug reactions (ADRs). We developed INTERcheck® in order to optimize drug prescribing in elderly people with complex co-morbidity and altered pharmacokinetics and pharmacodynamics.

- To evaluate the effectiveness of INTERcheck® to review pharmacological profile and reduce the use of potentially inappropriate medications (PIMs), potentially severe drug–drug interactions (DDIs) and the anticholinergic burden in daily practice, we conducted a prospective study on two samples of elderly patients hospitalized in an Italian geriatric ward. In the observational phase the number of patients exposed to at least one PIMs remained unchanged from admission (n=29, 39.1%) to discharge (n=28, 37.8%). In the intervention phase 25 patients (41.7%) were exposed to at least one PIMs at hospital admission and 7 (11.6%) at discharge (p<0.001). Similarly patients exposed to at least one potentially severe DDI decreased respectively from 27 (45.0%) to 20 (33.3%), p=0.703. The number of newly created potentially severe DDIs decreased from 37 (59.0%) of the observational phase to 9 (33.0%), p<0.001. Use of INTERCheck® was associated to a significant reduction in PIMs and potentially severe DDIs.

Drug information service for the elderly
A daily free of charge telephone service for drug and clinical information is available for physicians and elderly. Nearly 600 questions are answered each year.
DEPARTMENT OF CARDIOVASCULAR RESEARCH

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1994-1996 Director of European Coordinating Centre and Member of Steering Committee, Collaborative Organization for RheothRx Evaluation (CORE), McMaster University, Hamilton, Canada
from 1993 Member of Steering Committee, Studio GISSI-Prevenzione, Milano, Italy
from 2002 Member of “Fibrinolytic Therapy Trialists’s Collaboration”, Oxford, UK e del “Collaborative Group on Angiotensin Converting Enzyme Inhibitors Trials”, National Institutes of Health, Bethesda, Washington, USA
from 1989 Head of the Laboratory of Clinical Drug Evaluation, Istituto di Ricerche Farmacologiche "Mario Negri"
1985-1988 Head of the Clinical Drug Evaluation Unit of the Laboratory of Clinical Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri" from 1984 Member of the Scientific and Organising Secretariat, Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico (GISSI-1, GISSI-2, GISSI-3 studies) Milano, Italy
from 1975-1984 Researcher at the Laboratory of Clinical Pharmacology, Istituto di Ricerche Farmacologiche “Mario Negri”
from 1993 Researcher at the Laboratory of Clinical Drug Evaluation Unit of the Laboratory of Clinical Pharmacology, Istituto di Ricerche Farmacologiche “Mario Negri” and at the Regional Center for Drug Information of the Lombardy Region

Selected publications

- "Coronary Artery Disease (C4D) Genetics Consortium. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease Nat Genet 2011; 43: 339-344

ANNUAL REPORT 2013
Maria Carla Roncaglioni got her Biological Science degree in 1987 at the University of Milan.

**Education**

1987  Doctoral degree in Biological Sciences, University of Milan, Italy
1982-1983  “Research Fellow” at the Dept. of Biochemistry, Faculty of Medicine, Rijksuniversiteit of Limburg, Maastricht, The Netherland (Prof. C. Hemker);
1998-1999  “Visiting Scientist” at the Cardiovascular Research Unit, Hammersmith Hospital, London, UK (Prof. A. Maseri)

**Main fields of activity**

Coordination of multicenter clinical trials and observational studies in different cardiovascular areas (neurological, angiological, cardiological). Coordination of a network of more than 1000 GPs actively involved in epidemiological and experimental studies in the prevention of cardiovascular diseases.

**Position**

from 2001  Head of the Laboratory for General Practice Research, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy
from 1989  Senior Researcher in the Clinical Pharmacology Laboratory, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy
from 1974  Researcher in the Laboratory for the Study of Haemostasis and Thrombosis, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

**Selected publications**


Gianni Tognoni got his Medical Doctor degree in 1970, University of Milan.

**Main areas of methodology**

Randomized clinical trials; outcomes studies; pharmacoepidemiology; pharmacoconomics; epidemiological monitoring and assessment of health care systems, drug policy; genetic epidemiology; community epidemiology; transfer of technology; health and human rights.

**Main clinical areas**

Acute and chronic CV diseases; psychiatry; aging; intensive care; neurodegenerative disorders; haemotology.

**Position**

2004-2010  Member, Commission of Human Experimentation of the Italian Drug Agency (AIFA)
2001-2003  Member, Commissione Unica del Farmaco (CUF), Ministry of Health
from 2002  Director, Consorzio Mario Negri Sud, S. Maria Imbaro, Chieti.
1996-2002  Coordinator, Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche "Mario Negri", Milano
from 1990  Co-Director, Scuola Superiore di Ricerca in Medicina Generale (CSeRMEG)
from 1976  Founding member of the International Society of Drug Bulletins (ISDB)
Coordinator, Commission of Human Experimentation, Regione Lombardia
from 1983  Founder and in the Editorial Board of the nursing research Journal Rivista dell'Infermiere/Assistenza Infermieristica e Ricerca
from 1977 Consultant to WHO and other UN agencies for drug selection and policy; training in methods of clinical and epidemiological research in developing countries mainly in Latin America and Africa


1975-1984 Head, Regional Centre for Drug Information (CRIF), Regione Lombardia, Istituto di Ricerche Farmacologiche "Mario Negri", Milano

1969-1974 Research Assistant, Laboratory of Clinical Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri", Milano

Selected publications


Giovanna Balconi

Giovanna Balconi got her degree at the School for Technicians of Biomedical Institutes of the University of Milan, with a specialisation in Histology in the Pathological Anatomy Laboratory of the same University (1968).

Main fields of interest

Isolation, culture and characterization of peripheral blood circulating progenitor cells of patients with heart failure.

“In vitro” culture and characterization of stem cells for repair of myocardial infarction in experimental animal models.

Management of biobanks in clinical studies.

Positions

from July 2005 Head of Tissue Culture Unit, Cardiovascular Clinical Pharmacology Laboratory, Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, Italy

Oct 1995 - June 2005 Head of Tissue Culture Unit, Vascular Biology Laboratory, Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, Italy

Dec 1983 - Oct 1995 Head of Tissue Culture Unit, Anticancer Chemotherapy Laboratory, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

Oct 1968 - Nov 1983 Researcher, Anticancer Chemotherapy Laboratory, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

Selected publications


Paola Di Giulio got her Nursing Diploma at the Nursing School of Istituto Nazionale dei Tumori in Milano and her Master in Oncology Nursing at Guildford University (UK) in 1995.

**Main fields of activity**
Coordination of multicentre and observational studies in cardiology and palliative care. Coordination of nursing networks.

**Position**
from 2007 Lecturer and responsible (from 2008) of the Cure Area of the Health Department of the SUPSI (Scuola Universitaria delle Professioni Sanitarie della Svizzera Italiana)
from March 2001 “Assistenza Infermieristica e Ricerca”
from 1997 Responsible of the Nursing Research Unit
from 1995 Senior researcher of the Cardiovascular Research Department
from 1989 Consultant of the Clinical Pharmacology Laboratory

**Selected publications**

Fabio Fiordaliso got his Biological Science degree in 1995 at the University of Milan.

**Education**
1998 Postdoctoral degree in Pharmacological Research, Istituto di Ricerche Farmacologiche “Mario Negri”, Milan, Italy
1995 Doctoral degree in Biological Sciences, University of Milan, Italy

**Main fields of activity**
Therapeutical potential of stem cell and antioxidant treatments in experimental model of diabetic cardiomyopathy and in primary myocyte cultures exposed to hyperglycemia. Morphological and structural analysis of cells and tissue by optical, confocal and electron microscopy.

**Positions**
from 2007 Head of Bio-imaging Unit, Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche “Mario Negri”, Milan
from 2006 Member of the Heart Failure Association (HFA) of the European Society of Cardiology
from 2005 Member of the Working group on myocardial function (WG 4) of the European Society of Cardiology
from 2005  Member of the steering committee of the Consorazio of Microscopy and Image Analysis (MIA)
from 2001  Senior Research Scientist, Laboratory of Cardiovascular Clinical Pharmacology (Department of Cardiovascular Research), Istituto di Ricerche Farmacologiche “Mario Negri”, Milan
1997-2001  Post-Doctoral Research Fellow at Cardiovascular Research Institute (Department of Medicine), New York Medical College, Valhalla, New York
1994-1997  Research Fellow, Laboratory of Cardiovascular Clinical Pharmacology (Department of Cardiovascular Research), Istituto di Ricerche Farmacologiche “Mario Negri”, Milan
1992-1994  Research training, Institute of General Pathology, University of Milan (Italy)

Selected publications

Serge Masson obtained his doctorate (PhD) in Biochemistry and Cellular Biology in 1990 at the University of Marseilles (France), followed by a postdoctoral stay at the Panum Institute in Copenhagen (Denmark).

Education
- 1988-1990  Doctorate fellow, Faculty of Medicine, University of Aix-Marseilles, France
- 1990-1993  Post-doctoral Researcher, Panum Institute and Assistant Lecturer, University of Copenhagen, Denmark
- 1993  Research Scientist, NMR Laboratory, Hospital “San Raffaele”, Milan, Italy
from 1994  Research Scientist, Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, Italy

Main fields of activity:
Physiopathology, diagnostic and prognostic role of the activation of neuroendocrine systems in cardiovascular disease

Position
from 2002  Head of the Cardiovascular Endocrine Unit, responsible for Quality Assurance for the Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy
from 2011  Thesis Examiner for PhD of the Open University of London, UK
from 2002  Tutor of fellows of the School of Specialists in Pharmacological Research, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy
from 2002  Fellows of the American Heart Association (Basic Council) and the Working Group on Myocardial Function of the European Society of Cardiology

Selected publications


Enrico Bjørn Nicolis has attended the courses in Computer Science at the University of Milan.

Enrico Bjørn Nicolis has attended the courses in Computer Science at the University of Milan.

**Education**

1991-1999 “Research fellow”, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

**Main fields of activity**

Data management and analysis of randomized clinical trials. Developing of database and tools for studies of population genetics, particularly for linkage analysis.

**Position**

from 2001 Head of the Bioinformatics Unit, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

from 1999 Research fellow of the Laboratory of Clinical Drugs Evaluation

from 1997 System administrator at the EDP center, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

from 1991 Research fellow at the Medical Informatics and Applied Statistics Unit, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

**Selected publications**


**ACTIVITIES**

The areas of interest of the Department of Cardiovascular Research include the experimental, clinical, genetic, epidemiological aspects of acute myocardial infarction, cardiac failure, cardiac arrhythmias, cardiac arrest, as well as the clinical and epidemiological investigation of cardiovascular prevention, hypertension and stroke. Following the successful experience of the GISSI-trials (Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto), the activation of large collaborative networks in the setting of the National Health Service hospitals and in general practice has become a key characteristics of the Department, which can now rely on the
permanent collaboration of over 300 clinical groups and of several hundred general practitioners. Over the years, firm links have also been established with international leading research groups.

The activity in experimental research includes the pathophysiology, the pharmacological modulation and the prognostic role of the activation of the renin-angiotensin-aldosterone system, as well as other biohumoral systems, in myocardial infarction and heart failure. A model of cardiac arrest and cardiopulmonary resuscitation in rats and pigs has been recently set up and is being used for assessing the role of inflammation in cardiac and brain injury after cardiac arrest, and the protective effects of different interventions.

The activity in clinical research includes the clinical assessment of therapeutic strategies and of biomarkers of cardiovascular risk with large scale clinical trials in the field of acute coronary syndromes, congestive heart failure and atrial fibrillation. Several studies have been conducted in the area of clinical epidemiology and risk factors assessment of myocardial infarction. A recently developing area is the genetic epidemiology of myocardial infarction and heart failure. The collaboration with an European genetic network has allowed the participation to large GWAS (genome wide association studies) on coronary disease, myocardial infarction and stroke.

The collaboration with a large network of General Practitioners in the area of cardiovascular prevention allowed to test new hypotheses through large scale clinical trials and to evaluate the actual transferability of evidence based interventions in the every day practice through epidemiological or outcome research studies. Among the different activities, the Cardiovascular Research Department contributed to the accreditation of the Institute as a Contract Research Organization (CRO) for the conduction of clinical trials, mainly academic. The Department is able to arrange monitoring activities (counting on certified monitoring personnel) and it is also attested by EudraVigilance for the submission of online Safety Reports.

Pharmacoepidemiological studies through the analysis of a large sample of Local Health Units drug prescriptions were also performed. A research network of nurses has been developed with the main focus on the assessment of health-related quality of life of patients and on the epidemiology of nursing interventions and their implications for patients' well being and outcomes.

Participation to public ongoing projects funded on a competitive basis:
1. European projects (FP7):
   - FOCUS
   - HOMAGE
   - CREACTIVE
   - SHOCKCOMICS
2. Projects by the Italian Ministry of Health, Ricerca Finalizzata:
   - ICOS-ONE (call 2009)
   - Immune procoagulant and inflammatory responses in severe sepsis and septic shock (call 2011-2012)
   - Preclinical optimization of treatment with inhaled argon to improve neurological outcome and survival after cardiac arrest (Young Investigators call 2011-2012)

**MAIN FINDINGS**

A subgroup analysis of patients enrolled in the GISSI-AF trial has shown that the risk of incident atrial fibrillation is predicted by circulating cardiac markers (natriuretic peptides and troponin T) and by left atrial function as assessed by echocardiography in patients in sinus rhythm. Predictors of atrial fibrillation could help in treating or even preventing this arrhythmia.
which has a prevalence of 5-6% in the elderly and is associated with a 10-fold increase in risk of stroke.

A recent analysis on 7000 patients with chronic heart failure enrolled in the GISSI-HF trial has shown that an unintentional decrease in body weight of at least 2 kg over the first year after enrolment is a relevant risk factor. The body weight loss (cachexia) is independent from other risk factors. Studies are ongoing to better understand the mechanisms of this weight loss and how possibly it could be attenuated.

Experiments are ongoing on the cardio- and neuro-protective effects of the noble gas argon, administered after cardiac arrest. Preliminary results of experiments in the pig suggest that ventilation with argon 70% in oxygen started with the resuscitation manoeuvres improves the recovery of neurologic functions and reduces histological injury in the brain and in the heart. The PROCARDIS is part of the Coronary Artery Disease Genetics Consortium (C4D), that has reported a meta-analysis of genome-wide association studies for coronary artery disease (CAD) in discovery and replication cohorts including both European and South Asian studies. Five loci newly associated with CAD have been identified. This study showed that the effect sizes of previously unidentified CAD-associated genes discovered by GWAS (genome wide association studies) have become progressively smaller, suggesting that there may not be large-effect common variants remaining to be discovered, but rather that a large number of common variants of small effect may contribute to CAD risk.

Greater understanding of the genetic variants underlying CAD, and particularly the pathways involved, may lead to development of new therapeutic approaches to help address the world’s leading cause of death.

The Department has contributed to largest GWAS study of ischemic stroke conducted to date, as part of the Wellcome Trust Case Control Consortium 2 (WTCCC2). A new association with the HDAC9 gene region has been identified in large vessel stroke with an estimated effect size that is at the larger end for GWAS loci (OR = 1.38, 95% CI = 1.22–1.57, from replication data). The GWAS also replicated known associations with three other loci and showed genetic heterogeneity across subtypes of the disease for all four stroke loci. This genetic heterogeneity seems likely to reflect heterogeneity in the underlying pathogenic mechanisms and reinforces the need for the consideration of stroke subtypes separately in research and clinical contexts.

Results of the Risk & Prevention trial have been published (N Engl J Med 2013; 368: 1800-1808). The study aimed to verify whether a daily supplementation of 1 gr of polyunsaturated fatty acids (Omega-3) could reduce, as already documented in patients with a history of myocardial infarction, principal complications’ occurrence in a population at high cardiovascular risk. The Risk & prevention Study, (more than 12,000 patients involved with mean age 64 years, followed for more than 5 years) had highlighted that a pharmacological treatment with Omega-3 does not reduce cardiovascular mortality or hospitalizations for cardiovascular causes when this treatment is added to a good medical assistance as the one already delivered by the net of 860 Italian GPs participating to the study in their clinical practice.

NATIONAL COLLABORATIONS

AMD (Associazione Medici Diabetologi) - Lombardia
ANMCO (Associazione Nazionale Medici Cardiologi Ospedalieri)
AREU - Azienda Regionale Emergenza Urgenza - Lombardia
Azienda Ospedaliera CTO/Maria Adelaide, Torino
Centro Cardiologico Monzino IRCCS, Milano
Centro Emofilia e Trombosi Angelo Bianchi Bonomi, Fondazione Ca’ Granda - Ospedale Maggiore Policlinico, Milano
CINECA (Consorzio Interuniversitario per il Calcolo Automatico dell'Italia Nord-Orientale)
CSERMEG (Centro Studi e Ricerche in Medicina Generale)
Dipartimento Cardio-Vascolare ed Endocrino-Metabolico, Ospedale Casa Sollievo della
Sofferenza IRCCS, San Giovanni Rotondo (FG)
Dipartimento Cardiologico “A. De Gasperis” - Struttura Complessa di Cardiologia 2 -
Insufficienza Cardiaca e Trapianto, Azienda Ospedaliera Ospedale Niguarda Ca’ Granda,
Milano
Dipartimento di Cardiologia e UTIC, Istituto Clinico Humanitas IRCCS, Rozzano (MI)
Dipartimento di Immunologia, Istituto Clinico Humanitas IRCCS, Rozzano (MI)
Ematologia, Ospedale Sant’Anna, Torino
Fondazione Don Gnocchi IRCCS, Milano
Fondazione Istituto Neurologico “Carlo Besta”, Milano
Fondazione per il Tuo Cuore - Heart Care Foundation - ONLUS, Firenze
Fondazione Sestini, Bergamo
Gruppi organizzati di MMG (FIMMG, CoS, Ass.Cu.M.I., AMISI)
IEO - Istituto Europeo di Oncologia, Milano
IFOM-FIRC, Milano
IRC - Italian Resuscitation Council, Bologna
ISMETT Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, Palermo
Istituto di Anestesioologia e Rianimazione, IRCCS Ospedale Maggiore Policlinico, Mangiagalli,
Regina Elena, Milano
Istituto di Anestesia e Rianimazione, Ospedale San Gerardo, Monza (MI)
Istituto di Ricerca in Cure palliative Lino Maestroni, Cremona
Istituto Ortopedico Galeazzi, Milano
Istituto Ortopedico Rizzoli, Bologna
IRCCS Fondazione Cà Granda Ospedale Maggiore Policlinico, Milano
Laboratorio di Endocrinologia, Ospedale Luigi Sacco, Milano
Politecnico, Milano
Regione Emilia Romagna
Regione Lombardia
Regione Lazio, Dipartimento di Epidemiologia
Servizio Farmaceutico, USSL 20, Verona
SIBioC (Società Italiana di Biochimica Clinica e Biologia Molecolare)
Unità Operativa di Anatomia e Istologia Patologica, Ospedale Luigi Sacco, Milano
Unità Operativa Semplice di Neuroanestesia e Neuroriaminazione, Dipartimento di Medicina
Perioperatoria e Terapie Intensive, Ospedale San Gerardo, Monza (MI)
Unità Operativa Piede Diabetico, IRCCS Multimedica, Sesto San Giovanni (MI)
Università degli Studi di Milano, Dipartimento di Medicina Interna
Università degli Studi di Milano, Dipartimento di Scienze Farmacologiche
Università degli Studi di Milano, Polo Veterinario di Lodi (MI)
Università degli Studi di Milano Bicocca, Dipartimento di Biotecnologie e Bioscienze
Università degli Studi di Milano Bicocca, Dipartimento di Scienze della Salute, Centro di
Biostatistica per l’Epidemiologia Clinica
Università degli Studi di Catania, Dipartimento di Anestesia e Terapia Intensiva
Università degli Studi di Catania, Dipartimento di Scienze del Farmaco, Sezione di Biochimica
Università degli Studi di Palermo, Scuola di Specializzazione in Anestesia e Rinamizione
Università degli Studi di Torino, Dipartimento di Anatomia, Farmacologia e Medicina Forense
Università degli Studi di Torino, Dipartimento di Scienze della Sanità Pubblica e Pediatriche
Università degli Studi di Verona, Dipartimento di Sanità Pubblica
Università degli Studi di Verona, Istituto di Anatomia Umana
INTERNATIONAL COLLABORATIONS

Cecomet (Centro de Epidemiologia comunitaria y Medicina tropical, Esmeraldas) Ecuador
Cochrane Collaboration, Oxford, UK
Clinical Trial Research Unit, Auckland University, Nuova Zelanda
CNIC Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spagna
CTSU (Clinical Trial Service Unit)/ISIS (International Studies on Infarct Survival), Oxford, UK
Department of Cardiology, Italian Hospital of Buenos Aires, Argentina
Department of Epidemiology, Harvard School of Public Health, Boston, USA
DSAN SUPSI (Scuola Universitaria Professioni Sanitarie), Lugano, Svizzera
ECLA (Estudios Cardiologicos de Latino-America)
ECRIN (European Clinical Research Infrastructures Network)
Helsingborg Hospital, Sweden
Institut Lorrain du Coeur et des Vaisseaux Louis Mathieu, Vandoeuvre-les-Nancy, Francia
Institute of Clinical Medicine, Akershus University Hospital, Lorenskog, Norvegia
Karolinska Institutet, Stockholm, Svezia
Laerdal Foundation for Acute Medicine, Stavanger, Norway
Mayo Clinic, Cardiorenal Research Lab, Rochester, MN, USA
PHRI (Population Health Research Institute), McMaster University, Hamilton, Ontario, Canada
The Third Military University, Chong Qing, China
University of Cambridge, UK
University of Aachen, Germany
University of Helsinki, Central Hospital, Finland
University of Manchester, Medicine/Cardiology Manchester Royal Infirmary, UK
University of Minnesota, Minneapolis, USA
University Medical Center, Groningen, Olanda
Wellcome Trust Centre for Human Genetics, University of Oxford, UK
WONCA (World Organization of Family Doctors)

EDITORIAL BOARD MEMBERSHIP

Current Controlled Trials, Global Heart (Maria Grazia Franzosi)
Journal of Cardiac Failure, Journal of Cardiovascular Medicine (Roberto Latini)
Disease Markers (Serge Masson)
Open Access Critical Care, Resuscitation, The Scientific World Journal (Giuseppe Ristagno)
European Heart Journal, International Journal of Health Services, Journal of Cardiovascular Medicine (Gianni Tognoni)

PEER REVIEW ACTIVITIES

American Heart Journal, American Journal of Cardiology, American Journal of Medicine, Annali di Igiene, Archives of Medical Research, Atherosclerosis Thrombosis and Vascular Biology, Biomarkers in Medicine, BMC Cardiovascular Disorders, Canadian Medical Association Journal, Cardiology, Cardiovascular Drugs and Therapy, Cardiovascular Research, Circulation, Clinical Biochemistry, Clinical Pharmacology and Therapeutics, Critical Care

NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP

Comitato Etico della Provincia di Trento
Comitato Scientifico IRC - Italian Resuscitation Council, Bologna
Gruppo di Studio SIAARTI - Società Italiana Anestesia Analgesia Rianimazione Terapia Intensiva
Working Group Basic Life Support, European Resuscitation Council

EVENT ORGANIZATION

Investigator's Meeting - Incontro finale Ricercatori ALBIOS - ALBumin Italian Outcome Sepsis study
28/03/13, Aula Guasti, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

Investigator's Meeting - Riunione sullo stato di avanzamento dello studio REGIA - Rischio Emorragico Ginocchio e Anca
Studio osservazionale prospettico di coorte sull’incidenza degli eventi emorragici nei pazienti sottoposti ad interventi di sostituzione protesica di ginocchio ed anca
08/05/13, Aula E, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

Investigator's Meeting - Riunione sullo stato di avanzamento dello studio ICOS-ONE (International CardioOncology Society-ONE Trial)
14/05/13, Aula Poster, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

Investigator's Meeting - Riunione sullo stato di avanzamento dello studio CYCLE (Ciclosporina A nell’infarto miocardico acuto riperfuso)
30/05/13, Sala Giacomo Binda - Fortezza da Basso, Firenze

Investigator's Meeting - Riunione di avvio dello Studio FALCO (Sorveglianza dei pazienti con Fibrillazione Atriale in Lombardia trattati con Anticoagulanti Orali)
03/12/13, Aula E, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

Master di I° Livello in Ricerca Clinica dell’Università degli Studi di Milano, Facoltà di Medicina e Chirurgia (Anno Accademico 2013-2014)
05/11/13 Introduzione al corso
La ricerca clinica oggi: profit e no-profit
Corso di introduzione alla statistica medica
06/11/13 Elementi di statistica descrittiva
Il disegno dello studio in epidemiologia
07/11/13 Inferenza statistica 1: Stima e intervalli di confidenza
Esercitazioni di Inferenza Statistica - 1
11/11/13 Il disegno degli studi clinici
Metodi statistici per l’analisi dell’outcome. Le principali misure di rischio
13/11/13 Legislazione sulla sperimentazione clinica e ruolo dei Comitati Etrici - 1
Esercitazioni di Inferenza Statistica - 2
14/11/13 Legislazione sulla sperimentazione clinica e ruolo dei Comitati Etrici - 2
18/11/13 La dimensione del campione negli studi clinici
Dagli studi primari alla sintesi delle evidenze: revisioni sistematiche e metanalisi
19/11/13 Revisioni sistematiche e metanalisi
Sistemi free per la gestione delle referenze
Gestione della ricerca clinica in un IRCCS
20/11/13 Reazioni avverse e farmacovigilanza
Uso clinico dei biomarker in oncologia
21/11/13 Gestione della complessità clinico-terapeutica del paziente anziano ospedalizzato
Le interazioni tra farmaci
Monitoraggio delle sperimentazioni cliniche in medicina generale
Monitoraggio negli studi no-profit
25/11/13 Trial di non-inferiorità
Ricerca clinica nel campo dell’epilessia. Ricerca clinica nell’ictus
26/11/13 La ricerca bibliografica oggi
Internet e le nuove tecnologie per l'aggiornamento medico-scientifico
Ricerca Traslazionale
Outcome Research
27/11/13 Dalla preclinica alla clinica: sviluppo di nuovi farmaci cardiovascolari
Il monitoraggio dei Clinical Trial in Ricerca Cardiovascolare
28/11/13 Gestione della ricerca clinica in Azienda
La farmacovigilanza degli studi no profit: nuove direttive e prospettive future
02/12/13 Problemi aperti nella scoperta e nello sviluppo di farmaci
Ricerca in medicina generale
Monitoraggio degli studi clinici profit & report delle reazioni avverse
03/12/13 Il “discorso etico”: dalla linearità dei buoni principi alla provocazione del reale
05/12/13 Test diagnostici e Teorema di Bayes
Inferenza statistica 2: Test statistici
06/12/13 Analisi della sopravvivenza
Esercitazione
IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

CONFERENCE AND WORKSHOP CONTRIBUTIONS

Unità di Terapia Intensiva Cardiologica - Clinica Cardiologica, Dipartimento di Scienze Cardiologiche, Toraciche e Vascolari dell’Università di Padova. I° Congresso Nazionale di ipotermia terapeutica in cardiologia. Una nuova era nelle cure post-arresto cardiaco. 01/02/13, Crowne Plaza Hotel, Padova, Italy
- Fisiopatologia della sindrome post arresto cardiaco

ESC European Society of Cardiology - ESC Working Group on Cardiovascular Pharmacology and Drug Therapy. Biomarkers for innovative medicine in heart failure - Biomarker and clinical
decision making in CV disease: focus on heart failure. Eighth Annual Meeting: Transatlantic Heart Failure Biomarker Working Group. 20-21/04/13, Cannes, France
- Potential novel heart failure biomarkers
- Potential novel biomarkers of left ventricular hypertrophy in elderly people. The PREDICTOR Study

SMART - Organizing and Scientific Committee. 24° SMART - Simposio Mostra Anestesia, Rianimazione e Terapia Intensiva. Emergenza I. 08-10/05/13, MiCo-Milano Congressi Ala Nord, Milano, Italy
- New circulating biomarkers predictive of outcome after resuscitation from cardiac arrest

Istituto Clinico Humanitas IRCCS. Seminar: Inflammation in cardiovascular diseases: the case of heart failure and atrial fibrillation. 16/05/13, Rozzano MI, Italy
- Inflammation in cardiovascular diseases: the case of heart failure and atrial fibrillation

Università degli Studi di Palermo - Scuola di Specializzazione in Malattie dell’Apparato Cardiovascolare. Aggiornamenti della Scuola in tema di: Sindrome post-arresto cardiaco, Aritmologia interventistica, Supporti meccanici all’assistenza ventricolare, Risonanza magnetica e diagnosi cardioligica, Ischemia miocardica (Sessione: La sindrome post-arresto cardiaco). 17-18/05/13, Steri, Sala delle Capriate, Palermo, Italy
- Fisiopatologia della sindrome post arresto cardiaco

EFLM European Federation of Clinical Chemistry and Laboratory Medicine. EUROMEDLAB 2013, 20th IFCC-EFLM European Congress and 45th SIBioC Congress. 19-23/05/13, Milano, Italy
- Presepsin (sCD14-ST) is an early predictors of outcome in patients with severe sepsis or septic shock. Preliminary data from the ALBumin Italian Outcome Sepsis (ALBIOS) Study

AMD Associazione Medici Diabetologi. XIX Congresso Nazionale AMD. 29/05-01/06/13, Marriott Park Hotel, Roma, Italy
- ANNO 2010: viaggio nella prescrizione farmacologica del diabetico anziano in Lombardia

ANMCO Associazione Nazionale Medici Cardiologi Ospedalieri - Fondazione per il tuo Cuore ONLUS - Heart Care Foundation. 50 anni uniti nel cuore, 44° Congresso Nazionale di Cardiologia ANMCO. 30/05-01/06/13, Fortezza da Basso, Firenze, Italy
- Il modello di score del rischio GISSI-HF nei pazienti con scompenso cardiaco

European Atherosclerosis Society. 81st EAS Congress. Centro Congressi Cité Internationale, 02-05/06/13, Lyon, France
- Association of ADIPOQ gene variants and heart failure in an italian population

IRC Italian Resuscitation Council. Rispondere, Agire, Ricostruire. Scegliere la solidarietà. Congresso Nazionale IRC, 07-09/06/13, Pieve di Cento (BO), Italy
- Compessori meccanici per RCP
- Debriefing dopo RCP: cosa, come, quali dati
- Campagna VIVA! Iniziative realizzate e in via di sviluppo

ANMCO Associazione Nazionale Medici Cardiologi Ospedalieri - Fondazione per il tuo Cuore ONLUS - Heart Care Foundation. Corso avanzato di formazione su metodologia, strategie e tecniche della ricerca clinica. Edizione 2012-2013 - Introduzione alla ricerca Clinica, 11/06/13, Learning Centre ANMCO, Firenze, Italy
- Trial clinici. Laboratori centralizzati (core labs) per imaging e analisi di laboratorio. Sottoprogetti

ANMCO Associazione Nazionale Medici Cardiologi Ospedalieri - Fondazione per il tuo Cuore ONLUS - Heart Care Foundation. Corso avanzato di formazione su metodologia, strategie e tecniche della ricerca clinica. Edizione 2012-2013 Modulo 5 - Storia naturale di un farmaco. Dal laboratorio di ricerca alla pratica clinica, 26-28/06/13, Learning Centre ANMCO, Firenze, Italy
- Biomarkers in cardiology

Roche Diagnostics International Ltd. ProCardio Symposium, 9th International Symposium. The value of biomarkers in cardiovascular disease. 29-30/08/2013, Amsterdam, The Netherlands
- Monitoring risk in chronic heart failure: the GISSI and Val-HeFT study

European Society of Cardiology. ESC Congress 2013. 31/08-04/09/13, Amsterdam, The Netherlands
- The fibroblast growth factor-23/vitamin D axis, left ventricular mass and mortality in elderly people
- Reduction in body weight is an independent risk factor for mortality in chronic heart failure. Insights from GISSI-HF and Val-HeFT trial

German Sepsis Society. 6th International Congress. Sepsis and multiorgan dysfunction of cardiology. 04-06/09/13, Weimar, Germany
- Comparison of presepsin (sCD 14-ST) and procalcitonin for early prediction of outcome in severe septic shock. Preliminary findings from the Albumin Italian Outcome Sepsis (ALBIOS) study

Heart Failure Society. 17th Annual Scientific Meeting. 22-25/09/13, The Peabody Orlando Hotel, Orlando, Florida, USA
- The ANP genetic variant rs5068 is associated with higher circulating concentrations of natriuretic peptides in patients with chronic heart failure

ESICM European Society of Intensive Care Medicine. Lives 2013, 26th Annual Congress (President’s Session - Improving patient outcomes across the globe: insights from the latest randomized studies). 05-09/10/13, Paris, France
- Amplitude spectrum area to predict defibrillation outcome and survival during pre-hospital cardiopulmonary resuscitation in 1617 cardiac arrest patients in Lombardia, Italy

Maastricht University - Faculty of Health Medicine and Life Science - Department of Cardiology. Grand Rounds Lecture at the azM. 11/10/13, Maastricht University Medical Center, The Netherlands
- Inflammation, consequence or cause? The case of heart failure and atrial fibrillation

European Resuscitation Council - Polish Resuscitation Council Resuscitation. Scientific Symposium of the ERC on Outcomes (Session: Monitoring the efficiency of resuscitation). 25-26/10/13, Krakow, Poland
- Ventricular fibrillation analyses to guide CPR intervention and predict success of defibrillation"

GiViTI (Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva). 22° GiViTI Meeting - CREATIVE Kickoff Meeting. 13-15/11/13, Centro Congressi, Pesaro, Italy
- Imaging e biomarker nello studio CREATIVE
SITI - Società Italiana di Terapia Intensiva. 26° Congresso Nazionale SITI (Sessione: Nuove Tecnologie). 14-16/11/13, Firenze, Italy
- Novità nel campo della defibrillazione

American Heart Association - American Stroke Association. Annual Meeting ReSS. Resuscitation Science Symposium (Session: Best of the best oral abstract presentations). 16-17/11/13, Dallas, Texas, USA
- Activation of the kynurenine pathway in patients resuscitated from ventricular fibrillation out-of-hospital cardiac arrest

American Heart Association. AHA Annual Meeting. Scientific Session 2013 (Session: Cardiac arrest and resuscitation: mechanisms and laboratory studies and Session: Novel strategies to improve outcome from cardiac arrest). 16-20/11/13, Dallas, Texas, USA.
- Post-resuscitation treatment with argon improves neurological outcome in a porcine model of myocardial infarction and cardiac arrest
- Ventricular fibrillation waveform analysis to better guide resuscitation interventions

Boheringer Ingelheim. GLORIA-AF Global Registry on Long-Term Oral Antithombotic Treatment in Patients with Atrial Fibrillation. Investigators’ Meeting. 09/12/13, Boheringer Ingelheim, Milano, Italy.
- Presentazione del protocollo dello studio GLORIA-AF

Azienda Ospedaliera di Padova - GEPA (Gestione Elettromedicali Prodotti per Analisi). PreSEPSIn pathfast. Utilizzo di presepsin (sCD14-ST) quale marcatore di sepsi. 18/12/13, Aula Didattica, Istituto di Anestesia e Rianimazione, Padova, Italy
- Risultati trial ALBIOS: 100 Terapie Intensive in Italia
- La presepsina in pazienti con sepsi severa e shock settico: risultati dello studio ALBIOS. Una banca biologica rappresentativa

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**GRANTS AND CONTRACTS**

AIFA (Agenzia Italiana del Farmaco), Azienda Ospedaliera Ospedale Niguarda Ca’ Granda Milano, Azienda Ospedaliera San Gerardo Monza, Bluegreen Biotech Srl, Boehringer Ingelheim Italia Spa, Chiesi Farmaceutici, Centro Nacional de Investigaciones Cardiovasculares (CNIC) Madrid, Comunità Europea, Consorzio Mario Negri Sud Santa Maria Imbaro, Elior Ristorazione SpA, Fondazione Banca Popolare di Bergamo Onlus, Fondazione San Raffaele del Monte Tabor Milano, Fondazione Humanitas per la Ricerca Rozzano, Fondazione per il Tuo Cuore - Heart Care Foundation Onlus Firenze, Fondazione Livia Tonolli Pallanza, Fondazione Sestini Bergamo, Fondazione Veronesi, Health Diagnostic Laboratory, Inc., USA, Helsinki University - Central Hospital, Helsingborg Hospital, Institute of Clinical Medicine - Akershus University Hospital - Lørenskog, Istituto Europeo di Oncologia IRCCS Milano, Laerdal Foundation for Acute Medicine Stavanger, Ministero della Salute, Mitsubishi Chemical Europe, Novartis Pharma SpA, Perfetti Van Melle SpA, Prima Vera SpA, Population Health Research Institute-Mc Master University, Regione Lombardia, ROCHE Diagnostics, Sigma Tau SpA, SPA Società Prodotti Antibiotici SpA, Università degli Studi di Milano, Università degli Studi Milano Bicocca, University of Manchester, UK
SCIENTIFIC PUBLICATIONS (2013)

n-3 polyunsaturated fatty acids and atrial fibrillation in patients with chronic heart failure: the GISSI-HF trial
Eur J Heart Fail 2013; 15: 1289-1295

Unilateral acid aspiration augments the effects of ventilator lung injury in the contralateral lung
Anesthesiology 2013; 119: 642-651

The cardiokine secreted Frizzled-related protein 3, a modulator of Wnt signalling, in clinical and experimental heart failure
J Intern Med 2013; E-pub

Aspromonte N, Di Fusco SA, Latini R, Cruz DN, Masson S, Tubaro M, Palazzuoli A
Natriuretic peptides in acute chest pain and acute coronary syndrome: from pathophysiology to clinical and prognostic applications
Coron Artery Dis 2013; 24: 33-39

Predictors of mortality in 6975 patients with chronic heart failure in the Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico-Heart Failure Trial. Proposal for a Nomogram
Circ Heart Fail 2013; 6: 31-39

Mutant copper-zinc superoxide dismutase (SOD1) induces protein secretion pathway alterations and exosome release in astrocytes: implications for disease spreading and motor neuron pathology in amyotrophic lateral sclerosis
J Biol Chem  2013; 288: 15699-15711

A combination of untargeted and targeted metabolomics approaches unveils changes in the kynurenine pathway following cardiopulmonary resuscitation
Metabolomics 2013; 9: 839-852

Cerchiari EL, Greco N, Pellis T, Ristagno G, Scapigliati A, Semeraro F
Social networks as a tool to promote the week of cardiac arrest awareness “Viva!” in Italy
Resuscitation 2013; 84: e85-e86

Chen B, Yin C, Ristagno G, Quan W, Tan Q, Freeman G, Li Y
Retrospective evaluation of current-based impedance compensation defibrillation in out-of-hospital cardiac arrest
Resuscitation 2013; 84: 580-585

Left ventricular dysfunction and outcome at two-year follow-up in patients with type 2 diabetes: The DYDA study

The long term multi-center observational study of dabigatran treatment in patients with atrial fibrillation (RELYABLE) study
Circulation 2013; 128: 237-243
Tubular damage and worsening renal function in chronic heart failure
J Am Coll Cardiol HF 2013; 1: 417-424

Thromboembolic event rate in paroxysmal and persistent atrial fibrillation: Data from the GISSI-AF trial
BMC Cardiovasc Disord 2013; 13: 28

Incremental value of left ventricular systolic and diastolic function to determine outcome in patients with acute ST-Segment elevation myocardial infarction: The Echocardiographic Substudy of the OASIS-6 Trial
Ecocardiography 2013, E-pub

Individual patient data systematic review and meta-analysis of optic nerve sheath diameter ultrasonography for detecting raised intracranial pressure: protocol of the ONSD research group
Syst Rev 2013; 2: 62

The effects of apixaban on hospitalizations in patients with different types of atrial fibrillation: insights from the AVERROES trial
Eur Heart J 2013; 34: 2752–2759

Kette F, Locatelli A, Bozzola M, Zoli A, Li Y, Salmoiraghi M, Ristagno G, Andreassi A
Electrical features of eighteen automated external defibrillators: A systematic evaluation
Resuscitation 2013; 84: 1596-1603

Latini R, Masson S
Significance of measurable cardiac troponin by high-sensitivity assays in patients with chronic stable heart failure
Coron Artery Dis 2013; 24: 716-719

Incidence of atrial fibrillation in a population with impaired glucose tolerance: The contribution of glucose metabolism and other risk factors. A post hoc analysis of the Nateglinide and Valsartan in Impaired Glucose Tolerance Outcomes Research trial
Am Heart J 2013; 166: 935-940.e1

n-3PUFA and holter derived autonomic variables in patients with heart failure: Data from the Studio della Sopravvivenza nell'Insufficienza Cardiaca (GISSI-HF) Holter Substudy
Heart Rhythm 2013; 10: 226-232

Interaction between baseline and early worsening of renal function and efficacy of renin-angiotensin-aldosterone system blockade in patients with heart failure: insights from the Val-HeFT study
Eur J Heart Fail 2013; 15: 1236-1244

Uric acid: A cardiovascular risk factor in patients with recent myocardial infarction
Int J Cardiol 2013; 167: 262-269

Wine consumption and risk of cardiovascular events after myocardial infarction: Results from the GISSI-Prevenzione trial
Int J Cardiol 2013; 163: 282-287

Causes of death and influencing factors in patients with atrial fibrillation. A competing-risk analysis from the Randomized Evaluation of Long-Term Anticoagulant Therapy Study
Circulation 2013; 128: 2193-2201

Cardiovascular biomarkers, cardiac dysfunction, and outcomes in patients with type 2 diabetes: A prospective, multicenter study
Diabetes Care 2013; 36: e137-e138

High-sensitivity cardiac troponin T for detection of subtle abnormalities of cardiac phenotype in a general population of elderly individuals

Plasma n-3 polyunsaturated fatty acids in chronic heart failure in the GISSI-Heart Failure Trial: Relation with fish intake, circulating biomarkers, and mortality
Am Heart J 2013; 165: 208-215.e4

Elevated risk of death and major cardiovascular events in subjects with newly diagnosed diabetes: Findings from an administrative database.
Nutr Metab Cardiovasc Dis 2013 E-pub

Fish oil and post-operative atrial fibrillation. A meta-analysis of randomized controlled trials
J Am Coll Cardiol 2013; 61: 2194-2196

Evaluation of different strategies for identifying asymptomatic left ventricular dysfunction and pre-clinical (stage B) heart failure in the elderly. Results from "PREDICTOR", a population-based-study in central Italy
Eur J Heart Fail 2013; 15: 1102-1112

Nobili A, Pasina L, Latini R
Beta-adrenoceptor antagonists and antianginal drugs Chapter 18
In: Aronson JK, Side Effects of Drugs. Annual 35
Elsevier, Amsterdam 2013, In press

Revising the ECRIN standard requirements for information technology and data management in clinical trials
Trials 2013; 14: 97

Telomere/telomerase system impairment in circulating angiogenic cells of geriatric patients with heart failure
Int J Cardiol 2013; 164: 99-105

Palazzuoli A, Masson S, Ronco C, Maisel M
Clinical relevance of biomarkers in heart failure and cardiorenal syndrome: the role of natriuretic peptides and troponin
Heart Fail Rev 2013; Epub

Selective nanovector mediated treatment of activated proinflammatory microglia/macrophage in spinal cord injury
ACS Nano 2013; 7: 9881-9895

Lung stress and strain during mechanical ventilation: any difference between statics and dynamics?
Crit Care Med 2013; 41: 1046-1055

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An opto-structural method to estimate the stress-strain field induced by cell contraction on substrates of controlled stiffness in vitro
J Appl Biomater Funct Mater 2013; 11: 143-150

n-3 fatty acids in patients with multiple cardiovascular risk factors

Early kynurenine pathway activation following cardiac arrest in rats, pigs, and humans
Resuscitation 2013; 84: 1604-1610

Ristagno G, Gullo A, Lumb P
The First Weil Conference: A conference on cardiac arrest, shock, and trauma to address the state of the art and the goals of resuscitation science
J Crit Care 2013; 28: 113-115

Ristagno G, Li Y, Fumagalli F, Finzi A, Quan W
Amplitude spectrum area to guide resuscitation - A retrospective analysis during out-of-hospital cardiopulmonary resuscitation in 609 patients with ventricular fibrillation cardiac arrest
Resuscitation 2013; 84: 1697-1703

Ristagno G, Yu T, Quan W, Freeman G, Li Y
Current is better than energy as predictor of success for biphasic defibrillatory shocks in a porcine model of ventricular fibrillation
Resuscitation 2013; 84: 678-683

Roncaglioni MC, Tombesi M, Silletta MG
n-3 fatty acids in patients with cardiac risk factors

Control Consortium 2 (WTCCC2); C4D Consortium; CARDIoGRAM Consortium, Wallaschofski H, Smith NL, Tregouet D, Ridker PM, Tang W, Strachan DP, Hamsten A, O'Donnell CJ.
Multiethnic meta-analysis of Genome-Wide Association studies in > 100000 subjects identifies 23 fibrinogen-associated loci but no strong evidence of a causal association between circulating fibrinogen and cardiovascular disease
Circulation 2013; 128: 1310-1324

Santonocito C, Ristagno G, Gullo A, Weil MH
Do-not-resuscitate order: a view throughout the world
J Crit Care 2013; 28: 14-21

Scapigliati A, Ristagno G, Cavaliere F
The best timing for defibrillation in shockable cardiac arrest
Minerva Anestesiol 2013; 79: 92-101

Ibuprofen plus isosorbide dinitrate treatment in the mdx mice ameliorates dystrophic heart structure
Pharmacol Res 2013; 73: 35-43

Staszewsky L, Latini R
What is the atrium trying to tell us?
Eur Heart J 2013; 34: 255-257

Talan MI, Latini R
Myocardial infarction: cardioprotection by erythropoietin. Chapter 17

Common noncoding UMOD gene variants induce salt-sensitive hypertension and kidney damage by increasing uromodulin expression

CCL21 is associated with fatal outcomes in chronic heart failure: data from CORONA and GISSI-HF trials
Eur J Heart Fail 2013, 15: 747-755

Plasma phospholipid omega-3 fatty acids and incidence of postoperative atrial fibrillation in the OPERA Trial
J Am Heart Assoc 2013; 2: e000397

RESEARCH ACTIVITIES

Laboratory of Cardiovascular Clinical Pharmacology

Pilot study on microangiopathy in diabetic foot ulcer
Microangiopathy is considered one of the major complications in the diabetic foot, although the role of microvascular alterations in the etiopathogenesis and severity of the ulcer in diabetic foot are still unknown. The purpose of this study will be the assessment of microangiopathy determined by the increase of capillary basement membrane thickness and decrease of capillary lumen area by transmission electron microscopy in the foot ulcer of neuropathic and neuroischemic type 2 diabetic patients compared to healthy subjects. Furthermore, we will investigate the correlation between the presence of capillary and thrombosis with ischemic
parameters (TcPO2, ankle-brachial index) and between the presence of inflammatory infiltrate with blood inflammatory parameters. Nowadays all the neuroischemic and half of the neuropathic patients have been enrolled in the study.

Preclinical and clinical studies in cardiac arrest and cardiopulmonary resuscitation

Cardiovascular disease remains the leading cause of death in the Western world with 350,000 Americans and 700,000 Europeans sustaining cardiac arrest each year. Instead of the initial success of cardiopulmonary resuscitation, the majority victims die within 72 hours because of severe heart contractile failure due to post-resuscitation myocardial dysfunction. Furthermore, cardiac arrest and cardiopulmonary resuscitation represent a condition of systemic ischemia-reperfusion injury causing multi-organ damage.

For this purpose we are currently studying a preclinical model of cardiac arrest and cardiopulmonary resuscitation (CPR) in intact rats or in rats with metabolic syndrome (i.e. obesity, diabetes) and in pigs (in collaboration with University of Milan) aiming to: (a) evaluate inflammatory response and organ dysfunction after return of spontaneous circulation; (b) evaluate success of cardiopulmonary resuscitation manoeuvres and survival after new interventional approaches (i.e., hypercapnia, ranolazine, and ventilation with Argon). Particular interest on the metabolism of tryptophan, i.e. kynurenine pathway, as a route involved in post resuscitation dysfunctions and outcome is under investigation both experimentally and clinically.

Moreover, the severity of post-resuscitation myocardial dysfunction has been recognized to be related, partially, to the magnitude of the total electrical energy delivered with defibrillation. Consequently, the development of a non-invasive and real-time monitoring that allows prediction of outcome of the defibrillation attempt is therefore of great importance in decreasing the total defibrillation energy.

At present, we are evaluating a clinically applicable method based on electrocardiographic analysis of ventricular fibrillation waveform aiming to assess a non-invasive approach in order to guide the priority of interventions, namely chest compression or defibrillation (collaborating institutions: Emergency Department, San Gerardo Hospital, Monza and Azienda Regionale Emergenza Urgenza - Lombardia).

Albumin Italian Outcome Sepsis Study. The ALBIOS Study (AIFA)

ALBIOS is a multicenter, controlled, randomized clinical trial that compares the efficacy of human albumin and a crystalloid solution for volume replacement in patients with severe sepsis or septic shock. The primary endpoint is survival at 28 and 90 days after enrolment. Secondary endpoints include the number of organ dysfunctions, severity of organ dysfunction (SOFA scale), and lengths of stay in intensive care unit (ICU) and in hospital. More than 150 ICU in Italy have enrolled patients in this large study, coordinated by the Ospedale Maggiore Policlinico in Milan and the Consorzio Mario Negri Sud. A group of 50 ICUs participates to a biomarkers substudy, coordinated by the laboratory of Clinical Cardiovascular Pharmacology, and have collected serial blood samples from 1000 patients to measure biomarkers related to inflammation, infection, cardiac function and coagulation. Preliminary results on a new marker of bacterial infection and sepsis (one paper accepted for publication, a second in preparation) and on innate immunity (pentraxin-3 and circulating immunoglobulins) are now available and have been presented in international congresses Biomarkers of coagulation and fibrinolysis (in collaboration with the University of Bari) and cardiac stress (natriuretic peptide) or injury (troponin) are currently evaluated.

Prevalence of asymptomatic cardiac dysfunction and heart failure in a population of elderly subjects from Lazio. The PREDICTOR Study
This observational study evaluated the prevalence of asymptomatic cardiac dysfunction and heart failure in a random sample of elderly subjects from the Lazio area. The secondary objective was to identify clinical, biohumoral (natriuretic peptides) and non-invasive instrumental (echocardiography and ECG) markers of asymptomatic cardiac dysfunction and heart failure. The population under observation was a randomly selected sample of elderly subjects (age ranging from 65 to 84 years) resident in the area of 10 hospital cardiology centers. Blood samples have been collected from 2000 individuals and are stored in the biobank of the Laboratory of Clinical Cardiovascular Pharmacology. In a first paper (J Intern Med 2013; 273: 306-317), the association between left ventricular mass and two cardiac markers (troponin and natriuretic peptide) has been described. We have measured markers of inflammation (C-reactive protein), renal function (Cystatin C), phosphate metabolism (FGF-23 and vitamin D, submitted manuscript) and collagen metabolism (PINP). Novel markers of atrial fibrillation are under evaluation.

**OPERA: Omega-3 Fatty Acids for Prevention of Post-Operative Atrial Fibrillation**
Peri-operative administration of n-3 polyunsaturated fatty acids (PUFA) may significantly reduce the incidence of post-operative atrial fibrillation (AF) in patients undergoing cardiac surgery (CAS). The trial is concluded and showed that peri-operative administration of n-3 PUFA (8 g total pre-op and then 2 g/d for 14 days or until hospital discharge) did not reduce the incidence of AF in 1,516 patients undergoing CAS. A core laboratory is at Mario Negri, that coordinates the assay of cardiac (troponin and natriuretic peptide) and inflammatory markers (C-reactive protein). The main results of the clinical trial have been recently published (Mozaffarian et al., JAMA 2012). The predictive value for post-operative atrial fibrillation of cardiac biomarkers (natriuretic peptide and troponin, manuscript in preparation) and inflammatory markers (C-reactive protein and white blood cells count, ongoing statistical analyses) is under evaluation. In a relevant subgroup of patients, atrial tissue has been collected to evaluate, in collaboration with pathologists from the University of Parma, histological features (collagen deposition, myocytolysis, myocyte dimension) that may be associated with post-operative atrial fibrillation.

**Coronary Atherosclerosis in Outlier Subjects: Protective and Individual Risk Factor Evaluation. The GISSI-Outliers CAPIRE study**
The risk of developing clinical signs of ischemic cardiopathy is currently estimated with multivariable prediction models based on non-modifiable factors like age, sex and family history for early ischemic cardiopathy, and on conventional modifiable risk factors like hypertension, hypercholesterolemia, smoking and diabetes mellitus. However, there is a component of individual variability underlying the fact that a relevant number of individuals with multiple risk factors do not progress to coronary atherosclerosis or have clinical events, while others have such events or coronary disease in the absence of risk factors (= outliers). The purpose of the CAPIRE study is to identify possible novel protective or risk factors for coronary disease in outlier subjects and generate new etiological hypotheses and therapeutic targets for this disease. This is an observational, multicenter clinical study performed in 8 centers. Enrolment of the patients will last 2 years and each patient will be followed for 5 years with yearly clinical visit and phone contact every 6 months. The Laboratory of Clinical Cardiovascular Pharmacology is acting as a core laboratory for the evaluation of circulating biomarkers related to lipid profile, inflammation, metabolism and coagulation. A total of 544 patients have been enrolled, with 5-year follow-up ongoing. A paper on study objectives and design has been drafted and is under evaluation. Predefined circulation biomarkers have been assayed at study entry in all patients and statistical analyses under way.
Cyclosporin A in reperfused acute myocardial infarction – The CYCLE study

The final extent of myocardial infarction is the main determinant of prognosis in these patients. A preliminary study has shown that a single bolus of cyclosporin A (CsA), administered immediately before primary angioplasty, can reduce the final area of necrosis after a ST-segment elevation myocardial infarction (STEMI). The primary objective of this trial is to assess whether CsA can improve the outcome of a successfully reperfused STEMI, by favoring myocardial reperfusion. Male and female patients, older than 18 years, with a large STEMI will be enrolled within the first 6 hours from symptoms onset and with indication for primary angioplasty (PCI). The secondary objectives are a reduction of high sensitivity cardiac troponin T release 4 days after PCI, total heart failure mortality, cardiogenic shock or hospital admission for cardiovascular reasons within 6 months after randomization. By the end of December 2013, 314 patients have been enrolled in 26 centers; enrollment will be concluded by the end of April 2014. The study is conducted in collaboration with the Centro Studi ANMCO (Associazione Nazionale Medici Cardiologi Ospedalieri).

Prevention of anthracycline-induced cardiac toxicity: a multicenter randomized clinical study comparing two strategies - The ICOS-ONE study

Chemotherapy with anthracycline often induces a progressive and dose-dependent cardiac injury, reducing left ventricular output. The development of cardiac dysfunction, even if asymptomatic, may have a negative impact on the prognosis of a cancer patient. Measuring circulating cardiac troponin levels during chemotherapy with anthracycline allow to identify early cardiac injury, before the development of overt left ventricular dysfunction. Treatment with ACE inhibitors (ACEi) and beta-blockers (BB) before the elevation of circulating cardiac troponin levels during or after chemotherapy with anthracycline can protect the heart, as shown in a single-center study. Early prophylaxis with enalapril (ACEi) and possibly bisoprolol (BB) may further decrease the incidence of cardiovascular injury and thereby raising the probability of completing the chemotherapy. The primary objective of the ICOS-ONE study is to assess whether a treatment with enalapril given since the beginning of anthracyclin therapy is more efficient in preventing cardiac toxicity compared to the same treatment initiated at the first occurrence of raised troponin levels. Patients with an indication for treatment with anthracyclin for blood and solid cancer are being enrolled in this randomized multicenter clinical trial. In one arm, enalapril will be given at the beginning of chemotherapy (primary prevention) while it will be given only after the troponin elevation in the second arm (secondary prevention). Concomitant therapy with bisoprolol is recommended in both study groups in presence of different clinical or laboratory patterns. The patients are followed for 1 year from the end of chemotherapy with periodical clinical visits. By the end of December 2013, 109 patients have been enrolled in 15 centers; enrollment should be concluded by the end of January 2015. This trial is promoted by the IEO (Istituto Europeo di Oncologia) and coordinated in collaboration with the Laboratory of Clinical Drug Evaluation.

Biological markers in patients with traumatic brain injury. A European collaborative project in Intensive Care Units. CREACTIVE - Collaborative REsearch on ACute Traumatic brain Injury in intensiVe care medicine in Europe

Traumatic brain injury (TBI) is one of the main causes of death and disability in Western countries and the main cause of death for individuals below age 45 years. Most of the patients with mild-severe grades of TBI are admitted in Intensive care Units. PROSAFE is a recent collaborative network PROSAFE of ICUs in six European countries, under the coordination of the Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva (GiViTi). The
clinical study CREACTIVE, funded by a grant from the European Union (FP7–HEALTH–2013-INNOVATION-1), is a part of this collaborative network, with the following objectives: to better describe the epidemiology of mild-to-severe traumatic brain injury in 7 countries (Cyprus, Greece, Israel, Italy, Poland, Slovenia, Hungary); to collect and store in central repositories biological samples and clinical images, to evaluate their prognostic value; to build a prognostic model based on clinical and biological variables to predict short-term and long-term outcomes of TBI patients; to identify more effective therapeutic interventions in TBI; to identify centers of excellence in the treatment of mild-severe TBI. In a subgroup of ICUs, biological samples (blood and cerebrospinal fluid) from approximately 2000 patients will be collected and stored in a central repository. The laboratory of Cardiovascular Clinical Pharmacology will act as a core laboratory for the collection of biological samples and the assay of phenotypic markers associated with brain damage, inflammation, hypothalamic pituitary axis, and coagulation disorders.

Heart “Omics” in AGEing - HOMAGE
The concept of HOMAGE, a collaborative project financed by a European Union grant under the FP7 Health 2012.2.1.1-2 program, is that, in older people, “omics” based biomarkers can detect asymptomatic pathological processes that predict who will develop of heart failure and other common serious cardiovascular conditions and characterize distinct phenotype(s) more likely to respond to targeted preventive therapy that could efficiently promote active healthy ageing. The objectives of the HOMAGE project are (i) to identify “omics” based biomarkers that reflect specific pathological pathways (early diagnosis) leading to HF and other serious cardiovascular conditions that are also potential targets for therapy (stratification for personalized medicine), (ii) to validate the predictive value of these biomarkers for the development of HF and commonly associated co-morbid conditions, and (iii) to demonstrate the feasibility of an “omics” biomarkers-based approach to select patients for whom treatment will prevent or delay the onset of HF. The laboratory of Clinical Cardiovascular Pharmacology is contributing to the HOMAGE consortium by sharing selected biological samples and related clinical data from patients with type II diabetes (in collaboration with the ANMCO Research Center and the Italian Association of Clinical Diabetologists) and from elderly individuals (in collaboration with the Department of Epidemiology and cardiologist of the Lazio Region). The laboratory is also involved in a proof-of-concept clinical trial on ‘omics-derived biomarker-selected therapy in heart failure. Currently, the effort is put on the transfer to a central database and harmonization of clinical information from all cohorts of subjects and patients contributing to the HOMAGE consortium.

Multiscale approach to the identification of molecular biomarkers in acute heart failure induced by shock - ShockOmics
This project, funded by the European Union (FP7), coordinated by Giuseppe Baselli, Politecnico di Milano (Electronic, Information and Bioengineering Department, Bioengineering Section), aims at investigating physiopathologic mechanisms in different types of shock. The Mario Negri Unit, led by Giuseppe Ristagno, will set up and study the consequences of hemorrhagic shock in the pig, by multiple approaches, hemodynamics, echocardiography, circulating biomarkers, histology, immunohistochemistry. Within the same project, new circulating biomarkers in humans will be searched for by metabolomic techniques.

Laboratory of Clinical Drug Evaluation

BeTACTIC Study: Best Therapy After Cardiac Transplantation, the Italian Challenge
BeTACTIC is a multicenter, randomized, no-profit trial funded by the National Health Service.
The study compares the efficacy and safety of Everolimus (Ev) and Mycophenolate (MMF) in association with Cyclosporine (CyA) in patients with acute multiple/late rejection, cardiac allograft vasculopathy (CAV), renal dysfunction after cardiac transplantation (HTx). Survival after HTx has improved in the last years, while the attrition rate beyond the 1st year after HTx did not change substantially. CAV and cancer are the leading causes of death after HTx. Many factors as acute rejections and citomegalovirus infections are involved in CAV pathogenesis. Cancer shows higher incidence in immunosuppressed patients. Significant morbidity/mortality derive from renal insufficiency and vascular complications.

Ev and MMF were adopted due to better efficacy vs Azathioprine in de novo HTx. However, Ev and MMF have not been tested in a head to head comparison late after HTx in patients with CAV.

The planned length of the BeTACTIC study is 5 years. Patients will be enrolled at least 1 year after HTx. A total of 400 patients will be randomized in 12 Transplant Centers in Italy. BeTACTIC is promoted by the Cardiology Department, Trapianti e Insufficienza Cardiaca, Ospedale Niguarda Ca’ Granda, Milano and coordinated by the Laboratory of Clinical Drug Evaluation of the Istituto Mario Negri.

REGIA - Rischio Emorragico Ginocchio e Anca
Assessment of the hemorrhagic risk of treatment with low molecular weight heparins, oral anticoagulants, antiplatelet drugs in patients undergoing total hip or knee replacement surgery

Major orthopedic surgery is as a high-risk event for venous thromboembolism (VTE). The anticoagulant prophylaxis reduces the risk of postoperative VTE by 50 to 70%. Major bleeding is a possible complication of thromboprophylaxis with an estimated frequency of 1% to 3% in randomized clinical trials (RCT). However, in clinical practice, the estimates may be argued since: 1) bleeding rates are probably underestimated in RCT, due to frequent exclusion of the high risk patients; 2) definition of bleeding is non-standardized and can vary from study to study; 3) the type of intervention, of anesthesia, of prophylactic agent and the timing of administration in relation to surgery may influence bleeding rate. There is scarce information on the frequency of bleeding after hip or knee replacement in routine practice in Italy. The objectives of the study are the incidence of major and minor bleedings in the first three months after surgery.

The REGIA study is promoted by the Laboratory of Clinical Drug Evaluation and funded by the National Health Service. The study has collected data on bleedings in nearly 3000 patients admitted for hip or knee replacement in the four participating hospitals (Istituto Ortopedico Galeazzi, Milano; Istituto Rizzoli, Bologna; CTO Maria Adelaide, Torino, Policlinico Tor Vergata, Roma) for hip or knee replacement, during surgery, hospitalization, and their consequences at three month follow-up. Analysis of data is ongoing.

ICOS-ONE Study - Prevention of anthracycline-induced cardiotoxicity: a multicentre randomized trial comparing two therapeutic strategies

The background and the objectives of the ICOS-ONE study are summarized among the activities of the Laboratory of Cardiovascular Clinical Pharmacology. The Laboratory of Clinical Drug Evaluation is responsible of the organizational and regulatory activities, of the data management and bioinformatics and of the central and on site monitoring of the study.

MANAGE Study - Management of myocardial injury After NoncArdiac surGery Trial. A large, international, randomized, placebo-controlled trial to assess the impact of dabigatran (a direct thrombin inhibitor) and omeprazole (a proton-pump inhibitor) inpatients suffering myocardial injury after noncardiac surgery
The Population Health Research Institute (PHRI), McMaster University, Hamilton, Ontario, directed by Professor Salim Yusuf, is the coordinating center of a multinational network of cardiology clinics that collaborate to multicenter large scale clinical trials (nearly 40 Countries and more than 600 cardiology clinics). During the last 20 years the Laboratory of Clinical Drug Evaluation has been responsible for the scientific coordination in Italy of several of these trials (INTER-HEART, CURE, ACTIVE, CURRENT, OASIS-8 FUTURA, RE-LY, AVERROES, RE-LY Registry, RIVAL). The MANAGE study is a multicentre, international, blinded, randomized placebo controlled trial aiming to determine the impact of dabigatran on the risk of a major vascular complication and omeprazole on the risk of a major upper gastrointestinal complication in patients suffering myocardial injury after noncardiac surgery (MINS) and followed on average for 1 year. MINS is the most common major vascular complication after noncardiac surgery. Worldwide approximately 10 million adults annually suffer a perioperative myocardial injury. This figure for perioperative myocardial injury represents 15-20% of all cases of myocardial infarction in all settings. Myocardial injury after noncardiac surgery carries a poor prognosis and is an independent predictor of 30-day and 1-year mortality. The MANAGE Trial will enroll 3200 patients, 100 of whom in Italy.

GISSI-HF Genetic Substudy
The GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell’Insufficienza cardiaca) is a collaborative group endorsed by ANMCO (Associazione Nazionale Medici Cardiologi Ospedalieri) and by the Istituto Mario Negri, active from 25 years in the cardiovascular research field. The GISSI-HF was the fifth large scale clinical trial conducted by the Group and was a prospective, multicenter, randomized, double blind, placebo controlled study, with randomized allocation of patients with a clinical diagnosis of heart failure to n-3 PUFA and/or rosuvastatin to assess the effects of long-term administration of n-3 PUFA and/or rosuvastatin on all-cause mortality and cardiovascular hospitalizations. The study randomized more than 7000 patients with the participation of 357 departments of cardiology; results have been published (GISSI Investigators, Lancet 2008). Several substudies focus on possible mechanistic effects of the study treatments. Among them a genetic substudy conducted by nearly 100 Centres that have included 2500 patients, gives the opportunity to improve knowledge on the role of genetic factors involved in heart failure, through a collection of blood samples of a large population of patients, involving cases of heart failure of different etiologies, i.e. non-ischaemic and ischaemic heart disease. The role of genetic factors in causes, evolution, prognosis and treatment of heart failure is largely unexplored, with the exception of heart failure originated by specific cardiomyopathies (such as dilated, hypertrophic, arrhythmogenic right ventricular cardiomyopathies), for which the role of heritable gene mutations is increasingly well understood. Heart failure (HF) is a syndrome with different etiologies, and more than one half is caused by coronary heart disease (CHD). We are focusing on the relationship between the genetic variants of the candidate genes involved in the lipid metabolism and in the inflammatory response. In collaboration with the Laboratory of Cardiovascular Clinical Pharmacology the influence of some genetic variants on the circulating adiponectin and on the prognosis of diabetic patients with heart failure has been assessed. In addition, we conducted an association study between the occurrence of cardiac failure and the genetic variants of adiponectin gene. The results of the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) and of the European Genome Wide Association Study on five genetic variants associated with heart failure have been replicated in our cohort of patients.

GISSI-Prevenzione-Genetic Study
Myocardial infarction is a multifactorial disease. While the role of known risk factors on coronary heart disease susceptibility is well defined, the impact of the genetic components and
its interaction with environmental factors need investigation. The GISSI-Prevenzione trial investigated the effects of pharmacological treatments with n-3 PUFA and pravastatin on morbidity and mortality after myocardial infarction. During the study more than 8000 samples of a large population of patients affected by this disease have been collected and stored with the collaboration of SIBioC (Società Italiana di Biochimica Clinica e Biologia Molecolare). The GISSI-Prevenzione-Genetic Study investigates the role of genetic factors in ischaemic heart disease. The objectives of the project are 1) to assess the relationships between the polymorphisms of various candidate genes and the clinical outcome in patients enrolled in the large clinical trial GISSI-Prevenzione study; 2) to assess whether these relationships are modified by the pharmacological treatments. According to these objectives, we investigated the relationship between APOE, mortality and the response to treatment in 3300 myocardial infarction survivors randomized to pravastatin or no treatment. Association studies in the same population on the adiponectin gene variants, the CRP (C-reactive protein) gene variants, some genetic variants on Chromosome 9p21 have been conducted. Results on the role of genetic variants of PTX3 protein, a novel long pentraxin whose expression is induced by cytokines in endothelial and mononuclear cells, and involved in the atherogenesis process, has been recently published in collaboration with the Istituto Clinico Humanitas and the IRCCS San Giovanni Rotondo.

**Studio GISSI Outliers**

**CAPIRE - Coronary Atherosclerosis in outlier subjects: Protective and Individual Risk factor Evaluation.**

Valutazione dei meccanismi di protezione o di suscettibilità individuali nei confronti della malattia aterosclerotica delle arterie coronariche e delle relative manifestazioni cliniche - Genetica

The association between known risk factors and ischemic cardiopathy is currently estimated with multivariable prediction models. However, there is a component of individual variability underlying the fact that a relevant number of individuals with multiple risk factors do not progress to coronary atherosclerosis or have clinical events, while others have such events or coronary disease in the absence of risk factors (outliers). The purpose of the CAPIRE study is to identify possible novel protective or risk factors for coronary disease in outlier subjects and generate new etiological hypotheses and therapeutic targets for this disease. This is an observational, multicenter clinical study supported by the Heart Care Foundation in the framework of the GISSI-Outliers program. The Laboratory of Clinical Drug Evaluation is responsible of the assessment of the genetic profile of the outliers patients. The first step is the assessment of the variants of associated to the atherosclerotic disease (e.g. Chr 9p21).

**Studio GISSI Outliers**

**GISSI-VAR : Investigation of patients with BAV requiring valve and/or aortic repair. Correlation of surgical and ECO distinctive features with histologic and genetic findings in phenotypically homogeneous outlier cases**

Bicuspid aortic valve (BAV) is the most common congenital heart valve disorder, affecting up to 2% of the population. Only 20% of patients with a congenitally BAV will maintain a normally functioning valve throughout life, while more than 30% of patients will develop serious morbidity. Bicuspid valves are likely to be the result of a complex developmental process, not simply the fusion of two normal cusps. Several family-based studies have shown that BAV disease, either alone or in combination with other cardiovascular malformations, can be inherited in families, and is therefore likely to have a genetic basis. The aim of this prospective longitudinal study is to select homogeneous small groups of surgical patients with the same subtype of BAV and same aortic behaviour and identify
markers/predictors of favorable-unfavorable aortic wall evolution to evaluate if there is a BAV phenotype more likely to be considered at high risk for aortic degeneration. The study will focus on multiple aspects of BAV disease: morphology, genetics, histology. The study is supported by the Heart Care Foundation in the framework of the GISSI-Outliers program, with the participation of 11 cardiosurgery departments. The Laboratory of Clinical Drug Evaluation will be responsible of the assessment of the genetic profile of the BAV patients.

**Laboratory of General Practice Research**

**Risk and Prevention Study (R&P)**

R&P is a study on the optimization of cardiovascular prevention of subjects at high risk performed at national level by General Practitioners.

**Study objective and design**

- Controlled clinical trial, double-blind and randomised, of the efficacy of a n-3 PUFA treatment in reducing the incidence of cardiovascular events, both fatal and non-fatal, in a population defined as at high risk by participating GPs.
- Practicability and overall yield of the preventive interventions adopted (outcome study). The epidemiological and care history of this population shall form the object of a specific evaluation according to a plan of formal predefined analyses.

**Study population**

**Inclusion criteria**

- multiple risk factors (e.g. hypertension, hypercholesterolemia, diabetes, smoking, family history of myocardial infarction, obesity, sex and old age)
- previous cardio-cerebrovascular events or clinical manifestations of atherosclerotic disease (stroke, TIA, peripheral arteriopathy, previous arterial revascularisation procedures, angina pectoris).

**Exclusion criteria**

- serious co morbidity with an unfavourable prognosis over the short term (e.g. cancer)
- expected non-compliance over a long period of time; contraindications (known allergies to n-3 PUFA)
- indications (previous MI) for treatment with n-3 PUFA.

**Efficacy measures**

The primary objective was to evaluate if a long-term administration of n-3 PUFA (1 gr per day) was more effective than the corresponding placebo in reducing cardiovascular mortality and hospitalization for cardiovascular causes (primary end-point).

**The duration of follow-up** was 5 years.

**Up-date of the study**

From February 2004 to March 2007 12,521 patients have been enrolled by a network of 860 GPs. The Local Health Authorities involved are 57 and in each one investigator’s meeting has been organized.

The Risk & Prevention study ended the 31st of October 2011. During a median follow up of 5 years 1,468 cardiovascular events occurred (the minimum expected number reported in the protocol was 1,383 events). Only 62 GPs, out of a nationwide network of 860, withdrew from the study and 86 patients were lost to follow-up. The main results of this study, published in May 2013 on the New England Journal of Medicine, are two. The first one scientific and relevant for public health: a supplementation with n-3 PUFA on top of already recommended pharmacological and non-pharmacological treatments for cardiovascular prevention does not reduce fatal or non-fatal cardiovascular disease. The second result is as important as the first
one: for the first time, and with the biggest study in this field, Italian primary care has been recognized at the highest level of the scientific literature as a producer of knowledge that become international reference in a key area for cardiovascular prevention.

Epidemiological and clinical profile of diabetic patients in Lombardy Region using administrative databases.

The study is part of an on-going pharmaco-epidemiological project in collaboration with the Health Department of the Lombardy Region. Its main objective is the definition of a model to assess and control the use of health resources of diabetic patients by means of integrated administrative database.

Specific aims of the study are:

to describe prevalence, incidence, hospitalization and mortality of the diabetic population In particular to investigate:

- the prescriptions of both anti-diabetic and cardiovascular drugs;
- hospital admissions, prescriptions of laboratory test and specialist medical examinations as indicators of process of care;
- the incidence of major cardiovascular complications and mortality in the first years of follow-up in patients with newly diagnosed diabetes;
- sex differences in cardiovascular outcomes, pharmacological treatments and indicators of care in patients with newly diagnosed diabetes;
- prescribing changes in a elderly population from 2000 to 2010

The population analysed has been selected among the resident population of the Lombardy Region throughout 8 years of observation (between 2000 and 2007). Diabetic patients have been identified each year if they met one of the three following criteria: - a) at least a prescription of an A10 drug: insulin and/or oral glucose lowering agent; b) the occurrence of at least one hospitalization with Disease Related Group (DRG)=294 (diabetes in a subject > 35 years old) or DRG=295 (diabetes in a subject < 35 years old); c) presence of the exemption code number 013.250 indicating diabetes. On the basis of these criteria 10 different data sets have been created one for each year of observation. Data from prescription database, hospital admission and outpatient clinic visits and examinations were also included in the analysis via linkage to the personal identification number (national identifiers).

The analyses show that:

- Morbidity and mortality risk is high since the onset of diabetes and rose over time. Younger newly diagnosed diabetes (40-49 years old) had a risk of death or hospital admission for cardio-cerebrovascular events similar to subjects without diabetes ten years older. These data highlight the importance of prompt and comprehensive patients care including measures to reduce other cardiovascular risks since the onset of diabetes.
- Females with diabetes could be considered a separate risk category from those without, with the same risk profile as males. Diabetes is linked to a higher increase of mortality in females relative to males. This might reflect sex differences in the use of revascularization procedures or therapeutic regimens. Closer attention and implementation of standard care for females are necessary from the onset of diabetes.
- The drug prescription profile of elderly diabetic patients changed noticeably from 2000 to 2010, toward a tendency to recommended antidiabetic drugs and increased cardiovascular prevention (prescriptions of ACE inhibitors, statins and antiplatelet drugs). These changes might possibly be linked to the decrease in hospital admissions and mortality observed in oldest diabetic groups (65-74 and 75-84 years).

“GLICINE-SPIDER” Study

“Glicine-Spider” is an observational study carried out in the Coronary Care Units (CCU) of Lombardy. The protocol is a collaboration between the ANMCO (Italian Association of Hospital Cardiologists) Lombardia, AMD (Association of Medical Diabetologists) Lombardia
and the Mario Negri Institute. The study is coordinated by the General Practice Research Laboratory and the Clinical Drug Evaluation Laboratory.

Hyperglycemia at the onset of an acute coronary syndrome (ACS) constitutes a negative prognostic factor in diabetic and non-diabetic patients and a poor control of blood glucose in the early hours after hospital admission for ACS is an additional unfavourable prognostic factor. Recent guidelines, although recognizing the importance of controlling blood glucose in ACS, do not clearly define therapeutic strategies to apply and glycemic target values of the patients with and without diabetes hospitalized in CCU for a confirmed ACS.

The aim of the study is to describe in a large sample of patients hospitalized in CCU for an ACS:

- the prevalence of diabetes and hyperglycemia
- the type of treatment and blood glucose control during the acute phase
- the incidence of mortality and cardiovascular complications occurred during the hospitalization according to diagnosis and blood glucose level

From May 2009 to April 2010, 1,282 patients have been included from 31 CCUs. The data analysis is in progress.

FOCUS Study (Fixed Dose Combination Drug for Secondary Cardiovascular Prevention. Improving Equitable Access and Adherence to Secondary Prevention Therapy with a Fixed-Dose Combination Drug)

Several randomized controlled trials and metaanalyses have demonstrated that the long term administration of aspirin, statins, beta-blockers, and angiotensin converting enzyme inhibitors (ACE inhibitor) improve prognosis in high risk patients, particularly those recovering from an acute coronary event. However, wide variability in the pattern of prescription among physicians, limited access to expensive drugs in emerging countries, and poor adherence to medications limit the use of these drugs and the efficacy of cardiovascular prevention.

A Fixed Dose Combination (FDC) pill for cardiovascular prevention was first proposed by Wald and Law in 2000 and supported by the WHO. During the last few years this concept, particularly in the field of primary prevention has been questioned by some experts while the potential role of a polypill for secondary cardiovascular prevention is receiving increasing attention. However, a direct proof of the polypill effect on patients’ adherence is still lacking.

The global objective of the FOCUS consortium is to make FDC drugs for secondary cardiovascular prevention available throughout the world at a low price, in order to improve access to treatment in developing countries improving adherence to medication. The Centro Nacional de Investigaciones Cardiovasculares (CNIC) in Madrid is the coordinator of the FOCUS study and the leader of the consortium composed also by Istituto Mario Negri, DAMNIC Institute, Fundació Clinic per a la Recerca Biomèdica (FCRB), ARTTIC, the World heart Federation (WHF), the Instituto de Salud Carlos III (ISCIII), FERRER and the Federaciòn Argentina de Cardiologia (FAC).

The study is international, multicenter in two phases:

**Phase 1** is a descriptive, non-interventional study. Its aim is to provide a comprehensive analysis of factors precluding adequate secondary prevention, including health system characteristics, drugs affordability and availability, as well as patients’ characteristics.

**Phase 2** is an interventional, randomized, two-arm study. Patients are randomized to receive a FDC of ramipril, simvastatin and acetilsalycilic acid or the three medications separately. The primary objectives is to compare the adherence to treatment in post myocardial infarction patients receiving a FDC vs those with conventional treatment (3 drugs separately).

Secondary objectives are to evaluate the effect of a FDC on blood pressure control and lipid profile and the safety and tolerability of FDC treatment.

Two countries in Europe (Spain and Italy) and three in South America (Argentina, Brazil e Paraguay) are involved in the study.
Nowaday in Italy have been involved 666 patients in phase 1 and 227 in phase 2. The study will end on May 2014 and the result’s publication is expected for September 2014.

“Il Sale è meglio averlo in Zucca” project
The idea for this project originated from the awareness that Italian diet is excessively rich in salt and this can cause major cardiovascular diseases. Data available from previous studies showed that a partial reduction in dietary salt intake leads to a decreased incidence and a better control of hypertension. Reduction in dietary salt can, however, compromise food’s taste and therefore this could represent an unacceptable option for the population. It is possible to reduce salt supplement during food preparation without jeopardize its taste by substituting some foods with other adding up spices and aromatic plants or utilizing salt substitutes. This project, conduct in collaboration with the Laboratory of Nutrition Toxicology and Elior (an industrial catering company), had the aim to gather data on tricks useful to reduce salt in the diet without modify the food’s taste. Thirteen courses were selected from the winter menu of a company cafeteria and a sample for the assessment of sodium content was taken from each. The recepies were modified in order to reduce the sodium content of about 20% by adding for example spices or mixed herbs to keep the food’s taste acceptable. From these new recepies samples for the assessment of the sodium content were taken and the customers’ appreciation was recorded through specific questionnaires in which they were asked to individuate the modified recepies and if they needed to add some salt to the courses they’ve choosed. The results were encouraging, it has been demonstrated that it is feasible to reduce the sodium content in courses served in a company cafeteria and this does not modify customers’ appreciation because they are not induced to add more salt than what they would usually add.

Studio FALCO: Surveillance of patients with atrial fibrillation in Lombardy treated with oral anticoagulant drugs
The new direct oral anticoagulant drugs (DOAC) direct thrombin inhibitors and inhibitors of the activated X factor, are now available on the Italian market for the treatment of the thromboembolic complications in patients with Atrial Fibrillation (AF). Caracteristics of these new drugs are, with respect to so far recommended anticoagulant therapy warfarin and acenocumarol (AC), the rapidity of action, the low potential of interactions with other drugs or foods and a stable anticoagulant effect with no need of monitoring INR frequently. These drugs are dabigatran, rivaroxaban and apixaban (which will enter the Italian market soon). It is foreseeable that these caracteristics will lead to a rapid transition of the warfarin prescriptions to DOAC prescriptions. This switching will bring a change in the patients’ management (no need of INR monitoring) whom will still need to be monitored for adverse events once DOAC will be used in clinical practice.

This pilot study aims to start a surveillance of a representative sample of non valvular AF patients in Lombardy that will start a new oral anticoagulant therapy (either with DOAC or with AC).

Study population
Patients with non valvular AF that will start a new antithrombotic therapy will be consecutively selected. It will be possible to include:
- patients that start an antithrombotic therapy for the first time
- patients that switch from an antithrombotic therapy to another

Fortysix centers joined the study so far: 21 Cardiological unit, 14 Centers for the control of anticoagulant therapy, and 11 Internal Medicine/Geriatric units.

The stratification of global cardiovascular risk in hypertensive patients of the district of Borbon – Ecuador
The Laboratory is involved in a collaborative project with the Cecomet (Centro de Epidemiologia comunitaria y Medicina tropical) in Esmeralda, Ecuador, on the prevalence and
treatment of hypertension in the district of Borbon, a rural zone of Ecuador in the northern part of the country.

In this area, 36% of the adult population is affected by hypertension and more than half of hypertensive patients present blood pressure levels > 160/110 mmHg.

From 2001, in the District is ongoing an intensive follow-up of the hypertensive population with the following aims: to evaluate the global cardiovascular risk of the population, to better control blood pressure levels increasing the number of subjects treated with hypertensive therapy (in particular those at high cardiovascular risk) and monitoring of the clinical complications. Preliminary data show that:

- Patients treated with hypertensive therapy are increased from 39% to 59%
- Antihypertensive drugs are mainly prescribed to subjects with high blood pressure levels (80% of those with systolic blood pressure \( \geq 180 \text{mmHg} \) are actually under treatment) or at high cardiovascular risk (82%)
- Blood pressure control is improved (patients with systolic blood pressure levels \( \geq 180 \text{mmHg} \) decreased from 33% to 24% and those with levels <160-179 increased from 26% to 34%)
- The fraction of patients at high or very high cardiovascular risk is decreased from 40% to 33%

However, the compliance to antihypertensive treatment is still unsatisfactory since only half of the subjects are compliant with the prescribed therapy.

**Laboratory of Medical Statistics**

The Laboratory of Medical Statistics develops applied research in three main fields: controlled clinical trials, observational studies and genetic epidemiology.

**Controlled clinical trials**

The laboratory deals with planning, management and statistical analysis of controlled clinical trials, carried out in the different laboratories of the Department of Cardiovascular Research, by means of the GISSI trials experience.

At present, GISSI trials focus on GISSI-HF, GISSI-AF and OPERA clinical studies, concerning heart failure and atrial fibrillation and their subprojects aiming to assess the role of: biomarkers, levels of circulating fatty acids, echocardiographic parameters and body weight loss on the patients’ prognosis. Recently, two superiority trials have been activated: the BeTACTIC study that will randomize about 400 patients undergone heart transplantation and the CYCLE study that will recruit 444 patients in reperfused acute myocardial infarction.

It’s now active the multicenter trial of superiority in the cardio-oncology field: the ICOS-ONE study to test if two different therapeutic strategies can prevent anthracycline cardiotoxicity. The study plans to randomize 268 patients with cancer receiving chemotherapy based on anthracyclines.

Results regarding the large trial concerning cardiovascular prevention, Risk & Prevention study (Rischio & Prevenzione) which included more than 12500 patients have been presented.

The epidemiological history of this population is under evaluation according to pre-established statistical analysis.

Statistical methodology applied to clinical studies has a leading and developing role as far as methods are concerned (e.g.: missing data management; development of prognostic risk scores, methods for the assessment of competing risks, development of forecasting models for biomarkers based on Reclassification techniques and on Discriminations Indices etc.).
Moreover, clinical trial management implies the setup of data planning and screening methods, the ad interim analysis and the choice of the best study design (superiority, non-inferiority and equivalence studies).

**Observational studies**

The activation of observational studies allows to characterize the epidemiological profile of categories of patients followed in their natural clinical course. The prospective observational study GLICINE-SPIDER has evaluated the risk profile of 1300 patients with hyperglycemia at the onset of an acute coronary syndrome (ACS) in the hospitals of the Lombardia region. The cohort study REGIA, evaluated the incidence of major and minor hemorrhages and the characterization of the risk profile of about 3000 patients undergoing hip and knee replacement surgery. The results of the study have been presented to the researchers and will soon be published.

The study FALCO (Sorveglianza dei pazienti con Fibrillazione Atriale in Lombardia trattati con AntiCoagulanti Orali) has just been activated and provides randomization and observation of 800 patients in major Italian institutes.

**Genetic Epidemiology**

The laboratory has recently developed specific skills on genetic epidemiology analysis. These studies are carried out together with the laboratory of Clinical Drugs Evaluation. Statistical analysis techniques concerning cardiovascular genetics have been developed in the last five years.

The study of the genetic component of multifactorial diseases, such as the cardiovascular disease, has been dealt with in the PROCARDIS study, by means of the genome-wide screening. This technique aims at identifying genes that can cause coronary disease. PROCARDIS database gave the opportunity of studying some quantitative traits such as the level of lipids or body mass index.

During the second step of the PROCARDIS project, supported by the 6th Framework Program of EEC, a screening on the whole genome has been carried out by means of the “genome-wide association” technique. For this project about 1 million of polymorphisms (SNPs) have been analyzed in order to identify a possible relationship with coronary disease.

Recently, the C4D genetic Consortium, of which the PROCARDIS Consortium takes part, has demonstrated the existence of new susceptibility genes to coronary artery disease (CAD). Indeed CAD is caused by the occurrence of many genes as emerged from recent meta-analyses on GWAS.

Concerning the GISSI-Genetic Prevention study, the laboratory has developed statistics genetics techniques to analyze case control studies in order to assess the association of genetic variants linked to adiponectin, HsCRP, PTX3 with coronary disease. With regard to the GISSI-HF genetic substudy that has included about 2500 patients to evaluate the role of genetic variants involved in heart failure, the association of four polymorphisms of the adiponectin gene has been investigated by a case-control design.

It was also investigated the association of the genetic variants of rs5068 polymorphism with circulating levels of important biomarkers in chronic heart failure.

**Laboratory of Clinical Pharmacology**

**Quality of Life, Depression and Cognitive problems in heart failure patients (QDF-GISSI-HF)**

The QDF project is a sub-project of the GISSI-HF study. The aims of the study are 1) to describe the evolution of depression, cognitive problems and the quality of life in a sample of 1500 heart failure patients; 2) to assess the use of common instruments that measure QDF.
variables; 3) to compare the assessment of the instrument (Geriatric Depression scale, Mini Mental State Examination, Kansas City Cardiomiopathy Questionnaire) with the clinical perception of the nurses; 4) to describe if assessed or perceived patients' problems (low quality of life, high depression or compromised cognitive function) lead to any caring intervention.

The baseline clinical characteristics of the 1564 patients included in the QDF study are closely comparable with those of the GISSI-HF population. The study instruments could be validly administered to the greatest majority of patients (KCQQ 97.2%, GDS 94.9%, MMSE 80.6% of patients >70 years).

The nurses network nested in a major clinical trial has produced one of the largest prospective cohort of HF patients who are comprehensively assessed and prospectively monitored, to allow an integrated evaluation of the relevance and implications of QDF measurements also on the clinical outcomes of this population. Manuscripts on the study results are in preparation.
DEPARTMENT OF MOLECULAR BIOCHEMISTRY AND PHARMACOLOGY

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CURRICULA VITAE

Mario Salamina obtained his doctorate degree in Biochemistry and Food Technology at the University of Milan in 1971. His background is in biochemistry, biophysics and pharmacology. His scientific interests relate to problems of human and animal diseases originating from the aberrant folding of proteins. In this context, a major portion of his studies was devoted to the etiopathogenesis and therapy of prion diseases. He has published over 300 papers and 25 book chapters, the total number of citations of his papers is 9997 and his h factor is 50.

1971-1975 Research Fellow at the Laboratory of Biochemical Pharmacology, Mario Negri Institute
1976-1977 Post-doc at the Weizmann Institute for Science, Department of Biological Chemistry, Rehovot, Israel
1977-1997 Head, Laboratory of Enzymology, Mario Negri Institute
1986-1987 Visiting Scientist at the Weizmann Institute for Science, Department of Organic Chemistry, Rehovot, Israel
1995-2011 Dean of the Advanced School of Pharmacology and Responsible of Educational Activities, Mario Negri Institute
1995-present Member of the Board of Trustees of the Consortium “Mario Negri Sud”, Chieti, Italy
1997-present Head, Department Molecular Biochemistry and Pharmacology, Mario Negri Institute
1997-present Head, Laboratory of Biochemistry and Protein Chemistry, Mario Negri Institute
He has served in several national and international scientific committees, presently he is a component of the EU panel developing the project “The European Advanced Translational Research Infrastructure in Medicine” (EATRIS).

Selected publications

Gianfranco Bazzoni got his Medicine and Surgery degree in 1988 (at the University of Milan) and the specialisation in Pharmacological Research in 1992 (at the Mario Negri Institute, Milan). His area of expertise is cell biology, with focus on the processes of cell adhesion and migration.

1988-2000 Research Fellow, Mario Negri Institute
1993-1997 Post-doctoral Fellow, Dana Farber Cancer Institute and Harvard Medical School, Boston, MA
2000-2002 Research Scientist, Mario Negri Institute
2003 Head, Unit of Cell Adhesion, Mario Negri Institute
2004 to date Head, Laboratory of Systems Biology, Mario Negri Institute
2004 Regular Member of The American Physiological Society, Bethesda, MD
Referee for international scientific journals

Selected publications
- Martinez-Estrada OM, Manzi L, Tonetti P, Dejana E, Bazzoni G. Opposite effects of Tumor Necrosis Factor and
• Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. Physiol Rev 84: 869-901, 2004

Valentina Bonetto has got the degree in Pharmaceutical Chemistry and Technology at the University of Padua, Italy in 1993. She has got the Ph.D in Medical Biochemistry and Biophysics at Karolinska Institutet, Stockholm, Sweden.
Her principal lines of research are: 1) Study of the pathogenetic mechanisms at the basis of amyotrophic lateral sclerosis (ALS); 2) Identification of biomarkers of ALS; 3) Role of the oxidative modification in neurological disorders. These issues are investigated by different experimental approaches, including proteomics and mass spectrometry.
2000-2009 Research Scientist, Laboratory of Biochemistry and Protein Chemistry, Mario Negri Institute
2002-2009 also Assistant Telethon Scientist at Dulbecco Telethon Institute
2007-2009 Head, Unit of Medical Biochemistry, Laboratory of Biochemistry and Protein Chemistry, Mario Negri Institute
From 2009 to date, Head Laboratory of Translational Proteomics and Associate Telethon Scientist.
She is author of 35 publications from 1994 to 2011, in peer-reviewed journals. She is reviewer for scientific journals in the field of Proteomics and Neuroscience.

Selected publications

Lavinia Cantoni obtained her degree in Biological Sciences in 1973 at the University of Milan. Then she specialized in Pharmacological Research in 1977 at the Mario Negri Institute. Area of expertise: 1) biochemical-molecular mechanisms activated by oxidative stress 2) drug metabolism 3) porphyrias.
1974-1977 Research Fellow, Mario Negri Institute
1977-1978 Post-doctoral Fellow, Medical Research Council, Toxicology Unit, Carshalton, UK (Winner of a Welcome Trust Research Fellowship)
1979-1982 Research Scientist, Mario Negri Institute
1980-1990 Visiting Scientist, Toxicology Unit, Carshalton, UK, and Cornell Medical Center, New York, NY (short periods)
1983-1997 Head, Unit of Heme and Hemoprotein Metabolism, Mario Negri Institute
1998 to date, Head, Laboratory of Molecular Pathology, Mario Negri Institute
1975 to date Member of the National Roll of Biologists
1983 to date Member of the Italian Toxicology Society
She is reviewer for international scientific journals in the field of oxidative stress and neuroscience.

Selected publications


Enrico Garattini obtained his degree in Medicine and Surgery with full marks (110/110) in 1982 at the University of Milan. His scientific interests relate to problems of Cellular Biology and Molecular Biology.

1982-1990 Research Fellow of the National Research Council, Mario Negri Institute
1983-1987 Postdoctoral Researcher at the Roche Institute of Molecular Biology, Department of Neurosciences Nutley, New Jersey, US
1991-1997 Senior Researcher Regione Lombardia and Head of the Molecular Biology Unit, Mario Negri Institute
1997 to date Head, Laboratory of Molecular Biology, Mario Negri Institute

From 2005 Dean, Advanced School of Pharmacology (Philosophy Doctor), Mario Negri Institute
From 2011 Dean, Educational Activities, Mario Negri Institute

Selected publications


Marco Gobbi got his degree in Pharmacy at the University of Milan, Italy, in 1989.
His main fields of interest are: i) neurodegenerative diseases associated to misfolding and aggregation of peptides/proteins, such as beta-amyloid and prions; ii) alterations of synaptic transmission in the CNS, either due to diseases or to the effects of drugs on receptors and transporters; iii) nanoparticles for diagnostic and therapeutic purposes. These research fields are investigated by a close integration of pharmacodynamics (e.g. biomolecular interactions, mainly using surface plasmon resonance) and pharmacokinetics studies.

1981-1995 Researcher, Laboratory of Neuropharmacology and, from 1988, in the Laboratory of Receptor Pharmacology, Mario Negri Institute
1995-2010 Head, Unit of Synaptic Transmission, Mario Negri Institute
From 2010, Head, Laboratory of Pharmacodynamics and Pharmacokinetics
Co-author in more than 100 scientific publications on peer-reviewed international journals. First or last author in more than 50 of them.

Reviewer for international scientific journals operating in the Neuroscience/Neuropharmacology/Biochemistry fields.

Selected publications


Luisa Diomede is a Chemico-Biological Analysis Doctor (University “Carlo Bo”, Urbino, Ital) from 2007. Her main areas of interest are: i) the use of Caenorhabditis elegans as model organism to investigate the biochemical and molecular mechanisms underlying protein misfolding diseases; ii) the design and the validation of innovative therapeutic strategies for these pathologies.

1985-now Researcher at “Mario Negri” Institute for Pharmacological Research, Milan, Italy.
1992-now Permanent position at “Mario Negri” Institute for Pharmacological Research, Milan.
1992-2010 Senior Scientist, Laboratory of Biochemistry and Protein Chemistry.
2005-2011 Permanent position at “Mario Negri” Institute for Pharmacological Research, Milan.
2011-now Head of “Human Pathologies in Model Organisms” Unit.

Co-author in more than 70 scientific publications on international journals. Reviewer “ad hoc” for International journals.

Principali pubblicazioni

Maddalena Fratelli got her degree in Biological Sciences at the University of Pisa and at the Scuola Normale Superiore di Pisa in 1983. Then the specialization in Pharmacological Research at the Mario Negri Institute in 1986.

Her main fields of interest are: 1. High throughput genomic systems for the study of drug action and pharmacoresistance. 2. Redox regulation of protein function and gene expression: glutathionylation and gene expression profiling of glutathione dependent responses to oxidant challenge.

1988-1989 Postdoctoral Research Fellow in the Medical Research Council, Neurobiology Unit, Cambridge, UK.

Since 1995, Head, Unit of Mediators of inflammation, Laboratory of Neuroimmunology, Mario Negri Institute

Since 2005, Head, Unit of Pharmacogenomics, Laboratory of Molecular Biology, Mario Negri Institute

Selected publications


Mineko Terao obtained her doctorate degree in Pharmaceutical Science from the Kobe Women’s College of Pharmacy, Japan in 1978. Her scientific interests relate to problems of Cellular Biology and Molecular Biology.

1983 Ph.D in Molecular Biology, Kyoto University, Japan
1982-1983 Research Fellow, Department of Medical Chemistry, Kyoto University Faculty of Medicine, Japan
From 1987 Visiting Scientist of Mario Negri Institute
From 1998 Head of the Unit of Gene Structure and Regulation, Mario Negri Institute

Selected publications

- Villa R, Kurosaki M, Barzago M M, Kolek M, Bastone A, Colombo L, Salmona M, Terao M, Garattini E. Regulation and biochemistry of mouse molybdo-flavoenzymes. The DBA/2 mouse is selectively deficient in the expression of
aldehyde oxidase homologues 1 and 2 and represents a unique source for the purification and characterization of aldehyde oxidase. J Biol Chem 2004; 279: 8668-8683

ACTIVITIES

The Department comprises seven laboratories. Research is heterogeneous in terms of scientific interests and aims, but it is unified by the structural and functional study of specific, pharmacologically important gene products, using a common body of techniques. Classical biochemistry and molecular biology methods are used to define proteins that might be targets for the pharmacological activity of drugs. Potential direct interactions between drugs and proteins are studied at the molecular level by a variety of approaches ranging from animal studies to computer simulations.

MAIN FINDINGS

Development of new protocols for the preparation of β-amyloid. Use of Surface Plasmon Resonance (SPR) to study the elongation kinetics and the binding properties of the highly amyloidogenic Aβ 1–42 peptide. Characterization of the binding properties of a new peptide inhibitor of β-amyloid aggregation. A2V mutation favors the formation of toxic Aβ1-40 aggregates, in vitro and in vivo. Generation of new transgenic C. elegans strains pan neuronally expressing wild type or A2V-mutated human Aβ1-40. Identification of the molecular mechanisms responsible of oligomer formation of amyloidogenic proteins. Recognition of soluble oligomers by a new immunoassay based on surface plasmon resonance (SPR) and evaluation of oligomers toxicity by a new behavioral test on C. elegans. Epigallocatechin-gallate, a green tea bioactive polyphenol, prevents the formation of toxic Aβ oligomers. Characterization of the binding of β-amyloid oligomers to prion protein. Synthesis, biological and chemico-physical characterization of peptides deduced from prion protein sequence. A newly developed SPR-based epitope scanning indicates structural differences in brain-derived aggregated mutant prion proteins related to genetic prion diseases. The expression of a mutant prion protein (PG14) impairs glutamatergic transmission in mice cerebellum, an effect down-hill to a defect of voltage-gated calcium channels. Identification of tetracyclines as potential therapeutic agents for prion diseases. Doxycycline persistently accumulates in the brain of patients with Creutzfeldt –Jakob disease chronically treated with the drug. Plasma levels of doxycycline are not affected by haemodialysis in patients with Dialysis Related Amyloidosis (DRA), in which the chronic treatment with the drug was active in reducing articular disability. Generation of new transgenic C. elegans strains expressing different isoforms of human β2-microglobuline as models of Dialysis Related Amyloidosis (DRA). P32G mutation and ΔN6 truncation favours the formation of toxic β2-microglobuline oligomers, in vitro and in vivo. Characterization of SEPN1, a member of the selenocysteine-containing protein family localised in the membrane of the Endoplasmic Reticulum and whose genetic mutations have been identified as the cause of some congenital myopathies, and its involvement in the ER redox
homeostasis. Moreover the analysis of ascorbic acid as a potential treatment of the SEPN1-related-myopathies is ongoing. Treatment with a soluble TNF receptor in the wobbler mouse, reduces motor neuron degeneration and the phosphorylation of the two main stress kinases (p38 e JNK) activated by TNF receptors.

Identification of a panel of protein biomarkers in peripheral blood mononuclear cells of Amiotrophic Lateral Sclerosis (ALS) patients and a rat model of ALS. Identification of a novel pathogenic mechanism that may contribute to the spreading of the disease and motor neuron death in a mouse model of ALS. Development of constitutive and conditional motor neuronal cell models to unravel the toxicity of mutant G93A superoxide dismutase 1 (G93A-SOD1) responsible for some forms of familial ALS.

Mitochondrial damage due to mutant G93A-SOD1 occurs selectively in motor neurons. Endogenous levels of ubiquinol were higher in the CNS tissues of SOD1G93A mice, even at a presymptomatic stage. Chronic treatment with ubiquinone or ubiquinol has no effect on the disease progression and survival in SOD1G93A mice. The human mutant G93A-SOD1 protein is toxic to motor neuronal cells under hypoxic conditions.

Drugs or exogenous compounds impairing the electron transport chain are a risk factor to motor neurons of individuals carrying mutant forms of SOD1. Synthesis of glutathione, the main cellular antioxidant, and intracellular levels of glutamate, a component of glutathione and a neurotransmitter, are altered in a cell model of ALS. Identification and characterization of a novel class of retinoids endowed with strong and selective apoptogenic activity on the neoplastic cell. Pre-clinical development of these agents for the treatment of acute leukemia.


Development of knock-out animals for molybdo-flavoproteins: AOX1, AOH1, AOH2, AOH3. Creation of integrated instruments for the rationalization of Microarray analysis processes. Recombinant C1-inhibitor binds with high affinity with Mannose Binding Lectins, an interaction possibly underlying its superior anti-ischemic properties in animal models. Identification of a new synthetic MBL ligand, which proved to be neuroprotective in animal models of ischemia.

Evidence for the binding between C3 and P-selectin, in a collaborative study regarding the role of complement system in triggering microvascular thrombosis. Confirmation and characterization of the binding of pentraxin-3 to P-selectin, a new mechanism involved in the leukocyte recruitment at sites of inflammation.

Determination of the binding properties of new FGF-2 ligands with antiangiogenic activities. Development of new protocols to evaluate, by surface plasmon resonance (SPR), the interaction between nanoparticles and their putative targets: application to nanoparticles functionalized to bind β-amyloid. Development of new protocols to evaluate, by SPR, the formation of protein corona on the nanoparticles surface.

**NATIONAL COLLABORATIONS**

Advanced Biology Center, Genoa
INTERNATIONAL COLLABORATIONS

The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel
Boston College, Boston, MA, USA
Burke Medical Research Institute, White Plains, New York, USA
Case Western Reserve University, Cleveland, OH, USA
Dept. de Quimica-Fisica de Macromolecules Biologicas, CSIC, Madrid, Spain
Division of Biomedical and Life Sciences, School of Health and Medicine, Lancaster University, Lancaster LA1 4YQ, UK
Faculdad de Ciencias Medicas, Universidad de Santiago de Chile, Chile
ETH, Zurich, Switzerland
FMP, Berlin, Germany
Giessen Polyclinic University, Giessen, Germany
Group of Elegans New Investigators in Europe
Houston University, TX, USA
Keio University, Tokyo, Japan IBSN CNRS, Marseille, France
Indiana University, Indianapolis, IN, USA
Institut de Genetique et Biologie Moleculaire et Cellulaire, Strasbourg, France
Institute for Behavioral Genetics, University of Colorado, USA
Institut Pasteur, Paris, France
Laboratoire de Physico-Chimie, Pharmacotechnie et Biopharmacie, , Univ Paris-Sud 11, Chatenay- Malabry, France
John Innes Centre, Norwich, UK
Keio University, Tokyo, Japan
Lundbeck, USA
Max Planck Research Unit for Enzymology of Protein Folding, Halle, Germany
Mayo Clinic College of Medicine, Jacksonville, FL, USA
National Institute of Health, Bethesda, MD, USA Nippon University, Tokyo, Japan
Pepscan System BV, Lelystad, The Netherlands Polichem S.A., Lugano, Switzerland
Politecnico di Zurigo (ETH), Switzerland
Technical University Braunschweig, Germany
Trinity College, Dublin, Ireland
Universidad de La Laguna, Tenerife, Spain
Universidad Nova, Lisbon, Portugal
Universitat des Saarlandes, Hamburg, Germany
Universitat Freiburg, Germany
Université Paris, France
Université Victor Segalen Bordeaux 2, Bordeaux, France
University of Aberdeen, UK
University of Amsterdam, The Netherlands
University of Birmingham, UK
University of Cardiff, UK
University of Glasgow, UK
University of Gottingen, Germany
University of Muenster, Germany
University of Patras, Greece
University of Southampton, UK
University of Sussex, UK
University of Vienna, Austria
Vanderbilt University, Nashville, USA
Waring-Webb Institute, University of Colorado, Denver CO, USA Weizmann Institut, Rehovot, Israel
Westfaelische Wilhelms-Universitaet Muenster, Germany

EDITORIAL BOARD MEMBERSHIP

Current Opinion in Pharmacology (M. Gobbi)
European Journal of Cancer (E. Garattini)
BioMolecular Concepts (V. Bonetto)

PEER REVIEW ACTIVITIES


CONFERENCE AND WORKSHOP CONTRIBUTIONS

Conference: “Alzheimer’s and Parkinson’s Diseases: Mechanisms, Clinical Strategies, and Promising Treatments of Neurodegenerative Diseases, 11th International Conference AD/PD™, “Different mutations at codon 363 of the tau gene produce different structural and functional properties”, 6-10 March, Firenze, Italy


Congress: “ENCALS, European Network for the Cure of ALS”, “Cyclophilin A is a molecular linker between SOD1- and TDP-43-mediated pathologies in ALS”, 31 May - 2 June, Sheffield, Great Britain

Conference: “Inaugural EATRIS Conference”, “Translational medicine and small molecules”, 3-4 June, Amsterdam, Holland

Congress: “BIOMEDIA - la condivisione del sapere - 28° convegno di studio - il laboratorio nelle malattie del sistema nervoso”, “Biomarcatori multiproteici di sclerosi laterale amiotrofica”, 6-7 June, Vicenza, Italy


Meeting: “19th International C. elegans Meeting”, “C. elegans expressing human beta2-microglobulin: a novel model for studying the amyloid toxicity”, 26-30 June, Los Angeles, California, USA

Meeting: “VIIIth Joint Meeting of Medicinal Chemistry”, “Thieno[3,2-d]pyrimidin-4(3H)-one derivatives as novel 5-HT7 receptor ligands”, “New thienopyrimidine and quinazoline derivatives as potential 5-HT7 receptor ligands”, 30 June – 4 July, Lublin, Poland

Symposium: “17th European Carbohydrate Symposium”, “Synthesis and affinity for mannose binding lectin of multivalent saccharide compounds”, 7-11 July, Tel Aviv, Israel

Congress: “15th ICI - Immunitas Vis Naturae - Milan 2013 - International Congress of Immunology”, “Orchestration of tissue repair by the humoral pattern recognition molecule PTX3: linking microbe and matrix recognition”, 22-27 August, Milano, Italy

Symposium: “International Symposium on Cyclophilins and other Foldases: Cell Signaling Catalysts and Drug Targets”, “Cyclophilin A is a translational biomarker and a potential therapeutic target for amyotrophic lateral sclerosis”, 19-21 September, Hallen, Germany

Meeting: “3rd MS Food Day”, “A new method for the determination of BHA in chewing gums”, 9-11 October, Trento, Italy


Congress: “American Heart Association Scientific Sessions 2013”, “Activation of the kynurenine pathway in patients resuscitated from ventricular fibrillation out-of-hospital cardiac arrest”, 16-20 November, Dallas, Texas, USA
Meeting: “European ProteOn™ System User Meeting - Joining Information”, “SPR-based Scanning of Native Brain-extracted Prion Protein”, 19 November, Vienna, Austria

Symposium: “24th International Symposium on ALS/MND”, “Inhibition of extracellular cyclophilin A as a possible therapeutic target for ALS”, “Cyclophilin A interaction network perturbation is a converging patho-mechanism in different forms of amyotrophic lateral sclerosis”, 6-8 December, Milano, Italy

GRANTS AND CONTRACTS

Agenzia Italiana del Farmaco, Rome, Italy
Alzheimer’s Association, USA
Associazione Italiana Ricerca sul Cancro (AIRC), Milan, Italy
Biotecnologie BT - Perugia, Italy
Comunità Europea (EU), Bruxelles, Belgium
Consiglio Nazionale delle Ricerche (CNR), Palermo, Italy
Fondazione Don Gnocchi, Milan, Italy
Fondazione Cariplo, Milan, Italy
Fondazione Italiana di Ricerca per la Sclerosi Laterale Amiotrofica (AriSLA), Milan, Italy
Fondazione Mariani, Milan, Italy
Fondazione Monzino, Milan, Italy
Fondazione Weizmann-Pasteur-Negri, Paris, France
Indena, Milan, Italy
Istituto Auxologico Italiano, Milan, Italy
Istituto Nazionale Neurologico "C. Besta", Milan, Italy
Lundbeck A/S, Copenhagen, Denmark
Ministero della Salute, Roma, Italy
Ministero dell'Istruzione, Università e Ricerca Scientifica (MIUR), Rome, Italy
North Shore University Hospital, NY, USA
Perfetti-Van Melle, Lainate, Milan, Italy
Sigma Tau, Pomezia, Rome, Italy
Società Autostrade per l’Italia, Milan, Italy
Telethon, Milan, Italy
Università di Firenze, Italy
Università di Milano-Bicocca, Italy
Università di Siena, Italy

Zambon Group, Bresso (Mi), Italy

SCIENTIFIC PUBLICATIONS (2013)

Fluorescent amyloid β-peptide ligand derivatives as potential diagnostic tools for Alzheimer's disease
Pure Appl Chem E-pub: 2013

Mutant copper-zinc superoxide dismutase (SOD1) induces protein secretion pathway alterations and exosome release in astrocytes. Implications for disease spreading and motor neuron pathology in amyotrophic lateral sclerosis
J Biol Chem 2013 288: 15699-15711
Beeg M, Diomede L, Stravalaci M, Salmona M, Gobbi M
Novel approaches for studying amyloidogenic peptides/proteins
Curr Opin Pharmacol 2013 13: 797-801

A mutant prion protein sensitizes neurons to glutamate-induced excitotoxicity
J Neurosci 2013 33: 2408-2418

In vivo fate of Avidin-Nucleic Acid Nanoassemblies as multifunctional diagnostic tools
ACS Nano E-pub: 2013

Borgo F, Diomede L
The nematode Caenorhabditis elegans as an innovative tool for studying foodborne metabolites and emerging pathogens in the food industry
Nutrafoods E-pub: 2013

Cimini S, Rizzardin M, Biella G, Cantoni L
Hypoxia causes autophagic stress and derangement of metabolic adaptation in a cell model of amyotrophic lateral sclerosis
J Neurochem E-pub: 2013

Biocompatible fluorescent nanoparticles for in vivo stem cell tracking
Nanotechnology 2013 24: 245603

Diomede L, Rigacci S, Romeo M, Stefani M, Salmona M
Oleuropein aglycone protects transgenic C. elegans strains expressing Aβ42 by reducing plaque load and motor deficit
PLoS One 2013 8: e58893

An N-terminal fragment of the prion protein binds to amyloid-β oligomers and inhibits their neurotoxicity in vivo
J Biol Chem 2013 288: 7857-7866

New insights into the molecular mechanisms underlying sensitivity/resistance to the atypical retinoid ST1926 in acute myeloid leukaemia cells: The role of histone H2A.Z, cAMP-dependent protein kinase A and the proteasome
Eur J Cancer 2013 49: 1491-1500

6-methoxy7-benzofuranoxy and 6-methoxy-7-indolyloxy analogues of 2-[2-(2,6-dimethoxyphenoxy)ethyl]aminomethyl-1,4-benzodioxane (WB4101): discovery of a potent and selective α1D-adrenoceptor antagonist
J Med Chem 2013 56: 6402-6412

Garattini E, Terao M
Aldehyde oxidase and its importance in novel drug discovery: present and future challenges
Expert Opin Drug Discov 2013 8: 641-654
Gescher A, Gobbi M
Pharmacology in the high tech age - new developments, opportunities and limitations
Curr Opin Pharmacol 2013 13: 775-777

Structure and evolution of vertebrate aldehyde oxidases: from gene duplication to gene suppression.

Lesma G, Cecchi R, Cagnotto A, Gobbi M, Meneghetti F, Musolino M, Sacchetti A, Silvani A
Tetrahydro-β-carboline-based spirocyclic lactam as type II' β-turn: application to the synthesis and biological evaluation of somatostatine mimetics
J Org Chem 2013 78: 2600-2610

A mouse model of familial ALS has increased CNS levels of endogenous ubiquinol9/10 and does not benefit from exogenous administration of ubiquinol10
PLoS One 2013 8: e69540

Markoutsa E, Papadia K, Giannou A, Spellia M, Cagnotto A, Salmona M, Statopoulos G T, Antimisiaris S G
Mono and dually decorated nanoliposomes for brain targeting, in vitro and in vivo studies
Pharm Res E-pub: 2013

Serological Proteome Analysis (SERPA) as a tool for the identification of new candidate autoantigens in type 1 diabetes
J Proteomics 2013 82: 263-273

Benefit of doxycycline treatment on articular disability caused by dialysis related amyloidosis
Amyloid 2013 20: 173-178

Moser J M, Bigini P, Schmitt-John T
The wobbler mouse, an ALS animal model
Mol Genet Genomics 2013 288: 207-229

The GTPase-activating protein RN-tre controls focal adhesion turnover and cell migration.
Curr Biol. 2013 23: 2355-64

Selective nanovector mediated treatment of activated proinflammatory microglia/macrophages in spinal cord injury
ACS Nano 2013 11: 9881-9895

Transglutaminase 2 transamidation activity during first-phase insulin secretion: natural substrates in INS-1E
Acta Diabetol 2013 50: 61-72
RESEARCH ACTIVITIES

Laboratory of Biochemistry and Protein Chemistry

Development of new therapeutic strategies for the treatment of central and peripheral amyloidosis

The development of an effective strategy for the prevention and cure of Alzheimer disease and systemic amyloidosis is of great importance due to the absence of an effective therapy. The severity of the affects seriously impacts the lives of patients and their relatives. The formation of amyloid fibrils and their deposition in specific tissues were for a long-time considered the cause of the disease, however recent studies showed that soluble oligomeric species are the actual culprits of the toxicity. The kinetics of protein aggregation due to conformational modifications and the comprehension of genetic, biochemical and structural determinants at the basis of this transformation are very important for unveiling the pathogenic process and the development of therapeutic strategies. Aiming at developing simple models that enable monitoring of the conformational changes that preceds fibril deposition, we have designed and developed a variety of synthetic peptides as deduced...
from the primary sequence of human amyloidogenic proteins in their wild-type or mutated forms.

In collaboration with the Istituto Neurologico “Carlo Besta” of Milan we have identified a mutated form of beta-amyloid (A673V) that displays amazing biological features since it binds to wild-type beta-amyloid and inhibits amyloid formation and the onset of the disease. This observation opens new therapeutic perspectives both for genetic and sporadic forms of Alzheimer disease based upon the use of protein fragments containing this mutation or peptide-mimetic compounds. Moreover, we have synthesized several Abeta peptides containing the same mutation and we have evaluated its importance in the aggregation and amyloidogenic properties. Similar studies have been carried out with prion proteins and some amyloidogenic proteins responsible of peripheral amyloidosis. The first approach for the development of candidate drugs contemplates the development of molecules capable to interfere with oligomeric species following direct interaction with protein molecules disrupting its beta-sheet conformation or the fibrillar aggregates. This activity requires in vitro studies with cell free models to determine the conformational features of amyloidogenic peptides, their secondary structure, the hydrogen-deuterium exchange, the resistance to digestion by proteases, the aggregation propensity and amyloidogenic characteristics.

To understand the molecular and biochemical mechanisms of action underlying the cause of the cytotoxic action, peptides are used for in vitro studies in variety of cellular models trying to correlate their physical features and the biological effect. Moreover, the subcellular distribution of peptides and their molecular targets are also investigated. We have reported that tetracyclines are new candidates as anti-amyloidogenesis drugs, in particular they disrupt amyloid tangles and increase the sensitivity of PrP to proteinase K digestion. Tetracycline are able to inhibit neuronal cell death and astroglial proliferation induce by PrP peptides and, in animal model of disease, they prolong the survival of animals inoculated with PrP.

The use of nanoparticles in pharmacology: new systems for the diagnosis and therapy

The Laboratory of Biochemistry and protein chemistry has developed in the last years a large number of approaches to investigate the interaction between biological matrices and nanoparticles in different areas of Research, with great emphasis on the diagnosis, therapy and toxicology. These studies stem from the evidence that the clinical development of molecules with promising therapeutic activity for the treatment of diseases with poor prognosis is often limited by problems related to their poor bioavailability, the rapid clearance, the difficulty to cross biological barriers and, last but not least, the risk to generate serious side-effects. The understanding of the behavior of nanocarriers in biological systems at an increasing level of complexity (biological fluids, cells, healthy and pathological model of human disorders) is prerequisite for therapeutic development before drug loading and/or conjugation with contrast agents. In a project financed by the Italian Association for Cancer Research (AIRC 5x1000) an integrated platform was developed to verify and categorize the potential and risks of polymeric nanoparticles against triple negative breast cancer. In collaboration with the Department of Oncology and the Polytechnic of Milano, a series of biocompatible nanoparticles with different profiles biodegradability were tested in both cellular and animal models of this disease. The results obtained from that this combined series of experiments significantly contributed to select the most promising chemical-physical parameters (material , size, external charge, degree of pegylation) for the next steps aimed at developing nanomedicines after conjugation with the therapeutic molecules of interest.

This kind of approach has been recently extended to develop and characterize new nanomaterials with very high degree of biocompatibility to favor the loading and the release of therapeutic agents or contrast agents for therapy and early diagnostic evaluation in oncology,
cardiology, neurology and cardiovascular diseases. In collaboration with Dr. Margherita Morpurgo (University of Padua) our group characterized a nanoparticle with a pharmacokinetic profile extremely innovative and potentially able to give a significant boost to the diagnostic and drug therapy in humans. This nanoparticle stems from the original idea of linking the two proteins abundantly expressed in the egg (avidin & biotin) to a back-bone composed of inactivated nucleic acids in order to create a structure easily adaptable in terms of conjugation with many molecules of therapeutic interest, and highly tunable in terms of stoichiometry of binding. The analysis of the interaction of these Avidin - Nucleic Acid Nanoassemblies (ANANAS) in biological fluids, cells and tissues (after intravenous administration in healthy animals) confirmed the huge potential for their future development as efficient transporters of molecules for diagnosis and therapy in many areas of research.

In collaboration with the group of Professor Masserini (University of Milan Bicocca) and Professor Futermann (Weizmann Institute, Israeli) we are characterizing nanoparticles to enable the passage through the blood brain barrier (BBB) of therapeutic enzymes already approved for the treatment of lysosomal storage disorders. In particular, our group is involved on the optimization of the loading of the enzyme glucocerebrosidase (that is mutated in children with Gaucher Disease) to liposomes already functionalized with peptides that improve the connection with the endothelia of brain vessels. This project is tightly connected with important collaborations developed within the European project "Nanoparticles for therapy and diagnosis of Alzheimer's Disease (NAD 2015)." In this multicenter study, our laboratory is evaluating the ability of different polymeric nanoparticles, and liposomes to overcome the blood-brain barrier to deliver anti-amyloidogenic drugs in the brain.

**Preclinical imaging to facilitate the process of translational from the animal to the clinic in the field of cell therapy**

The ability to standardize the criteria for preclinical and clinical analysis is one of the main objectives of pharmacological research. The non-invasive diagnostic imaging (MRI, ultrasound, CT) takes on increasing importance in many clinical settings. This spread has generated great development of non-invasive screening tools dedicated to preclinical studies. The Department of Biochemistry and Molecular Pharmacology is developing a series of combined procedures to correlate the information obtained from non-invasive imaging tools with the data obtained by traditional methods of histopathology and immunohistochemistry. The integration among these different, but highly complementary, techniques can be identified with the term "preclinical imaging".

In close association with the activities described above, our group of preclinical imaging has developed and optimized a number of protocols to the analysis of longitudinal tracking of stem cells by preincubation with biocompatible nanoparticles properly functionalized with fluorescent contrast agents. In particular we focus our attention on the fate of stem cells in mice depending on: 1) the type of cell injected ; 2) the number of cells ; 3) the route of administration (intravenous – IV-, intracerebroventricular – ICV- ); 4) the frequency of administration; 5) the time from administration; 6) the status of the experimental subject (healthy or diseased animal). This study is crucial to design the potential and the risk of stem cells for human therapy. Through analysis of magnetic resonance imaging (MRI), we evaluated biodistribution, persistence, accumulation of human amniotic mesenchymal cells and muscle. To be tracked, cells were previously incubated with superparamagnetic iron oxide nanoparticles (SPION) and then administered in mice in the lateral ventricles. We have compared the behavior of cells in both healthy mice and specific models of motor neurodegenerative disorders. This study is crucial to hypothesize the possible interaction of cells in future clinical trials in patients with amyotrophic lateral sclerosis (ALS). Our studies have shown that the cells remain within the ventricular system for at least 4 weeks, do not proliferate, do not migrate into the brain parenchyma but diffuse into the cerebrospinal fluid also in the areas contiguous to the area pathological (the brainstem and the cervical region of the spinal cord). This study of traceability
enableds to give greater substance to the encouraging results of pharmacological effects, carried out in collaboration with Dr. Canzi (Neurologico Carlo Besta Institute) and Dr. Bendotti of the Department of Neuroscience. Also in the context of a multicenter collaboration we have developed an original method of tracking fetal stem cells by internalizing biocompatible polymeric nanoparticles linked to a fluorophore. In this case cells were tracked by fluorescence molecular tomography (FMT). FMT is a non-invasive technique complementary to MRI. In particular, we evaluated the fate of cells of the wall of the umbilical cord in a other model of ALS (the SOD-1 G93A) after IV or ICV respectively.

Our results suggest that: 1) the use of these new fluorescent nanoparticles as cell markers, is a valid and reliable system for tracking cell; 2) the implanted cells have a similar response, in terms of location and permanence in the tissues; 3) the injected cells by ICV stay for a longer time, but remain confined to the ventricular whereas cells injected systemically migrate almost selectively in the lung but, in some small part, reach the brain areas. These results represent a reliable starting point for planning a study of efficacy and, possibly, to respond to potential or limits of the processing.

We have recently developed a third type of analysis that will take advantage of dual nanoparticles with a fluorescent component and a paramagnetic contrast agent. Nowadays our group is testing the possible interaction between the particles and the stem cells (to rule out, as it did for the two previous studies, the possible toxicity of nano- particles to be injected into the cell). We are aimed at combining MRI to FMT to merge the resolution of the first method with the sensitivity of the second. This will likely enable us to reduce the study to intermediate times by histological analysis and therefore in compliance with the guidelines of the European community on the need to reduce the use of animals used in research (3R rule). In addition, this technique, if possible, could provide a method of inquiry easily translatable to clinical practice.

The nematode Caenorhabditis elegans as experimental model to investigate in vivo the molecular mechanisms underlying the aggregation of amyloidogenic proteins

The description of the molecular events underlying the in vivo amyloidogenesis is crucial for the design of effective therapeutic strategies. To this end, in our laboratory we use Caenorhabditis elegans as experimental model since it offers the unique opportunity to analyze the genetic and molecular functions of human disease-related genes in vivo. In particular, using this system, it is possible to correlate the phenotype of the transgene with the disease insurgence, the degeneration, the protein expression and its aggregation into the oligomeric or fibrillar forms.

Different transgenic strains expressing various fragments of human β amyloid (Aβ) in neurons or in muscles are available in our laboratory. We also developed new transgenic strains expressing Aβ A-V or A-T mutated peptides in position 2 under a neuronal promoter, to evaluate for the first time, the in vivo effects. The expression of these peptides results in the cytoplasmic inclusion and in the appearance of specific phenotypes, such as the progressive paralysis. The amyloid aggregates observed in worms are similar to those observed in the brain of patients with Alzheimer’s disease or in muscles of patients with sporadic forms of Inclusion Body Myositis, the most common myopathy. These models were already used to study the relationship between protein sequence, the kinetic of amyloid formation and toxicity. A transgenic C. elegans strain producing only the oligomeric form of the Aβ protein was also available representing a good predictive model for the investigation of drugs specifically interfering with oligomers. C. elegans is also applied to investigate the molecular mechanisms underlying some systemic amyloidosis, like those caused by tissue deposition of immunoglobulin light chains or β2 microglobuline. Using this multidisciplinary genomic and molecular integrated approach, we will obtain important information for the development and validation of innovative therapeutic
strategies and for the comprehension of the in vivo molecular functions of genes related to human amyloidosis.

In addition, we are generating new transgenic worms expressing different isoforms of tau protein, which is involved in the insurgence of Alzheimer’s disease and frontotemporal disease (FTD), an heterogeneous group of neurodegenerative diseases called tauopathies. Tau play a crucial role in regulating the dynamic and morphogenesis of neuronal microtubules of the central nervous system and is fundamental for the regulation of axonal transport and neuronal function. In the last years we demonstrated that some mutations of tau can affect the pathogenesis of FTD and that these alterations are mainly due to defects in the protein’s ability to interact with microtubules and form insoluble aggregates. In collaboration with the Istituto Neurologico “C. Besta” of Milan, we performed a genetic screening on patients suffering from FTD and identified new tau mutations. These protein, were expressed in bacteria, purified and the biochemical and biophysical studies indicated that the presence of mutations specifically increased the ability of tau to promote microtubules polymerization and oligomers’ formation. The generation of transgenic *C. elegans* strains expressing the wild type or the mutated tau proteins will offer an innovative system for the investigation of the neurotoxic properties of these mutated tau and the elucidation of the pathogenetic mechanisms underlying tauopathies.

**Laboratory of Molecular Biology**

**The family of molybdo-enzymes**

Molybdo-enzymes are proteins requiring a molybdo-pterin cofactor (molybdenum-cofactor, MoCo) for their catalytic activity. Until a few years ago, it was believed that the family of molybdo-enzymes consisted only of three members: sulfite oxidase, aldehyde oxidase and xanthine oxidoreductase. In the last few years of research, our laboratory has determined the structure of the genes coding for different molybdoenzymes in rodents and humans. In particular, we demonstrated that rodents are endowed with four different aldehyde oxidase (AOX1, AOX3, AOX4 and AOX3L1) characterized by remarkable structural and functional similarity. The physiological substrate(s) and the physiological function(s) of this group of protein have not yet been identified, although it is known that aldehyde oxidases can oxidize aliphatic and aromatic aldehydes into the corresponding carboxylic acids and to hydroxylate different types of n-heterocyclic aromatic rings. The four different aldehyde oxidases of rats and mice are the product of an equivalent number of genes located at the short distance one from the other on the same chromosome. These genes originated through a number of a synchronous gene duplication events. Our studies aimed at the determination of the evolutionary processes underlying the development of the genes coding for aldehyde oxidases allowed us to establish that the natural history of this gene family is made of duplication and suppression events. These evolutionary processes resulted in the presence of variable number of aldehyde oxidases in different genomes. Man is characterized by the presence of a single active gene (AOX1) and two inactive pseudo genes clustered on chromosome 2. In the last years we have focused on the functional definition of the different mouse aldehyde oxidases and our long term aim is to establish the reasons underlying the disparity in the number of these enzymes between humans and rodents. To this purpose, we generated two knockout animals for the AOX4 and AOX3L1 genes. The AOX4 knockout mouse was characterized phenotypically demonstrating minimal alterations of the epidermis. Indeed, the AOX4 knockout animal shows epidermal hypertrophy, which is associated with a peculiar fragility of the corneal layer. At the biochemical level, we observed a deficiency in the synthesis of retinoic acid in the two organs where AOX4 is present in significant amounts (skin and Harderian glands). This observation is in line with the idea that AOX4 may have a role in the metabolism of
retinaldehyde to retinoic acid, the active metabolite of vitamine A. Recently we gathered novel data indicating a role for AOX4 in the control of the adipose tissue homeostasis. The observation is of particular importance also in man as human AOX1 seems to exert a similar effect in the synthesis and deposition of lipids. Currently we are performing similar studies in a knockout mouse for AOX3L1.

**Retinoids in the treatment and chemoprevention of myeloid leukemia and mammary carcinoma**

Our laboratory has a long standing interest in defining the therapeutic potential of natural and synthetic derivatives of retinoic acid, the active metabolite of vitamin A. These compounds, commonly defined as retinoids, are characterized by cyto-differentiating, anti-proliferative and apoptotic effects which are at the bases of their therapeutic activity in the context of myeloid leukemia and mammary carcinoma. Retinoids are very active therapeutic agents, although they are endowed with dose limiting side effects, particularly chronic administration. A rational clinical use of retinoids calls for a better knowledge of the mechanisms of action underlying the anti-neoplastic action exerted by these compounds. In-depth knowledge is of fundamental value for the design of novel retinoid-based treatment strategies characterized by increased therapeutic index. We have a long-standing interest in the definition of the molecular mechanisms regulating the activity of retinoic acid nuclear receptors, as they may lead to the identification of pharmacological targets to be modulated in a specific manner. Indeed, we believe that knowledge in this field may lead to the development of rational combinations between retinoids and other pharmacologically active agents to be used in the treatment of different tumor types. Such an approach has led us to the recent identification of the prolyl-isomerase, PIN1 as a negative regulator of the retinoic acid receptor, RARα. Pharmacological inhibitors PIN1 proved to be particularly effective in sensitizing the leukemic cell to the anti-neoplastic activity of retinoids. These results open up the possibility to develop combinations based on PIN1 inhibitors and retinoids for the treatment of acute myeloid leukemia. Following the same type of logic, we have recently demonstrated that the inhibition of the microRNA, miR21 in mammary carcinomas positive for estrogen receptor is of the utmost importance in potentiating the anti-proliferative activity of retinoids in this particular type of tumor. Finally, we observed that the peculiar subgroup of mammary cancer positive for HER2 may benefit from retinoid-based treatment or associations between retinoids and inhibitors of HER2 receptor tyrosine kinase activity.

Currently, we are conducting a series of studies aimed at defining the cellular and molecular determinants of the sensitivity/resistance to retinoids operating in breast carcinoma, using an approach which integrates the high-throughput genomic methodologies and the molecular pharmacology of retinoids. To this aim, we are in the process of defining the gene-expression profiles of retinoid responses in a panel consisting of more than 40 breast carcinoma cell lines characterized for basal profile of gene-expression, gene copy number variations (CNV) and the presence of genetic polymorphisms. In addition, we have set up an in vitro methodology for the short-term incubation of tissue slices obtained from surgical samples deriving from patients suffering from different types of breast cancer.

**Laboratory of Pharmacodynamics and Pharmacokinetics**

**Misfolding proteins and related diseases**

One of the laboratory’s main research fields regards the diseases associated with protein “misfolding”, i.e. the formation of aberrant tertiary conformations of proteins or peptides,
as a consequence of mutations, stress or aging. Besides the loss of the protein’s physiological properties, the misfolding often results in new biochemical properties, particularly the propensity to aggregate and form amyloid-like deposits. We are particularly interested in Alzheimer’s disease (AD), in which there is aggregation of amyloid-β (Aβ) peptides (Aβ1-40 and Aβ1-42, detectable in the amyloid plaques typical of AD brain), and in spongiform encephalopathies, due to misfolding and aggregation of the prion protein (PrP). Recent studies suggest that misfolding and the consequent propensity to form toxic aggregates is common to different proteins and results in different diseases (e.g. alpha-synuclein for Parkinson disease, poly-Q expansions for Huntington disease, superoxide dismutase in amiotrophic lateral sclerosis, transthyretin in systemic amyloidosis). Better knowledge of the molecular and cellular mechanisms involved in these events is needed for the development of useful therapeutic strategies.

Our activities are mainly dedicated to the analysis of the aggregation features of different proteins, in different experimental conditions, with the final aim to identify/develop compounds interfering with the formation of toxic assemblies. For that, we use different approaches including in silico computational simulations, in vitro chemical-physical and biochemical techniques and some in vivo studies in collaboration with other groups (in particular studies in C. elegans with Dr. L. Diomede of the “Biochemistry and Protein Chemistry” lab). As regards in vitro studies, in particular, we obtained interesting results by using Surface Plasmon resonance (SPR), a well known and a powerful method to study molecular interactions. Thus, we have developed SPR protocols to analyze the polymerization kinetics of PrP or Aβ1-42 amyloid fibrils, or for a specific recognition of toxic Aβ oligomers. These protocols have been conveniently applied to evaluate the effects of mutations, for screening molecules with potential anti-amyloidogenic activities, or for investigating potential binding targets of aggregated species, enabling, for example, to describe the interaction between Aβ1-42 oligomers and PrP. SPR has also been applied, within the European FP7 project “NAD” (Nanoparticles for Alzheimer’s Disease) to test functionalized nanoparticles for their binding to Aβ assemblies. Nanoparticles may conveniently carry drugs and/or imaging agents at the site of interest (e.g. Aβ aggregates), thus representing new potential diagnostic and therapeutic opportunities.

A number of observations suggest that the neuronal degeneration observed in some misfolding diseases is preceded by subtle cellular dysfunctions of synaptic transmission (synaptopathies), in many cases caused by the toxic oligomeric aggregates. In collaboration with the “Neurobiology of Prions” lab (Dr. R. Chiesa), and using biochemical methods to study neurotransmitter’s release and uptake, we have recently found that the expression of a mutant PrP (PG14) impairs voltage-gated calcium channels and, in turn, glutamatergic transmission in mice cerebellum.

We are also involved in other projects related to misfolding diseases, in charge of the analytical determination of drugs levels in biological samples (e.g. plasma or brain tissues), after in vivo treatments. Within the NAD project (see above), we evaluate in mice the pharmacokinetic profile of the molecules carried by nanoparticles, looking in particular at the passage of the blood-brain- barrier. The laboratory is also a partner in an integrated project (PHARMACOG, IMI) aiming to develop and validate new strategies for the identification of effective therapies for AD. Our task, in particular, is to analyse the pharmacokinetics of old and new potential anti-AD drugs, to be integrated with the pharmacodynamic properties and the drug’s activities in new preclinical and clinical models.

Finally, we are also in charge of the pharmacokinetics studies included in clinical trials, coordinated at the Istituto Neurologico Besta (Milan) and Ospedale San Matteo (Pavia), aiming at evaluating the effects of doxycycline for the treatment of Creuzfeldt-Jacob diseases (PrP disease) or peripheral amyloidosis (dialysis-related or transthyretin-related amyloidosis).
Q10 coenzyme in amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease involving brainstem and spinal cord motoneurons, leading to complete paralysis of skeletal muscles and early death, usually from respiratory failure. Mutations in the copper-zinc superoxide dismutase (SOD1) gene are responsible for some forms of familial ALS, and on this basis transgenic rodents have been generated, contributing significantly to our understanding of the pathogenic mechanisms of the disease. For example, transgenic mice carrying the mutant SOD1, and showing motoneuron degeneration, have increased oxidative stress associated with mitochondrial damage. The Q10 coenzyme (CoQ10, or ubiquinone) is a major component of the mitochondrial respiratory chain, and its reduced form ubiquinol has antioxidant properties. We have therefore investigated whether there is a correlation between plasmatic/CNS CoQ10 levels and the disease progression in mutant SOD1 transgenic mice, and the effects of a chronic treatment with ubiquinol in the same mice. These studies have been carried out in collaboration with the Laboratory of Molecular Neurobiology (Dr. C. Bendotti).

Glutamatergic and serotoninergic neuropharmacology

Our laboratory has long experience in neuropharmacological studies, particularly on the glutamatergic and serotoninergic neurotransmission systems, and the instrumentation to analyze the main mechanisms of synaptic transmission (neurotransmitter release, binding to receptors and reuptake by specific transporters). On this ground we have different collaboration with academic Chemico-Pharmaceutical departments for the characterization of new synthetic molecules acting on these mechanisms. Moreover, we have collaborated with the laboratory of Experimental neurology (Dr. A. Vezzani), studying the expression and localization of glutamatergic NDMA receptors during epileptogenesis.

Nanotechnologies

Nanotechnologies represent one of the main research endeavors of the 21st century, with potential applications in many fields. With regard to biomedical applications, great interest is currently being devoted to the development of nanoparticles (NPs) as suitable carriers for imaging probes and therapeutic agents. We are applying our analytical expertise to evaluate the *in vitro* kinetics of the release of compounds from nanoparticles, and to evaluate the pharmacokinetic and biodistribution profile of the carried molecule after in vivo treatment, in particular for the passage of the blood-brain-barrier. We have also developed new approaches, based on Surface Plasmon Resonance (SPR), for rapid and quantitative analyses of the interaction between NPs—functionalized with specific ligands—and their putative biological targets. Moreover, we showed that SPR can provide important details on the formation and the role of the protein “corona”, *i.e.*, the protein layer which coats NPs once they come into contact with biological fluids. These novel applications of SPR sensors may be very useful to characterize, screen and develop nanodevices for biomedical purposes.

Molecular interactions

SPR, an advanced technique specifically developed for the study of molecular interactions, enables us to contribute to different projects in collaboration with other laboratories. In particular, one of these projects, carried out in collaboration with the Inflammation and Nervous System Disease Laboratory (Dr. M.G. De Simoni) and the Department of Organic and Industrial Chemistry, University of Milan (Dr. A. Bernardi) is investigating the hypothesis that MBL play a role in ischemia-induced damage, and that MBL inhibitors might have significant anti-ischemic effects. Studies include the synthesis of new potential MBL ligands,
the evaluation of their ability to interact with MBL in vitro (through SPR studies in our laboratory) and their anti-ischemic effects in vivo.

We are also collaborating with laboratories of the “Mario Negri ” Institute in Bergamo (Dr. M. Morigi and Dr. M. Noris), for studies regarding new molecular mechanisms involved in the haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). We are using SPR to investigate whether new protein-protein interactions may contribute to the link between thrombosis and complement cascade activation.

The laboratory is a partner in a multicentre project entitled “Miniaturized System for Molecular Diagnostic and Proteomic of Sepsis Based on Integration of Surface Plasmon Resonance”, aiming at identifying new biomarkers of sepsis and exploiting new SPR systems as low cost and rapid diagnostic tools. Our laboratory is in charge of the identification and validation of suitable biomarkers, measuring them in plasma with our conventional SPR system.

**Laboratory of Molecular Pathology**

**In vitro models for investigating motor neuron pathologies**

Mutant forms of specific proteins play a key role in many neurodegenerative diseases. Experimental models in vivo and in vitro are sorely needed to study the effects of these toxic proteins. The motor neuronal cell line NSC-34, a widely used model to study motor neuron degeneration, is available in the laboratory. We have applied the pTet-Off system to control gene expression through the level of tetracyclines to the NSC-34 cell line establishing a new cell line (NSC-34 tTA40) that stably expresses the transactivating protein tTA. This cell line is suitable to study the pathogenic mechanisms of motor neuron diseases after transient/stable transfection with genes of interest for these pathologies.

The NSC-34 and the NSC-34 tTA40 cell lines were used to obtain in vitro models to study the pathogenic mechanisms of amyotrophic lateral sclerosis (ALS). Mutant forms of superoxide dismutase 1 are responsible for some of the familial forms of ALS. We developed NSC-34-based cell lines expressing constitutively or conditionally human G93A mutant superoxide dismutase 1 (G93ASOD1).

**Novel intracellular targets in the selective degeneration of motor neurons in amyotrophic lateral sclerosis**

Amyotrophic lateral sclerosis (ALS) is a rapidly fatal neurodegenerative disease characterized by loss of motor neurons. The management of this disease remains essentially supportive and symptomatic. Understanding the mechanisms underlying the disease is a way to favor more efficient therapeutic strategies. We utilized our cell models to investigate the biochemical-molecular mechanisms underlying the alterations of mitochondrial morphology observed in the early stages of the disease in the motor nerve terminals of ALS patients and in the murine models of the disease. We showed that motor neurons are selectively susceptible to mitochondrial damage induced by a mutant form of human superoxide dismutase 1 (G93ASOD1) and that this damage was modulated by the extent of expression of the mutant protein.

Furthermore the expression of G93ASOD1 protein increased the susceptibility of motor neurons to inhibitors of the electron transport chain (ETC) and to oxidants. Exposure to drugs or exogenous compounds impairing the ETC could thus be a risk factor to motor neurons of individuals carrying mutant superoxide dismutase 1.

We have shown that in motor neuronal cells the activity of glutamate cysteine ligase, the rate limiting enzyme for the synthesis of glutathione, the main cellular antioxidant, was
modulated by the level of G93ASOD1. A higher expression level of mutant SOD1 produces a decrease of glutathione level. This effect is associated to a lower level of glutamate, an amino acid which is a precursor of glutathione and a neurotransmitter. Furthermore the glutamine/glutamate mitochondrial metabolism is impaired and this evidentiates a new aspect of mitochondrial damage due to mutant SOD1.

A variation in the level of glutathione may influence the formation of nitrated proteins, a pathogenic mechanism in ALS, which was investigated in collaboration with the laboratory of Translational Proteomics.

**Cytochrome P-450 superfamily**
Cytochrome(s) P-450 have evolved into a large superfamily which plays a major role in the metabolism of drugs and other chemicals. The majority of existing drugs depends on the P-450 system for terminating their biological effects or for side effects or adverse reaction. The laboratory has a long-standing interest in the induction/degradation mechanisms of specific cytochrome P-450 families due to drug administration or to disease states.

**Activation of enzymes of the heme metabolic pathway (heme oxygenase system, biliverdin reductase) as a protective response to stress**
The enzymatic system of heme oxygenase (HO) is devoted to cellular degradation of heme containing molecules, like cytochromes and hemoglobin, and to recycling of iron. Products formed by the catalytic activity of HO - carbon monoxide and bile pigments - are important regulating factors in the cell. An increase of HO activity (which is usually sustained by activation of the inducible form HO-1) is now considered a protective mechanism against untoward stimuli particularly when oxidative stress is involved. In the past, the laboratory of Molecular Pathology identified cytokines as inducers of HO activity and as transcriptional activators of the HO-1 gene. We are currently investigating the functional significance of HO-1 activation in neurodegeneration.

**Laboratory of Translational Proteomics**
Identification of protein biomarkers of ALS in peripheral blood mononuclear cells (PBMC) of patients and a rat model.
A biomarker is a molecule that underlines the physiological or pathological state of an organism. A disease biomarker is potentially an important tool in clinical studies because it can support prompt diagnosis, monitor disease progression and help to evaluate the efficacy of any new therapy. Proteins, the most desirable biomarkers, can help in identifying the molecular mechanisms at the basis of the disease and therefore support research in developing new and more effective therapeutic approaches. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motor neurons, the cells that control movement. Generally there is a progressive loss of the ability to control voluntary movement up to respiratory muscle paralysis and death. To date for ALS there is no effective therapy. Moreover, there is no test or procedure to ultimately establish the diagnosis of ALS. It is through a clinical examination and series of diagnostic tests, often ruling out other diseases that mimic ALS, that a diagnosis can be established. Therefore it would be important to identify validated biomarkers, i.e. biomarkers verified in a large population of patients and controls. The search of biomarkers for neurodegenerative diseases such as ALS it has been focusing principally in the cerebrospinal fluid (CSF). CSF, the fluid that surrounds the central nervous system and reflect its metabolic changes, is considered the source of excellence for biomarker discovery in neurological
diseases. Unfortunately, although the advancements in the analysis of proteins (proteomics), the analysis of CSF is still complex. Moreover, the withdrawal of CSF is highly invasive and not easily feasible in large-scale validation or longitudinal studies.

In collaboration with the Laboratory of Molecular Neurobiology and the Laboratory of Methodology for the Biomedical Research at the Mario Negri Institute, “Fondazione Salvatore Maugeri”, IRCCS, Milano, and NEuroMuscular Omnicentre (NEMO), Niguarda Ca’ Granda Hospital, Milano we are conducting a series of studies with the aim to identify biomarkers of ALS.

We look for biomarkers in peripheral blood mononuclear cells (PBMC), i.e. lymphocytes and monocytes, easily isolated from peripheral blood and easily analyzed by proteomics if compared with CSF. The rationale for this analysis is that ALS is now recognized as extending beyond motor neurons, so it can be regarded as a multi-cellular/multi-systemic disease. In particular, PBMC display traits of the disease such as down-regulation of Bcl-2, increased nitrative stress, intracellular calcium dysregulation and glutamatergic dysfunction, suggesting that they can be a useful source of disease biomarkers. By a two-dimensional difference in gel electrophoresis approach we identified a panel of protein biomarkers in PBMC that are closely associated with ALS, such as chloride intracellular channel protein 1 (CLIC1), heterogeneous nuclear ribonucleoprotein A2/B1 (ROA2), and tyrosine nitrate actin that can distinguish with a high discriminatory power ALS patients from healthy controls, interleukin-1 receptor-associated kinase 4 (IRAK4) and cyclophilin A (CypA) that can distinguish with a high discriminatory power ALS patients from other neurological disorders. We demonstrated also that CypA, protein disulfide isomerase A3 e TDP-43 associate with disease progression in a longitudinal study. Translational biomarkers, that link responses between human and animal model, are of particular interest because their role in the pathogenesis can be investigated in detail in the animal model where they can also offer important preliminary information for clinical trials. We found that CypA, CLIC1, tyrosine nitrate actin, glutathione S-transferase omega-1 and far upstream element-binding protein 1 are translational biomarkers since they are similarly regulated in ALS patients and in a rat model of ALS already at a presymptomatic stage of the disease, suggesting a possible involvement in pathways that trigger the disease. Further mechanistic studies in the animal models with these proteins are now warranted. We are planning to validate such PBMC candidate biomarkers in a large population of patients and controls by immunochemical methods.

Protein secretion pathway alterations and exosome release in astrocytes from a mouse model of ALS: Implications for disease spreading and motor neuron pathology

The mechanisms leading to the selective motor neuron vulnerability in ALS are still not known. The interplay between motor neurons and astrocytes seems to be crucial in the outcome of the disease. Astrocytes, the most abundant glial cell type in the central nervous system, are responsible for major protective functions for motor neurons, such as releasing trophic factors and limiting motor neuron firing by clearing glutamate from the synaptic cleft. However, astrocytes can also adopt an activated state that is becoming increasingly appreciated as contributing to ALS. We therefore compared the proteome of the astrocytes from mice overexpressing mutant copper-zinc superoxide dismutase (G93A SOD1), the best characterized mouse model of familial ALS, with those from mice overexpressing human wild-type (WT) SOD1. The goal was to identify altered pathways induced by the expression of the mutant protein that may contribute to the disease. We showed that overexpression of G93A SOD1 in primary astrocyte cultures is associated with decreased levels of proteins involved in secretory pathways. This is linked to a general reduction of total secreted proteins, except for specific enrichment in a number of proteins in the media, such as mutant SOD1 and valosin-containing protein (VCP)/p97. Because there was also an increase in exosome release, we could deduce that astrocytes expressing mutant SOD1 activate unconventional secretory pathways, possibly
as a protective mechanism. This may help limit the formation of intracellular aggregates and overcome mutant SOD1 toxicity. We also found that astrocyte-derived exosomes efficiently transfer mutant SOD1 to spinal neurons and induce selective motor neuron death. We conclude that the expression of mutant SOD1 has a substantial impact on astrocyte protein secretion pathways, contributing to motor neuron pathology and disease spread.

**Laboratory for the Study of Biological Systems**

System-level analysis of protein interactions in the epithelial junctional complex

Inter-cellular junctions form the apical junctional complex and mediate adhesion between adjacent cells, thus representing the cellular basis for tissue cohesion (for instance, the epithelial lining of the intestine). In order to acquire system-level understanding of the apical junctional complex, we have studied (using a methodological approach of ‘network analysis’) all the protein interactions that have been described at the junctions in epithelial cells of human origin. We also found that proper ‘hubs’ (i.e., very rare proteins with an exceedingly high number of interactions with other proteins) were absent from the junctional network. Nevertheless, we observed that the most connected (albeit non-hub) proteins were also essential proteins. In addition, we have detected modules within the junctional networks (i.e., densely inter-connected groups of proteins). Analysis of the modules has highlighted general organizing principles of the junctional complex, thus confirming the usefulness of network analysis for studying the components and the interactions of the cell.

**Laboratory of Signal Transduction**

Dissecting the complex interplay between ER redox homeostasis and muscle patho-physiology

Many studies have highlighted the connection between redox homeostasis and muscle physiology, and shown that disturbed redox signalling affects protein synthesis, folding and proteolysis in skeletal muscle. Moreover, redox changes in the endoplasmic reticulum (ER) have been associated with altered calcium handling and muscle dysfunction. However, because of the difficulties in handling redox, little is known about the molecular steps and components linking redox homeostasis and muscle physiology.

We have recently discovered that ER redox homeostasis is affected by the balance between the concentration of ROS (Reactive Oxigen Species) and the fast kinetics underlying oxidative protein folding, and have analysed the main oxido-reductases contributing to this homeostasis. Taking advantage of these findings, we would like to use an unprecedented multi-facetted approach in order to acquire a complete picture of the biological components of the complex relationship between redox homeostasis and muscle physiology. The main objectives of this proposal are: 1) to dissect the molecular mechanisms linking redox-sensitive proteins with muscle physiology by genetically manipulating the ER oxido-reductases; and 2) to modulate muscle redox function in vivo and test the impact of enhanced cell redox capacity on muscle performance and physiology.

Finally, the proposed study may bring to light new targets for pharmacological interventions for muscle diseases caused by an excess of ROS.
DEPARTMENT OF EPIDEMIOLOGY

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Carlo La Vecchia received his medical degree from the University of Milan and a Master of Science degree in Clinical Epidemiology from Oxford University. He is recognized worldwide as a leading authority in cancer etiology and epidemiology.

Work experiences: Presently, he is Chief of Epidemiology at the Mario Negri Institute in Milan, Italy and Professor of Epidemiology at the School of Medicine at the University of Milan. Dr. La Vecchia serves as an editor for numerous clinical and epidemiologic journals. He is among the most renowned and productive epidemiologists in the field with over 1,770 peer-reviewed papers in the literature and he is among the most highly cited medical researchers in the world, according to ISIHighlyCited.comsm, the developer and publisher of the Science Citation Index (H-index 105). Dr. La Vecchia is an Adjunct Professor of Medicine at Vanderbilt Medical Center and the Vanderbilt-Ingram Cancer Center and of Epidemiology at the University of Lausanne, CH.

Dr. La Vecchia is a temporary advisor at the International Agency for Research on Cancer IARC/WHO and at the World Health Organization in Geneva, and a registered journalist in Milan. He was Adjunct Associate Professor of Epidemiology at Harvard School of Public Health between 1996 and 2001.

Areas of interest: Dr. La Vecchia’s main fields of interest include cancer epidemiology and the risk related to diet, tobacco, oral contraceptive use and occupational or environmental exposure to toxic substances; and analysis of temporal trends and geographical distribution of mortality from cancer, cardiovascular diseases, perinatal and other selected conditions.

Eva Negri got a degree in Mathematics in 1985 at the University of Milan, School of Mathematics.

Work experiences: Since 2007: Laboratory Chief, Unit of Epidemiologic Methods, Department of Epidemiology; 1992-2006: Unit Chief, Unit of Epidemiologic Methods, Laboratory Epidemiology; since 1990-1992: Researcher at the Laboratory of Epidemiology; 1984-1990: Collaborator of the Laboratory of Epidemiology.

Areas of interest: Design, conduction and analysis of epidemiologic studies on chronic diseases (e.g. cancer and myocardial infarction) and injuries, analysis of mortality of cohorts of workers, analysis of temporal trends and geographic distribution of mortality from cancer, cardiovascular disease, injuries and other selected conditions, analysis of national health surveys, application of linear modeling techniques to the analysis of epidemiological data, collaborative re-analyses and meta-analyses of epidemiological studies.


Selected publications

Alessandra Tavani - degree in Biological Sciences, University of Milan, Italy (July 1977); Pharmacological Research Specialist, “Mario Negri” Institute for Pharmacological Research, Milan, Italy (July 1979).

Areas of interest: Epidemiology of cancer and coronary heart disease. Organization of case-control studies and cohort studies on cancer and coronary heart disease, including biological sample collection. Analyses of risk factors related to genetic factors and lifestyles, particularly coffee, diet, physical activity.


Selected publications


Eugenio Santoro

got his degree in Computer Science in 1990 at the Milan University. He started to work at the “Mario Negri” Institute in 1985 as a research fellow. He was Head of the Applied Statistics and Informatics Unit and of the Applied Statistics and Informatics laboratory, which was part of the Department of Cardiovascular Research. Since 2001 he is Head of the Laboratory of Medical Informatics that is currently part of the Department of Epidemiology. His main areas of interest have been biostatistics and clinical informatics with the development of software for data management and data analyses of large scale clinical trials in cardiology, such as the GISSI studies (Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardico). His main current area of interest is the Internet, and more recently the web 2.0, the social media, and their application in the medical field, in clinical research, and in medical education through the development of health related websites. He is author or co-author of more than 200 scientific papers published in peer reviewed journals, and of more than 70 scientific abstracts submitted to the main international meetings in the cardiology and in the computer science fields. He is also author of three books (available in Italian) about the use of the Internet in medicine ("Web 2.0 and medicine", “Guida alla medicina in rete” and “Internet in medicina. Guida all’uso e applicazioni pratiche”, published by the Pensiero Scientifico Editore, Rome) and of one section about Internet and medicine, included in one of the most important italian medical encyclopedia (“Enciclopedia Medica Italiana”, UTET 2007). He also collaborates to the publication of the Italian National Bioethics Committee’s guidelines about ethics, health, and the new information technologies.

Selected publications

Cristina Bosetti got her degree in Mathematics in 1994 at the University of Milan, School of Mathematics, and the Post-Graduate Diploma in Pharmacological Research in 1999 at the “Mario Negri” Institute for Pharmacological Research in Milan.

**Work experiences:** She is Head of the Unit of Cancer Epidemiology, Department of Epidemiology, IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri”, Milan since 2005. Previous work experiences include: Visiting scientist at “Life style and cancer group” of the International Agency for Research on Cancer (IARC), Lyon, France (Oct 2009); Collaboration with the “International Epidemiology Institute”, Rockville, MD, USA (2002-2009); Visiting scientist at the Unit of “Field and intervention studies”, IARC, Lyon, France (Sept. 2000/Jun 2001); Visiting scientist at the Department of Epidemiology, Harvard School of Public Health, Boston, MA (Sept-Nov 1998); Researcher at the Laboratory of Epidemiology, Istituto di Ricerche Farmacologiche “Mario Negri”, Milan (1998-2005); Researcher at the Laboratory of Mother and Child Health, Istituto di Ricerche Farmacologiche “Mario Negri”, Milan (1996-1997).

**Areas of interest:** Epidemiology of cancer, cardiovascular diseases and other chronic conditions. In particular case-control studies on cancers of the upper respiratory and digestive sites, thyroid, breast, hormone-related cancers, and on ischemic heart disease; analysis of risk related to diet, nutrition, alcohol, tobacco, diabetes, aspirin, occupational and environmental exposure to toxic substances, through the application of generalized linear models; meta-analysis and systematic reviews of the epidemiologic evidence on cancer risk in relation to various (environmental) exposures.

She authored/coauthored about 290 publications on peer-reviewed scientific Journals cited in PubMed/MEDLINE. Mean Impact Factor: 4.3. H-index: 44.

**Selected publications:**


**Areas of interest:** Epidemiological studies on obstetric diseases. Dermato-epidemiology. Cancer epidemiology (case-control studies on cancers of the breast, female genital tract). Analysis of temporal trends and geographical distribution of perinatal, infant mortality, cancer and other selected conditions (over 150 publications on these topics, 1993-2005).
Areas of interest: Dermatoepidemiologia, cancer epidemiology (case-control studies). Analysis of temporal trends and geographical distribution of perinatal mortality, cancer and other conditions.

Author / co-author of over 140 publications in peer-reviewed scientific journals listed in PubMed / MEDLINE. I.F. average: 2.9, excluding letters to the editor in journals with IF> 16: average IF = 2.4. H-index: 30 (Google Scholar or SCOPUS).

From 2007 to 2009, member of the Ethics Committee of the "Azienda ospedaliera Valtellina and Valchiavenna"

Selected publications

Silvano Gallus was born in Milan on the 20th of November 1970, and got his degree in Computer Science in 1999 at the University of Milan.

Work experiences: Chief of the Unit of Epidemiology for Clinical Research of the Department of Epidemiology (since 2006); computer analyst, graphic designer, and statistical and epidemiological consultant, Milan and Bergamo (since 2002); researcher at the Laboratory of Epidemiology (since 1997); creator, designer and webmaster of the website of one of a major Italian public hospital, Milan (1999-2002).

Areas of interest: Monitoring of prevalence and trends of smoking habit and obesity in Italy and Europe. Design, data managing, and statistical analyses of case-control studies on the associations between several risk factors (including in particular tobacco smoking, alcohol drinking and Mediterranean diet) and risk of cancer, coronary heart disease and several other conditions. Analyses of occupational cohort studies.

Since 2008, Dr Gallus is Associate Editor (Deputy Section Editor in 2010-2012) of the journal BMC Public Health, and is member of the editorial board of the following journals: The Open Obesiry Journal (since 2008), The Open Demography Journal (since 2009), World Journal of Gastrointestinal Oncology (since 2009), World Journal of Dermatology (since 2010).

He is referee for several journals, including BMJ, JAMA, JNCI and Tobacco Control.

In 2012 he received the European Research Advisory Board (ERAB) Publications Award.

He authored/coauthored more than 200 publications on peer-reviewed scientific Journals cited in PubMed/MEDLINE. H-index: 30 (Web of Knowledge).

Selected publications
Claudio Pelucchi got his degree in Statistical Science at the University of Milan-Bicocca, Italy, in 2003. Work experiences: Head of Unit of Analytic Epidemiology, Department of Epidemiology, “Mario Negri” Institute for Pharmacological Research (since 2011); Researcher at the Department of Epidemiology (1999-2010). Other work experiences: collaborations with the Institute of Pediatrics of the University of Milan, Italy (since 2006); with the Department of Traumatology, Orthopaedics and Industrial Medicine of the University of Turin, Italy (since 2003); with the International Prevention Research Institute, Lyon, France (2010-2011); with the European Society of Clinical Microbiology and Infectious Diseases (2009-2010).


Selected publications


ACTIVITIES

The Department of Epidemiology is involved in the epidemiology of several common cancers (including cancers of the breast, female genital tract, respiratory and digestive sites, prostate and urinary organs, sarcomas, lymphoid malignancies, etc.) and of cardiovascular diseases, both through a descriptive and an analytical approach. Among the activities of descriptive epidemiology are the analysis of temporal trends and geographical distribution of mortality from cancer, cardiovascular diseases, and other selected conditions, in Italy and Europe; the analysis of trends in tobacco consumption in the Italian and European populations, and the corresponding effects on incidence and mortality from lung and other tobacco-related neoplasms; the analysis of trend of obesity prevalence in Italy. The analytic epidemiology activities include the conduction and analysis of case-control studies, aimed at identifying and better quantifying the association between genetic factors (family history), selected lifestyle habits (diet, tobacco, alcohol, coffee, diabetes, etc.), use of exogenous hormones and exposure to various substances and the development of various forms of cancers and cardiovascular diseases. In particular, the Department works on the analysis of dietary correlates of cancer and cardiovascular disease risk; quantification of health effects of tobacco smoking, alcohol consumption, coffee drinking and implications for prevention; epidemiological studies on the risk related to oral contraceptive and hormone replacement therapy use; evaluation of the impact of screening in the early diagnosis and prevention of cancer. Other activities include: the conduction of quantitative reviews and meta-analysis of published data on alcohol, coffee, aspirin and other selected data; the re-analysis of original data from epidemiological studies of cancers of the oral cavity and pharynx, pancreas, stomach, thyroid, breast, ovary, cervix and bladder; the analysis of historical cohort studies of occupational exposures to aromatic amines, asbestos, herbicides and other known or potential carcinogens; the study of the role of infections in the etiology of atopic diseases (“Hygiene hypothesis”); and the evaluation and monitoring of human papillomavirus (HPV) in women at high risk of cervical cancer. Moreover, the
Department of Epidemiology collaborates in epidemiological and clinical studies in pediatrics and oncology with other Italian and European groups. In particular Professor La Vecchia is co-PI of a project of European Research Council (ERC), in collaboration with Prof Bach of the Institut National de la Santé et de la Recherche Médicale (INSERM) on the analysis of postulates of the hygiene hypothesis, which ascribes a protective role to the exposure to microbial agents (direct or indirect) in the development of atopy in early childhood. Another Department’s activity is related to the development of medical websites, the study of the quality of medical information available on the Internet, and the training and research on issues related to medical informatics and those concerning the use in the medical field of the Internet, the social media, and the web 2.0 applications.

**MAIN FINDINGS**

Our study on nasopharyngeal cancer (NPC) showed that, also in low-risk populations, vegetable consumption is a protective factor against this neoplasm. The stronger effect for yellow or red-pigmented vegetables is in agreement with the inverse association reported for carotenoids intake.

Our case-control study on NPC suggested that dietary intake of soluble and insoluble fibers is inversely related to NPC risk in a nonendemic southern European population.

A case-control study of oral cavity and pharyngeal (OCP) cancer confirms and further quantifies that a diet rich in fruits and vegetables and poor in meat and products of animal origin has a favourable role against OCP cancer. Combinations of low consumption of fruits and vegetables, and high consumption of meat with high tobacco and alcohol, led to 10- to over 20-fold excess risk of OCP cancer.

A case-control study conducted in Italy showed no association between dietary glycemic index (GI) and glycemic load (GL) and the risk of endometrial cancer, whereas a meta-analysis of published studies on the issue supported an increased risk for high GL, but not GI.

Closer adherence to the Mediterranean diet appears to be protective against hepatocellular carcinoma, particularly for patients chronically infected with hepatitis B and/or C viruses.

The metabolic syndrome is positively associated to hepatocellular carcinoma risk in subjects not chronically infected with hepatitis B and/or C viruses.

Overweight and obesity are strongly directly related to esophageal and gastric cardia adenocarcinoma, particularly esophageal adenocarcinoma.

In a study based on data from two large Italian case-control studies of gastric cancer, we found a favorable effect of high adherence to the Mediterranean diet on this neoplasm.

A low GL diet that also adequately adheres to the principles of the traditional Mediterranean diet may reduce the incidence of type 2 diabetes.

There is supportive evidence for a protective role of isoflavones and a suggestion for a protective role of proanthocyanidins against endometrial cancer.
Consistent inverse relations between dietary total antioxidant capacity and colorectal cancer have been reported for the first time in a case-control study from Italy.

We reported stronger associations for positive hormonal (particularly estrogen) receptor breast cancers than for negative hormonal receptor breast cancers with selected menstrual and reproductive factors (i.e., parity, increased age at first birth, post-menopause, increased age at menopause) and family history of breast cancer.

Our large case-control study on young-onset (≤45 years) colorectal cancer confirms that several recognized risk factors for colorectal cancer (e.g., high consumption of alcohol and meat, low consumption of fruit and vegetables) are also relevant determinants of young-onset colorectal cancer. Family history of colorectal cancer is a stronger risk factor in young subjects, as compared to middle-age and elderly ones.

In two combined case-control study conducted in Italy, we provided evidence that a priori-defined scores measuring adherence to the Mediterranean diet are favourably associated with pancreatic cancer risk.

An analysis of a posteriori dietary patterns showed that a diet characterized by a high consumption of meat and other animal products, as well as of (refined) cereals and sugars, is positively associated with pancreatic cancer risk, whereas a diet rich in fruit and vegetables is inversely associated.

In a network of case-control studies on several forms of cancer conducted in Italy and Switzerland, a family history of cancer increased the risk of other members of the family developing the same cancer and also a different cancer.

Consumption of red meat has been related to increased risk of several cancers. Cooking methods could modify the magnitude of this association, as production of chemicals depends on the temperature and duration of cooking. In a network of case-control studies conducted in Italy and Switzerland, we confirmed that red meat consumption is a risk factor for several cancer sites, while there is a limited impact of cooking methods.

In a pooled analysis of 24 case-control studies within the International Head and Neck Cancer Epidemiology (INHANCE) Consortium, we found that adult height is inversely associated with head and neck cancer (HNC) risk. As height can be considered a marker of childhood illness and low energy intake, the inverse association is consistent with prior studies showing that HNC occur more frequently among deprived individuals.

An analysis within the INHANCE study showed that cigar and pipe smoking are independently associated with increased risk of HNC.

A uniquely large collaborative study within the Pancreatic Cancer Case-control Consortium (PanC4), including 4717 pancreatic cancer cases, did not support the hypothesis that peptic ulcer and its treatment materially affect pancreatic cancer risk.

In the PanC4 data, we found that hay fever and allergy to animals were inversely related to pancreatic cancer risk, while there was no association with other allergies or asthma.

A meta-analysis of 17 studies on over 8500 cases found no increased bladder cancer risk among personal users of hair dyes.
A meta-analysis on 2100 cases suggested a lack of association between coffee intake and risk of glioma, and a tendency, if any, to a lower risk for tea and coffee plus tea.

A meta-analysis of adult brain cancer reported no association with alcohol drinking overall. However, a moderate increase in risk (+35%) was found at high doses of alcohol consumption.

We evaluated the association between light alcohol drinking (i.e., up to 1 drink/day) and several cancers through a meta-analytic approach. Light alcohol drinking increases the risk of cancer of oral cavity and pharynx, esophagus and female breast.

The alcohol-related relative risks of oral and pharyngeal cancer are similar with respect to sex, geographic area and type of alcoholic beverage. The association of alcohol is stronger in smokers than in non-smokers.

In a systematic review and meta-analysis of the literature on the relation between dietary patterns and risk of gastric cancer, we reported an almost 2-fold difference in gastric cancer risk between a “Prudent/healthy” diet rich in fruits and vegetables, and a “Western/unhealthy” diet-rich in starchy foods, meat and fats.

A meta-analysis of prospective studies provided quantitative evidence that coffee intake is inversely related to all cause and, probably, cardiovascular diseases mortality. No association was found for mortality for coronary/ischemic heart disease, stroke and cancer.

A meta-analysis indicated that the risk of hepatocellular carcinoma is reduced by 40% for coffee consumption vs no consumption. The inverse association might partly or largely exist because patients with liver and digestive diseases reduce their coffee intake.

A review and meta-analysis of the literature indicated that there is adequate evidence excluding an overall excess cancer risk in thiazolidinediones users within a few years after starting treatment. However, there is a modest excess risk of bladder cancer, particularly with reference to pioglitazone.

In a meta-analysis of epidemiological studies, we reported a favorable effect of exposure to dogs and pets on the risk of atopic dermatitis (AD) in infants or children, whereas no association emerged with exposure to cats. The favorable effect of dog exposure on AD might be explained by the role of contact with microbial agents during early life, affecting the development of the immune system. Our findings thus provide support to the hygiene hypothesis.

Within the Pricing Policies and Control of Tobacco in Europe (PPACTE) project, we analyzed smoking patterns among the elderly (≥65 years) in 17 European countries. Smoking prevalence was higher men and in countries with low implementation of tobacco control activities.

Using data from the repeated Behavioural Risk Factor Surveillance System (BRFSS) surveys in pre-crisis (2005–2007) and post-crisis (2009–2010) periods on a total of 1,981,607 US adults, we found that the 2008 economic crisis had a weak effect on smoking prevalence in the USA.

An ecological study of 27 countries of the European Union (EU) reported that the implementation of smoke-free legislation in workplaces and public places is not correlated with increase smoking prevalence in private venues (house and cars).
Using the legal sales data of different tobacco products in Italy, we found a substantial increase in non-manufactured cigarette combustible products (particularly hand-rolled tobacco) sales and a decrease in manufactured cigarettes sales between 2004 and 2012.

Smoking prevalence in Italy was 22.7% in 2011 and 20.8% in 2012; the latter is the lowest smoking prevalence reported over the last decades. The use of hand-rolled cigarettes is increasing in time and was particularly high in men and among young people.

We analyzed the motivation to start and quit smoking in the general Italian population, between 2005 and 2010. Most ever smokers started smoking because of the influence of friends and most ex-smokers quit because of tobacco-related health conditions, while only a minority of ex-smokers quit to avoid future illness.

We did not find unfavorable trends in overweight and obesity prevalence between 2006 and 2010 in a representative sample of Italian adults. There are, however, specific subgroups of the population with elevated prevalence of overweight and obesity, mainly adults from southern Italy and less educated ones.

An analysis of cancer mortality data in Europe in 2005-2009 showed that, with the major exceptions of female lung cancer and pancreatic cancer in both sexes, cancer mortality has moderately but steadily declined across Europe. However, substantial differences across countries persist, requiring targeted interventions on risk factor control, early diagnosis, and improved management and pharmacological treatment for selected cancer sites.

We updated and compared mortality trends from primary liver cancer (PLC) and intrahepatic cholangiocarcinoma (ICC) in Europe between 1990 and 2010. PLC mortality has become more uniform across Europe over recent years, with an overall decline; in contrast, ICC mortality has substantially increased in most Europe.

In an updated analysis of trends in mortality from leukemia in Europe over the period 1970–2009, we observed declines over the last decades, particularly in children and the young, mainly due to therapeutic advancements. The declines, however, were later (since the mid-late 1990s) and smaller in central and eastern European countries, when compared with western ones, particularly in middle-age adults. No decline was observed over age 70, when therapy may be less effective.

Lung cancer mortality in the EU peaked in the late 1980s at an age standardized (world standardized population) rate of nearly 55/100,000 men and declined to about 41/100 000 in the in 2005-09. Declines were stronger in the young. Geographic differences were also observed: over most recent calendar rates for Western European countries were around 35-49 /100,000 men while they were around 50/100,000 in Eastern Europe.

Total projected number of deaths for the year 2013 in the EU was 1,314,296 (737,747 men and 576,489 women), this means a projected 6% age standardized mortality rate fall since 2009 in men reaching 140.1/100,000 men and 4% fall reaching 85.3/100,000 in women. Recent trends for selected cancers were favourable with the exceptions of female lung cancer and pancreatic cancer in both sexes. Pancreatic cancer has become the fourth cause of cancer death overall, while lung cancer is expected to become the first cause of cancer mortality in women in the EU over the next few years.
We analyzed mortality data for hepatocellular carcinoma between 1988 and 2009 in the metropolitan area of Naples in Southern Italy. We observed significantly decreasing trends in the most recent recorded periods in both sexes for all ages and the 35-64 years age group, in spite of the high hepatitis C virus prevalence in the area.

We analyzed mortality data for Hodgkin’s disease (HD), non-Hodgkin lymphoma (nHL) and multiple myeloma (MM), for the metropolitan area of Naples in Southern Italy. These cancers are associated with the hepatitis C virus (HCV) which is known to have a high prevalence rate in this area. We found that HD had a favourable trend, while MM and male nHL had no significant trend, and female nHL had a significant rising trend.

We analyzed overall and site-specific cancer mortality between 1988 and 2009 in the metropolitan area of Naples and Caserta in southern Italy and compared them to Italian national data. Total cancer mortality showed favourable trends in both sexes in both Naples and Caserta, but in men the declining rate was smaller than the national one, this difference being greater when examining the elderly.

Healthcare administrative data on a large Italian cohort of early breast cancer women suggest that the incidence of short-term severe cardiotoxicity (not only congestive heart failure) of trastuzumab in clinical practice is higher than that recorded in clinical trials testing the same regimen. Age and history of cardiac disease are strong predictors of cardiotoxicity.

**NATIONAL COLLABORATIONS**

Associazione Italiana di Oncologia Medica (AIOM)
Associazione Medici Diabetologi – Regione Lombardia
Accademia Nazionale di Medicina, Genova
Agenzia giornalismo scientifico Zadig, Milano
Arcispedale S. Maria Nuova, Reggio Emilia
ASL di Bergamo
Associazione Nazionale dei Medici Cardiologi Ospedalieri (ANMCO)
Azienda Ospedaliera Niguarda Ca’ Granda, Milano
Azienda Ospedaliera San Gerardo, Monza
Azienda Ospedaliero-Universitaria San Giovanni Battista Le Molinette, Torino
Azienda Ospedaliera Universitaria Santa Maria della Misericordia, Udine
Azienda Unità Sanitaria Locale di Ravenna
Centro Cardiologico Monzino, Milano
Centro Studi Comunicazione sul Farmaco, Milano
Centro di Riferimento Oncologico, Servizio di Epidemiologia e Biostatistica, Aviano (PN)
Comune di Milano, Direzione centrale salute, Settore politiche per la Salute
Federazione Italiana delle Associazioni di Volontariato in Oncologia (FAVO)
Federazione Italiana Medici Medicina Generale (FIMMG)
Festival Internazionale del Giornalismo, Perugia
Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milano
Fondazione LuVI
Fondazione Politecnico di Milano
Fondazione SmithKline, Milano
Gruppo Italiano per lo Studio della Sopravivenza nell’Infarto miocardico (GISSI)
Gruppo Italiano Studi Epidemiologici in Dermatologia GISED, Bergamo
Gruppo Italiano Documentalisti dell’Industria Farmaceutica e degli Istituti di Ricerca Biomedica
Gruppo Studi Tumori Urologici (GSTU)
International Centre for Pesticides and Health Risk Prevention, Milano
Istituto Auxologico Italiano, Divisione Malattie Metaboliche III, IRCCS, Piancavallo (VB)
Istituto Auxologico Italiano, Laboratorio Sperimentale di Ricerche Endocrinologiche (LSRE), IRCCS, Milano
Istituto DOXA, Milano
Istituto Europeo di Oncologia, Divisione di Epidemiologia e Biostatistica, Milano
Istituto Europeo di Oncologia, Divisione di Chirurgia Cervico Facciale, Milano
Istituto Europeo di Oncologia, Divisione Melanomi e Sarcomi Muscolo Cutanei
Istituto di Fisiologia Clinica CNR, Sezione di Milano, Milano
Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (INRAN), Roma
Istituto Nazionale Neurologico “Carlo Besta”, Milano
Istituto Nazionale per lo Studio e la Cura dei Tumori, Dipartimento di Chirurgia Toracica, Oncologia
Istituto Nazionale per lo Studio e la Cura dei Tumori, Struttura Complessa di Chirurgia Generale Indirizzo Oncologico 4 (Melanomi e Sarcomi) Sperimentale, Unità di Eredità Poligenica, Milano
Istituto Oncologico Romagno
Istituto Ortopedico Gaetano Pini, Centro di Chirurgia Ortopedica Oncologica, Milano
Istituto Superiore di Sanità, Osservatorio Fumo Alcol Droga, Roma
Istituto Tumori “Fondazione Pascale”, Servizio di Epidemiologia, Napoli
Novartis Vaccines SpA, Siena
Ordine dei Medici della Provincia di Bari
Ospedale Casa Sollievo della Sofferenza San Giovanni Rotondo
Ospedali Riuniti di Bergamo
Ospedale Alessandro Manzoni, Unità di Gastroenterologia, Lecco (LC)
Ospedale “Luigi Sacco” Azienda Ospedaliera – Polo Universitario
Policlinico di Monza, Unità Operativa di Endoscopia I, Monza (MB)
Prima Clinica Ostetrico Ginecologica, Mangiagalli, Milano
Regione Lombardia, U.O. Governo dei servizi sanitari territoriali e politiche di appropriatezza e controllo Scuola Internazionale Superiore di Studi Avanzati (SISSA)
Società Italiana Attività Regolatorie
Società Italiana di Cure Palliative (SICP)
Società Italiana di Urologia (SIU)
Struttura Sistemi di remunerazione e Osservatorio Epidemiologico Direzione Generale Sanità
Unione Nazionale dei Giornalisti Scientifici Italiani
Unione Nazionale Medico Scientifica di Informazione (UNAMSI)
Università Bocconi di Milano, Dipartimento di Analisi Istituzionale e Management Pubblico, Milano
Università Bicocca Milano, Dipartimento di Informatica Sistemistica e Comunicazione, Milano
Università Cattolica del Sacro Cuore, Unità di Epidemiologia genetica e Biologia Molecolare, Istituto di Igiene, Roma
Università di Ferrara, Dipartimento di Studi Umanistici
Università di Milano-Bicocca, Dipartimento di Statistica, Milano
Università di Milano-Bicocca, I Clinica Otorinolaringoiatria, DNTB, Monza
Università degli Studi Arezzo, Dipartimento di Scienze della formazione
Università degli Studi di Firenze, Dipartimento di Scienze Politiche e Sociali
Università degli Studi di Milano, Dipartimento di Scienze Materne e Pediatriche, Milano
Università degli Studi di Milano, Dipartimento di Scienze Cliniche e di Comunità, Milano
Università degli Studi di Milano, Prima Clinica Ostetrico Ginecologica, Milano
INTERNATIONAL COLLABORATIONS

Aichi Cancer Center Research Institute, Division of Epidemiology and Prevention and Nagoya University Graduate School of Medicine, Nagoya, Japan
Catalan Institute of Oncology, Institut d’Investigació Biomèdica de Bellvitge (IDIBELL), Cancer Prevention and Control Unit, L’Hospitalet de Llobregat, Spain
Center of Oncology, Dept. of Epidemiology and Cancer Prevention, Varsavia, Poland
Centre for Research in Environmental Epidemiology (CREAL) and Municipal Institute of Medical Research (IMIM), Barcellona, Spain
European Public Health Association (EUPHA)
Evidence and Risk Assessment Division, Centre for Chronic Disease Prevention and Control, Public Health Agency of Canada, Ottawa, Ontario, Canada
Harvard School of Public Health, Department of Epidemiology, Boston, USA
Harvard School of Public Health, Department of Nutrition, Boston, USA
Hellenic Health Foundation
Hôpital Necker - Enfants Malades, Centre of the Association Claude Bernard on Auto-immunes diseases, Parigi, France
Institute de Academie des Sciences, Paris, France
International Agency for Research on Cancer, Lione, France
International Epidemiology Institute (IEI), Rockville, USA
International Life Science Institute (ILSI), Bruxelles, Belgium
International Prevention Research Institute (IPRI), Lyon, France
Karolinska Institute, Department of Medical Epidemiology and Biostatistics, Stockholm, Sweden
National Cancer Institute, Environmental Studies Section, Bethesda, USA
National School of Public Health, WHO, Atene, Greece
NUTRIM School for Nutrition, Toxicology and Metabolism, Department of Complex Genetics, Cluster of Genetics and Cell Biology, Maastricht University Medical Centre, Maastricht, The Netherlands.
Registre Vaudois des Tumeurs, Institut Universitaire de Médecine Sociale et Préventive, Losanna, Svizzera
Senologic International Society
Society for Internet in Medicine
The Tisch Cancer Institute and Institute for Translational Epidemiology, Mount Sinai School of Medicine, New York, NY, USA
Tobacco Free Research Institute, Dublino, Ireland
UNDP/UNFPA/WHO/WORLD Bank special programme of research development and research training in human reproduction, Ginevra, Switzerland
Universitat Pompeu Fabra, Department of Experimental and Health Sciences, Barcellona, Spain
University of Athens Medical School, Department of Hygiene and Epidemiology, Atene, Greece
University of Cordoba, Faculty of Medical Diseases, Cordoba, Argentina
University of Las Palmas de Gran Canaria, Department of Clinical Sciences, Las Palmas de Gran Canaria, Spain
EDITORIAL BOARD MEMBERSHIP

Advances in Therapy (Eva Negri)
Alimentazione e Prevenzione (Carlo La Vecchia)
Annals of Oncology (Carlo La Vecchia, Associate Editor)
Archives of Medical Science (Carlo La Vecchia)
BMC Public Health (Silvano Gallus, Associate Editor)
Cancer Epidemiol Biomark & Prev (Carlo La Vecchia)
Cancer Letter (Carlo La Vecchia, Associate Editor)
Current Cancer Therapy Reviews (Carlo La Vecchia)
Dermatology Research and Practice (Carlo La Vecchia)
Digestive and Liver Disease (Carlo La Vecchia)
Economia Politica del Farmaco (Carlo La Vecchia)
Epidemiology, Biostatistics and Public Health (Carlo La Vecchia, Editor)
European Journal of Cancer (Cristina Bosetti)
European Journal of Cancer Prevention (Carlo La Vecchia, Associate Editor)
European Journal of Clinical Nutrition (Carlo La Vecchia)
European Journal of Nutrition (Carlo La Vecchia)
Family Planning (Carlo La Vecchia)
In Scope Oncology & Haematology (Carlo La Vecchia)
Journal of Family Planning and Reproductive Health Care (Carlo La Vecchia)
ISRN Cardiology (Eugenio Santoro)
Maturitas (Carlo La Vecchia)
Nutrition and Cancer (Carlo La Vecchia)
Open Cancer Journal (Carlo La Vecchia)
Portale Partecipasalute.it – http://www.partecipasalute.it (Eugenio Santoro)
Revisiones en Ginecologia y Obstetricia (Carlo La Vecchia)
Revista Española de Nutrición Comunitaria (Carlo La Vecchia)
Revue d’Épidémiologie et de Santé Publique (Carlo La Vecchia)
Società Italiana Attività Regolatorie News, SIARNews (Eugenio Santoro)
The Breast (Eva Negri, Associated editor)
The Open Demography Journal (Silvano Gallus)
The Open Obesity Journal (Silvano Gallus)
The Scientific World Journal (Cristina Bosetti)
Tumori (Carlo La Vecchia)
World Journal of Dermatology (Silvano Gallus)
World Journal of Gastrointestinal Oncology (Silvano Gallus)

PEER REVIEW ACTIVITIES

Acta Dermato-Venereologica; Acta Psychiutrica Scandinavica; Acta Oto-Rhino-Laryngologica Italica; Alcohol and Alcoholism; Alcologia; American Journal of Clinical Nutrition; American Journal of Epidemiology; Annals of Epidemiology; Annals of Oncology; Appetite; Archives of Internal Medicine; BMC Public Health; British Journal of Cancer; British Journal of Nutrition; British Medical Journal; BMJ Open; Bulletin of the World Health Organization; Canadian
Journal of Physiology and Pharmacology; Cancer; Cancer Causes and Control; Cancer Detection and Prevention; Cancer Epidemiology Biomarkers and Prevention; Computer Methods and Programs in Biomedicine; Diabetes/Metabolism Research and Reviews; Digestive and Liver Disease; Epidemiologia & Prevenzione; Epidemiology; Epidemiology & Biostatistic; Epidemiology, Biostatistics and Public Health; European Heart Journal; European Journal of Cancer; European Journal of Cancer Prevention; European Journal of Clinical Nutrition; European Journal of Epidemiology; European Journal of Public Health; Evidence-Based Healthcare and Public Health; Food and Chemical Toxicology; Gynecological Endocrinology; Gut; Hearth; Hepatology; Human Reproduction; International Journal of Cancer; International Journal of Environmental Research and Public Health; International Journal of Epidemiology; International Journal of Food Sciences and Nutrition; International Journal of Hygiene and Environmental Health; International Journal of Obesity; ISRN Public Health; JAMA; Journal of American College of Nutrition; Journal of Clinical Endocrinology and Metabolism; Journal of Clinical Epidemiology; Journal of Epidemiology and Community Health; Journal of Investigative Dermatology; Journal of Medical Economics; Journal of Medical Internet Research; Journal of the National Cancer Institute; Journal of Women's Health; Lancet Oncology; Lung Cancer; Maturitas; Melanoma Research; Nature Reviews Urology; Nicotine & Tobacco Research; Nutrition and Cancer; Nutrition Journal; Nutrition, Metabolism Cardiovascular Disease; Obstetrics and Gynecology; Oncology; PLoS Medicine; PLoS ONE; Preventive Medicine; Public Health; Public Health Nutrition; QJM; Radiation Research; Recent Patents on Anti-Cancer Drug Discovery; Appetite; Revue d’Épidémiologie et de Santé Publique; The Breast; The Cancer Journal; The Lancet; The Open Obesity Journal; The Scientific World Journal; Tobacco Control; Tumori; World Journal of Gastroenterology.

NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP

Comitato Scientifico del Gruppo Italiano Studi Epidemiologici in Dermatologia
Comitato Scientifico della Società Italiana di Colposcopia e Patologia Cervico Vaginale
Comitato Scientifico del portale www.familyhealth.it
Data and Safety Monitoring Board of the “Phase II therapeutic trial with a humanized nonmitogenic CD3 (ChAgly CD3) monoclonal antibody in recently diagnosed type I diabetic patients” Executive Committee, International Head and Neck Cancer Epidemiology (INHANCE) consortium
Giuria del Premio Nazionale Comunicazione, Marketing e Informazione per la Salute – Festival Internazionale del Giornalismo
Ministero della Salute, Sottocomitato fumo
Scientific Review Committee del UND/WHO/World Bank Human Reproduction Programme

EVENT ORGANIZATION

First project meeting. IRCCS Istituto di Ricerche Farmacologiche Mario Negri. Stomach Cancer Pooling (STOP) project. Milano, Italy. 11/6/2013

Corso ECM "Internet al servizio della formazione e dell'aggiornamento del pediatra", organizzato in collaborazione con la ASL Provincia di Bergamo, Bergamo 20 april 2013

Corso ECM " Corso avanzato sull'impiego di PubMed e metodi di valutazione della ricerca biomedica ", Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, 29 may 2013
Corso ECM "Web 2.0, social media e apps per l’aggiornamento del medico e dell’operatore sanitario: corso base", Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, 4 june 2013

Corso ECM "Web 2.0, social media e apps per l’aggiornamento del medico e dell’operatore sanitario: corso avanzato", Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, 5 june 2013

Corso “PubMed, Twitter e i nuovi social media per il reperimento e la diffusione dell’informazione medico-scientifica”, organizzato in collaborazione con Unione Nazionale Medico Scientifica di Informazione (UNAMSI), Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, 11 may 2013

CONFERENCE AND WORKSHOP CONTRIBUTIONS

Master Universitario di secondo livello in epidemiologia. Università degli studi di Torino e fondazione ISI Torino. “Medical factors and risk of adult bone sarcomas: a multicentric case-control study in Europe”. Torino, Italy. 9-11/1/2013

Commissione giudicatrice. Dottorato Epidemiologia e Biostatistica, 25° ciclo. Monza, Italy. 28/1/2013

ECRIN-IA Methodology Workshop. “The methodological challenges”. Milano, Italy. 6-7/2/2013

Incontro Gruppo 2003 con Giorgio Napolitano. Roma, Italy. 20/2/2013


ETRMA-project. Rubber Industry cohort study at iPRI. Lyon. France. 9/4/2013


International Faculty Committee on Incretin Therapies and Pancreatic Safety. SANOFI Diabetes. Member of the Panel. New York, NY, USA. 13/5/2013


International Scientific Consensus Summit. Glycemic Index (GI), Glycemic Load (GL) and Glycemic Response (GR). “GI/GL and risk of major cancers: what can we conclude based on epidemiological evidence?”. “GI/GL and risk of major cancers: what can we conclude based on epidemiological evidence?”. Stresa, Italy. 6-7/6/2013


Seminari Biologici. Università Cattolica del Sacro Cuore. “Alcool e tumori. La questione della dose”.
Master di Epidemiologia e Biostatistica. “Epidemiologia dei tumori”
Roma, Italy. 21/6/2013

Comitato Nazionale per la Biosicurezza le Biotecnologie e le Scienze della vita. Riunione Plenaria. Palazzo Chigi. Roma, Italy. 24/6/2013

Meeting ESHRE. STIs, Sexual behaviour and contraception a European perspective.
“Contraceptive use, HPV and cervical cancer”. Capri, Italy. 30-31/8/2013

VII Congresso Nazionale SISMEC (Società Italiana di Statistica Medica ed Epidemiologia Clinica”. Scoperte scientifiche. Evidenze cliniche. Misure e metodi. “Quale contributo si aspetta la Consulta delle società scientifiche per la riduzione del rischio cardiovascolare (CSCV) dagli statistici medici e dagli epidemiologi clinici?”. Roma, Italy. 27/9/2013


Corso Comune -Tumori del torace. “Epidemiologia delle neoplasie polmonari”. Milano, Italy. 9/10/2013

Esperto per il Progetto “Revisione sulla qualità dell’assistenza sanitaria in Italia” (Expert for the project “Revision on quality of health care in Italy”). AGE.NA.S., OCSE, Rome. 24/10/2013


Breaking News in Interventistica Cardiovascolare. 5° Convegno Medici. Milano, Italy 30/11/2013
Seminario “Web 2.0 e medicina di montagna”, presso CAI Bergamo, 11 marzo 2013


Congresso “Pneumotrieste 2013. La salute del respiro”, “Facebook e Twitter: strumenti di lavoro in medicina”, Trieste 8-10 aprile 2013

Master in giornalismo scientifico digitale, Scuola Internazionale Superiore di Studi Avanzati (SISSA) di Trieste, anno accademico 2012-2013. Ruolo di docenze nel modulo “Medicina”, Trieste 9 aprile 2013

Corso avanzato di formazione su metodologia, strategie e tecniche della ricerca clinica, promosso dalla Associazione Nazionale Medici Cardiologi Ospedalieri, Firenze 1 marzo 2013

Festival Internazionale del Giornalismo, convegno “Comunicazione, marketing e informazione per la salute”, Perugia, 26 aprile 2013.

Corso “I nuovi social media per l’aggiornamento del medico”, Fondazione Biblioteca Biomedica Biellese, Biella 2 ottobre 2013

Corso “INTERNET PER L’AGGIORNAMENTO MEDICO E DELL’OPERATORE SANITARIO”, Fondazione Biblioteca Biomedica Biellese, Biella 8 maggio 2013

Master Universitario di II° livello in Statistica Medica e Metodi Statistici per l’Epidemiologia, Università degli Studi di Milano, Facoltà di Medicina e Chirurgia, anno accademico 2012-2013. Ruolo di docenze nel modulo “Internet e le nuove tecnologie per la ricerca clinica”, Milano 9-11 novembre 2013

Master Universitario di I° livello in Ricerca Clinica, Università degli Studi di Milano, anno accademico 2012-2013. Ruolo di docenze nel modulo “Internet e le nuove tecnologie per l’aggiornamento medico-scientifico”, Milano 26 novembre 2012

Corso “Internet, web 2.0 e social media al servizio della formazione e dell’aggiornamento del medico e dell’operatore sanitario: corso avanzato” promosso dalla Scuola Umbra di Amministrazione Pubblica, Perugia 16-17 maggio, 23-24 maggio, 13-14 giugno 2013

44° Congresso Nazionale di Cardiologia ANMCO, Titolo relazione “Potenzialità dei social network in ambito medico/cardioologico”, Firenze 1 giugno 2013

Corso “Social Media Strategies. Strumenti e tecniche”. Titolo relazione “I social media al servizio del medico e dell’operatore sanitario”, Università degli Studi di Firenze, Dipartimento di Scienze Politiche e Sociali, Firenze 29 giugno 2013


Workshop dal titolo “Virtualmente informati: scrivere di salute sul web” condotto nell’ambito del festival di Internazionale, organizzato dal Dipartimento di Studi Umanistici, Master in giornalismo e comunicazione istituzionale della scienza, Università di Ferrara, Ferrara 4-6 ottobre 2013

Corso “Internet e social media: nuovi strumenti per l’aggiornamento medico”, Provincia Autonoma di Bolzano, Bolzano 11 ottobre 2013


“European Master in Sustainable Regional Health Systems: Erasmus Mundus” in collaboration with the University of Verona, “Social media, social networks and medapps: applications to healthcare and social diseases”, Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, 23 ottobre 2013

Convegno “#GoodMedia”, “I social media come strumento di promozione della salute” organizzato dall’Ospedale Galliera di Genova, Genova 25 ottobre 2013

Convegno “Sonna - Bioetica e social network: esperienze in ambito scolastico e sanitario”, “Facebook, Twitter e medicina: potenzialità degli strumenti web 2.0 on ambio sanitario” Università degli Studi Areezo, Dipartimento di Scienze della formazione, scienze umane e della comunicazione interculturale, Arezzo 6 dicembre 2013

GRANTS AND CONTRACTS

AIFA
Arcispedale Santa Maria Nuova, Azienda Ospedaliera di Reggio Emilia
ASL Bergamo
Associazione Italiana Oncologia Medica
Associazione Italiana per la Ricerca sul Cancro (AIRC)
Azienda Ospedaliera San Gerardo di Monza
Centro di Ricerche sulla Gestione dell’Assistenza Sanitaria e Sociale (CERGAS)
Centro Cardiologico Monzino
Lega Italiana Lotta contro i Tumori (LILT)
Eli Lilly Italia Spa
ECRIN-IA
European Commission (FP7)
European Research Council (ERC)
Federazione Italiana Medici di Medicina Generale – Provincia Milano
Federazione Medico Sportiva Italiana – Regione Puglia
Fondazione Biblioteca Biomedica Biellese 3Bi
Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milano
Fondazione IRCCS Istituto Nazionale dei Tumori, Milano
Fondazione Politecnico di Milano
Fondazione Umberto Veronesi
GISED
Istituto Oncologico Romagnolo
Ministero della Salute
Ospedale “Luigi Sacco” Azienda Ospedaliera – Polo Universitario
Osservatorio Permanente sui Giovani e l’Alcol
Pfizer Italia Srl
Regione Lombardia
Weber Shandwich
ISA
Perfetti Van Melle
Provincia Autonoma di Bolzano
Roche S.p.A.
Regione Lombardia
Scuola Internazionale Superiore di Studi Avanzati (SISSA)
Scuola Umbra di Amministrazione Pubblica
UNAMSI
Unione Nazionale dei Giornalisti Scientifici Italiani
Università di Torino

SCIENTIFIC PUBLICATIONS (2013)


20: Bosetti C, Lucenteforte E, Bracchi PM, Negri E, Neale RE, Risch HA, Olson SH, Gallinger S, Miller AB, Bueno-de-Mesquita HB, Talamini R, Polese J, Ghadiriiran P, Baghurst PA,


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Santoro E. Slideshare per l'aggiornamento professionale in medicina. Ricerca & Pratica 2013; n.171: 121-122

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Santoro E. FDA: le linee guida per lo sviluppo delle apps mediche. Ricerca & Pratica 2013; n.174: 264-265
Santoro E. Condivisione dei dati, trasparenza della ricerca. Ricerca & Pratica 2013; n.173: 225

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Santoro E. Quintaliani G. Web 2.0 e social media: nuovi strumenti al servizio del nefrologo. G Ital Nefrol 2013; 30 (1)

Santoro E. I social media, le apps e la trasformazione della comunicazione, della formazione e dell’assistenza in sanità. Recenti Prog Med 2013;104(5):179-180

OTHER PUBLICATIONS (2013)


RESEARCH ACTIVITIES

Laboratory of General Epidemiology

CASE-CONTROL STUDIES OF LIFESTYLE, GENETIC FACTORS AND CANCER RISK
The Laboratory of Epidemiology has developed an integrated series of case-control studies of several cancer sites, which has been a uniquely productive resource for epidemiological research and risk quantification in Italy, with over 1,000 publications over the last 30 years. The study integrates newer studies (generally more sophisticated, including also biological material) with earlier datasets (including over 22,000 cases and a comparable number of controls) and allows to study key cancer risk factors (tobacco, alcohol, overweight, selected dietary factors, hormones) on a uniquely large dataset, as well as to understand their changing role over time. The Laboratory has also developed and integrated various sources of cancer epidemiology research, including questionnaire data, biobanks and record linkage systems, in order to quantify the associations between exposure and risk of major selected cancers in Italy, to test newer hypotheses and to prioritize primary and secondary prevention.

Among aspects investigated in the network of case-control studies are:
1. Nutrition and diet, including various measures of overweight and their implications on metabolic aspects on cancer risk, the separate and integrated role (e.g., dietary patterns) of food groups and nutrients, with focus on several specific diet components (e.g., flavonoids).
2. Alcohol and tobacco, with a focus on low doses for alcohol, time-risk relations after stopping smoking and drinking, and meta- and pooled-analyses with other datasets worldwide.
3. Familial and genetic factors, given the availability of history of any cancer in relatives (and age at cancer diagnosis), with the possibility to obtain lifetime-risk of familial cancer, as well as of biological samples to analyze genetic polymorphisms.

4. Hormonal factors, not only for recognized hormone-related cancers in collaborative re-analyses, but also for cancers of the pancreas, liver, lymphomas and sarcomas, where the role of hormones is open to discussion.

5. Other environmental factors, including, among others, disinfection-by-products (DBPs) and colorectal cancer, infections, hair dyes and occupational exposures and bladder cancer, hepatitis C and B and lymphomas, viruses and polychlorinated biphenyls (PCBs) and sarcomas.

6. Cohort studies on factors associated with cancer risk, survival and mortality, by linking our database with local and national (administrative) data.

7. Meta- and pooled-analyses. The project is part of a series of collaborative re-analysis conducted in Europe and worldwide on cancers of the upper digestive and respiratory tract, pancreas, breast and female genital tract, thyroid and lymphomas.

8. Food composition database, to include additional food components (e.g., proanthocyanidines, glutathione, total antioxidant capacity) and update the existing one.

META-ANALYSIS OF ALCOHOL CONSUMPTION AND CANCER RISK
Cancer sites causally related to alcohol consumption are those of the oral cavity and pharynx, esophagus (squamous cell carcinoma), larynx, liver, colorectum, and breast. For many other cancers the evidence is inconsistent and still open to discussion. Further, selected aspects of alcohol consumption on cancer risk need clarification, particularly the dose-risk relation and the heterogeneity of results across different populations. In this project, we investigated the relation between alcohol drinking and risk of cancer using a meta-analytical approach. The study scheme was based on an already available database of 235 epidemiological studies published from 1966 to 2000 and investigating 18 different cancer sites, integrated with new papers published until the end of 2011. Primary aims of this project were to estimate the parameters of the dose-response functions relating alcohol consumption to the risk of several types of cancer, using various meta-regression models and an ad hoc developed SAS macro software, and to identify the sources of heterogeneity (e.g., drinking pattern, geographical area, etc.) in the parameter estimates. For sites where the role of alcohol is still debated, the association with exposure to alcohol was investigated, regardless of the dose. All cancer sites were considered together in a single paper, in order to overview the strength of the evidence on the association between alcohol and cancer. We considered not only common cancers, but also rarer neoplasms, for which sparse information is available. Further, all cancer sites have been examined in another investigation, aimed to quantify the role of low doses of alcohol consumption and to elucidate whether there is any threshold in intake below which no effect on cancer is evident. Besides meta-analyses of all neoplasms, we investigated in-depth the effect of alcohol on the risk of several cancers, including oral cavity and pharynx, esophagus (adenocarcinoma) and gastric cardia, stomach, lung, ovary, kidney, bladder, brain, and lymphomas, considering results for different anatomic subsites and/or histological types and explored several potential sources of heterogeneity of results. The project has relevant prevention and public health implications, particularly the analysis focused on low doses.

INTERNATIONAL HEAD AND NECK CANCER EPIDEMIOLOGY (INHANCE) STUDY
The International Head and Neck Cancer Epidemiology (INHANCE) Consortium was established in 2004, based on the collaboration of research groups leading large molecular epidemiology studies of head & neck cancer that are on-going or have been recently completed. When taken collectively, questionnaire data on over 26,000 cases and 34,000 controls, and biological samples from a majority of the study population would be available. The 35
epidemiological studies included in the consortium have been conducted in various regions of the world. Worldwide, an estimated more than half a million head & neck cancer cases and 320,000 deaths due to head & neck cancer occurred in the year 2008. Head and neck cancers are a related group of cancers that involve the oral cavity, pharynx and larynx. While it is well-established that tobacco and alcohol account for at least 75% of head & neck cancers, important etiologic questions remain to be addressed: (i) the role of low penetrance genetic susceptibility factors (e.g. SNPs) and their interactions with environmental factors, (ii) etiology in rare subgroups including young age at onset, and nonsmokers and nondrinkers, (iii) the effect of human papillomavirus (HPV), particularly with respect to cancer subsite. The INHANCE consortium conducted pooled analyses of lifestyle risk factors such as alcohol beverage type and concentration, and also pooled analyses in rare groups such as early onset head and neck cancer cases, and nonsmokers/nondrinkers. Working groups have been formed for research topics such as HPV, genetics/ DNA repair, nonsmokers/nondrinkers, early onset cases and occupational factors. Future directions for the consortium will be to coordinate genotyping from a list of priority SNPs and to assess the effect of HPV infection. We anticipate that the INHANCE consortium will be a major step toward improving our understanding of the causes and mechanisms of head & neck cancers and the beginning of a long-standing cooperation. To date, 35 articles on INHANCE data consortium were published. Our Department is actively involved in the scientific collaboration and analyzed data on several modifiable and non-modifiable risk factors for cancer including family history of cancer, coffee and tea intake and dietary patterns. Under the supervision of our Department, several other dietetic aspects have been analyzed during 2013.

INTERNATIONAL PANCREATIC CANCER CASE-CONTROL CONSORTIUM (PANC4)
The Pancreatic Cancer Case Control Consortium (PanC4) has been created by a group of scientists from diverse biomedical disciplines (Epidemiology, Genetics, Biostatistics, Bioinformatics, Molecular Biology, Gastroenterology, Surgery) across the world who have joined together to improve our understanding of the causes of pancreatic cancer through joint, or pooled analyses of data. The PanC4 consortium includes over 15 case-control studies of pancreatic cancer conducted in North America, Europe, China, and Australia, besides the IARC-coordinated Surveillance of Environmental Aspects Related to Cancer in Humans (SEARCH) study from Canada, Europe and Australia, and includes overall over 800 cases of adenocarcinoma of the exocrine pancreas and about 14,000 corresponding controls. The original datasets were restructured either by the original study investigators or by the central coordinators using a uniform format for data harmonization. Among the risk factors already analyzed within PanC4 are cigarette smoking, smoking of other tobacco products, alcohol intake, and selected medical conditions (allergy, pancreatitis, ulcer and gastrectomy) and reproductive factors. New analyses are on-going for selected dietary items (including acrylamide, vitamin D, …), early onset pancreatic cancers, and history of diabetes.

TOTAL ANTIOXIDANT CAPACITY, FLAVONOIDS AND CANCER RISK
There are suggestions that a diet rich in fruit and vegetables has a favorable role on (digestive tract) cancers. It is however unclear which (micro) nutrients or bioactive compounds in fruit and vegetables may be responsible for such favorable effect. Flavonoids and proanthocyanidins have shown beneficial effects on cancer in experimental animals and in vitro. In previous investigations, we found favorable effects of flavonoids on stomach, liver, and breast cancer risk in the Greek population, and on cancers of the upper aerodigestive tract, stomach, colorectal, breast, endometrium, ovary, and kidney in Italy. Recently, the total antioxidant capacity (TAC) from diet has also been investigated, and inverse relations were reported for colorectal and
gastric cancer risk. The goal of the project is to examine the role of flavonoids, including proanthocyanidins, and TAC on the risk of selected respiratory and digestive tract, as well as hormone related and urinary tract neoplasms. The project is based on a network of case-control studies conducted in Italy and Greece on various cancers that include more than 15,000 cases and 20,000 controls.

DIABETES AND CANCER RISK
Diabetes mellitus has been related to the risk of colorectal, liver, pancreatic, (postmenopausal) breast, and endometrial cancer, though the quantification of this association in various populations remains open to discussion. Diabetes is also possibly directly related to bladder and inversely related to prostate cancer risk, whereas data are inconsistent for other major cancer sites, for which further information is thus necessary. It is also of interest to investigate cancer relation with the metabolic syndrome (MetS), a combination of abdominal obesity, diabetes, dyslipidemia, and hypertension, which has been identified as a risk factor for cardiovascular diseases, and more recently for various common cancers. Medications prescribed for the treatment of type 2 diabetes have also been hypothesized to influence the risk of cancer, although data are inconsistent. A project is on-going aims to: i) better assess and further quantify the association of diabetes and the risk of specific cancers; ii) add relevant evidence of the role of MetS on cancer risk; iii) provide additional data on the role of different classes of anti-diabetic drugs on the risk of cancer. The project includes following three phases: 1) analysis of diabetes, metabolic syndrome and cancer risk in a network of case-control studies, using data from a uniquely large and detailed database from a network of ongoing case-control studies from Italy and French speaking Switzerland, and using data from international collaborative groups; 2) systematic review and meta-analysis of epidemiological evidence on antidiabetic drugs and cancer risk; 3) investigations of the role of diabetes, antidiabetic drugs and cancer risk in a retrospective cohort study from electronic health databases.

TOBACCO CONTROL IN ITALY
Tobacco smoking remains the leading global cause of preventable disease and death, and is responsible for approximately 6 million deaths worldwide every year. In order to plan strategies to control tobacco in one country, it is important to systematically collect data on smoking prevalence and trends, using surveys conducted with standardized methods on representative samples of a country’s population. This allows to implement the most efficient interventions to control tobacco. Besides collecting and storing data on smoking, it is also crucial to promptly interpret them to provide to policy makers updated recommendations on which tobacco control strategy is more urgent, feasible and efficient. In order to monitor smoking prevalence in Italy, since 2001, in collaboration with the National Institute of Health and DOXA, we annually conduct a face-to-face survey on more than 3000 individuals representative of the general Italian population aged 15 years and over. Each year we update the standardized questionnaire in order to study specific issues on tobacco control in Italy. In 2013 we added a few questions on the emerging phenomenon of electronic cigarettes, a type of nicotine delivery system whose use was negligible only a few years ago. We observed that more than 45 million Italians (91.1%) have heard about e-cigarettes, 3.5 million (6.8%) have already tried, and more than 600,000 Italians (1.2%) regularly use it. Three out of 4 e-cigarette users reported to have favourably modified their smoking habit. However, 90% of users did not quit smoking as a consequence of starting vaping e-cigarettes. Almost 900,000 Italian never smokers, particularly the young, have tried at least once this new and potentially addictive product.
TOBACCO CONTROL IN EUROPE (FP7-PPACTE PROJECT)
Despite the favourable trends of smoking prevalence over the last few decades in high-income countries, tobacco remains the first cause of disease and death in North America and Europe. A collaborative project entitled Pricing Policies And Control of Tobacco in Europe (PPACTE), was conducted to provide a comprehensive analysis of tobacco pricing policy, which is considered the most effective intervention to control tobacco. Within the PPACTE project, in 2010 we conducted a face-to-face representative survey on smoking in 18 European countries (~18,000 adults). We showed that overall 27.2% of participants were current smokers (30.6% of men and 24.1% of women). Smoking prevalence among the elderly (adults ≥65 years old) was 11.1% (15.3% in men and 8.6% in women). There are substantial differences across Europe in smoking prevalence, and male-to-female and current-to-ex smoking prevalence ratios. Eastern European countries, lower income countries and those with less advanced tobacco control policies have less favourable smoking patterns and are at an earlier stage of the tobacco epidemic. In Europe, 10.4% of current smokers (12.9% of men and 7.5% of women) were “predominant" RYO users” (i.e., >50% of cigarettes smoked). This proportion was highest in England (27.3%), France (16.5%) and Finland (13.6).

EFFECTS OF ECONOMIC CRISIS ON SMOKING PREVALENCE
Scanty and controversial information is available on the impact of macroeconomic fluctuations on smoking behavior. We conducted a study to investigate the effects of the 2007-2008 economic crisis on smoking prevalence and number of smokers in the USA, using data from the repeated Behavioural Risk Factor Surveillance System (BRFSS) surveys in pre-crisis (2005-2007) and post-crisis (2009-2010) periods taking into account the demographic growth of the US population, the secular smoking prevalence trends and the changes in sociodemographic characteristics. The 2008 financial crisis had a weak effect on smoking prevalence. The crisis resulted in an increase in the number of smokers in the US by 0.6 million. This is largely due to an unexpected decrease of 1.7 million smokers among employed and an increase of 2.4 million smokers among unemployed individuals, whose smoking prevalence also remains extremely high in the post-crisis period (32.6%).

THE ROLE OF REGULATORY AGENCY TO CONTROL PUBLICATION BIAS (FP7-OPEN PROJECT)
During 2013, we have been involved in the project Overcome the Failure to Publish Negative Findings (OPEN), financed by the European Commission within the Seventh Framework Programme (FP7). Our work package aimed to evaluate the role of the main regulatory agencies, including in particular the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), on controlling failure to publish negative findings from clinical trials. We found that, although FDA has the most advanced policies to control publication bias worldwide, it does not provide sufficient regulations to fight against failure to publish negative findings from CTs. Currently, EMA has even less adequate procedures to control publication bias, but it recently announced a plan to improve transparency, through policies providing public access to CT results. Learning from limitations, knowledge gaps and loopholes of FDA policies, EMA has the opportunity to create a set of regulations more efficient to control publication bias.

THE HYGIENE HYPOTHESIS: REVISITING THE CONCEPT BY INTEGRATING EPIDEMIOLOGY AND MECHANISTIC STUDIES (FP7 ERC PROJECT)
The hygiene hypothesis postulating the paradoxical protective role of infections on immune-mediated diseases including atopy (i.e. atopic dermatitis, rhinitis, asthma) and more recently
autoimmune diseases has been the matter of extensive investigation. Aim of the present project is to validate this hypothesis integrating epidemiological and experimental studies, the latter being performed by another research group in Paris. Our epidemiological section includes both a systematic review approach, i.e., meta-analyses of studies of direct and indirect markers of infections and atopic diseases, and an original case-control study, to analyze the association between infections and atopy using atopic dermatitis as a prototypic model.

In particular 460 cases and 420 controls were recruited to date, and we expect to achieve the quota of 500 cases and 500 controls during 2014. With reference to systematic reviews, we conducted in 2012 a first meta-analysis on probiotics supplementation during pregnancy and childhood for the prevention of atopic dermatitis in which moderate protection was identified (20%). Two other meta-analyses of observational studies are currently in progress to assess whether exposure to infectious agents (including indirect markers) may influence the development of atopic dermatitis in childhood. The first of these, focused on exposure to pets was published in 2013.

EVALUATION AND MONITORING OF HPV INFECTION AND RELATED DISEASES IN WOMEN AT HIGH RISK OF CERVICAL CANCER - VALHIDATE STUDY

Infection from human papillomavirus (HPV) is a necessary cause of cervical cancer, which represents the second cause of death from total cancer in women worldwide. The Valhidate Study is an ongoing multicenter, prospective cohort study funded by the Health General Direction, Lombardy Region for the period November 2010 - November 2014. It aims to evaluate, in a cross-sectional study, and to monitor, in a prospective cohort study, HPV infection and cervical related diseases in high risk women, from HIV-infected women (DHIV), recent migrant women (DDRI), girls aged 13-18 years recruited through pediatric visit (D1318P) and young women aged 13–25 years (D1325), compared to one control group of women attending a spontaneous screening program (DASS). Adult participants undergo conventional cervical cytology, HPV DNA screening and genotyping. Pediatric participants undergo HPV DNA testing and genotyping of urine samples. HPV DNA, cytological abnormalities and HPV types will be analyzed according to demographic, epidemiological, behavioral, and clinical data collected in an electronic case report form. The follow up timing was defined by specific algorithms based on cytology and biomolecular results. The results from this study will allow to define specific strategies of primary and secondary prevention of the cervical cancer in the studied population. Between November 2010 and December 2013, 760 women were enrolled in the DHIV cohort, 393 in the DDRI, 1252 in the D1318P, 501 in the D1325G, and 1368 in the DASS, for a total of 4,274. Of these, 655 had at least one follow-up.

GENETIC VARIANTS AND SUSCEPTIBILITY TO SEVERE AND/OR RECURRENT LOWER RESPIRATORY TRACT INFECTIONS WITH WHEEZING IN CHILDREN

Lower respiratory tract infections (LRTIs) with wheezing are common in young children, and have a cumulative prevalence of up to 40% in the first six years of life. They are a major cause of morbidity and reduce the affected children's health-related quality of life because they are often severe and/or highly recurrent, and because up to 50% of children experiencing recurrent virus-induced wheezing in infancy later develop chronic asthma. Almost all LRTIs are due to viruses, the most frequent being respiratory syncytial virus (RSV), rhinovirus, parainfluenza virus and human metapneumovirus. The main aim of this project – that started in 2012 and will end in 2015, conducted by the Pediatric Clinic of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, with the collaboration of our Department – is to analyze possible
correlations between specific genetic defects in innate immunity (such as TLR mutations) and/or cytokine production and the risk of developing severe and/or recurrent LRTIs with wheezing in children. We also investigate the relative importance of the different viruses capable of causing LRTIs with wheezing in determining severity and recurrences. Finally, as inhaled steroids can significantly modify the outcome of wheezing episodes, in this project we assess the importance of steroid prophylaxis in reducing the risk of recurrences in genetically predisposed children.

**SPIRAL COMPUTED TOMOGRAPHY AND MIRNA ASSOCIATED TO A PRIMARY PREVENTION PROGRAM FOR THE EARLY DETECTION OF LUNG CANCER**

The MILD study conducted by the Istituto Nazionale per lo Studio e la Cura dei Tumori (INT), Milan aims to assess the efficacy of lung cancer screening in reducing the mortality in heavy smokers by using spiral-computed tomography (CT). Preliminary result on 4000 subjects showed that there was no favourable effect in terms of mortality in subjects who carried out a CT scan every year or every two years compared to those who did not carry CT after 6 years of follow-up. We are also collaborating in new prospective study conducted by INT which aims to determine whether microRNAs in plasma are able to improve the effectiveness of spiral CT in the early diagnosis of lung cancer in high-risk individuals. This study will assess the effectiveness of the test with plasma miRNAs as first-line examination, non-invasive, low-cost screening of lung cancer in high-risk individuals and the choice of optimal treatment.

**PUBLIC HEALTH PREVENTION AND INFORMATION**

The major products of our activity have also been published in the lay press, in order to increase the project impact on prevention and public health.

**Laboratory of Epidemiological Methods**

**CANCER MORTALITY IN EUROPE**

The Laboratory of Epidemiologic Methods has developed an integrated system for monitoring, modeling and interpreting cancer mortality statistics in Europe. Since its beginning in 1992, the project has had a considerable scientific production in spite of its low costs, and the Laboratory has acquired new tools and expertise, and collaborations with Italian and international research groups have been established. At the core of the project there is the European database on cancer mortality that the Laboratory has built and periodically updated, which derives from the WHO raw mortality data, integrated by other sources, whenever required. The database includes numbers of cancer deaths by country, cause, period, sex and age in Europe and selected other countries, together with estimates of the resident population. The aim of the project is to: i) periodically update the project’s database with data for more recent years; ii) update the systematic analysis of cancer mortality in Europe, and verify if the forecasts of a continuing fall in cancer mortality in Europe are met; iii) apply age-period-cohort models to help in the interpretation of rates, and assist in projection of trends; iv) monitor cancer mortality in Central and Eastern Europe and in selected middle income countries of the world, where delays in the adoption of effective strategies for cancer prevention, management and treatment have been apparent; v) further monitor tobacco-related mortality in Europe, highlighting successes and failures in smoking prevention efforts in various populations, with specific focus on women; vi) evaluate to what extent mortality statistics can contribute to the current scientific debate on the effectiveness of (organized) screening programs for cancers of the breast, prostate and colorectum; vii) quantify the burden and investigate trends of cancer mortality in older people;
and vii) develop and test a system to obtain short-term projections of cancer mortality. The project is not merely descriptive, since specific effort is devoted to the interpretation of the observed data in the light of epidemiological knowledge, highlighting information that can generate new hypotheses on cancer etiology. It offers a unique opportunity for the continuous exploitation of vital statistics in Europe, with the primary aim of monitoring and improving cancer prevention.

NOVEL HIGH COST CHEMOTHERAPIES: CLINICAL USE, SAFETY AND EFFECTIVENESS AFTER MARKETING APPROVAL IN ONCOLOGY PRACTICE

The objective of this project is to provide a detailed description of clinical use of selected new “targeted” high cost drugs in the Lombardy oncology practice, including the time trends of prescriptions, the physician compliance to Italian Medicine Agency (AIFA) approval indications, the evaluation of the frequency of major side effects, and the evaluation of the survival after treatment through healthcare databases. An additional objective is to investigate the clinical effectiveness of the therapies of interest on selected cancers, including colorectal, breast, and lung.

First publications investigated the clinical use of bevacizumab in patients with metastatic colorectal cancer and of trastuzumab in women with early or metastatic breast cancer, with focus on their frequency of serious adverse events. There was a gap between bevacizumab approval indication and clinical practice patterns. The frequency of serious adverse events and the survival rates of metastatic colorectal cancer were similar to the results reported in experimental clinical trials leading to drug approval. For trastuzumab, the incidence of short-term severe cardiotoxicity (not only congestive heart failure) in clinical practice was higher than that recorded in clinical trials testing the same regimen. Age and history of cardiac disease were strong predictors of cardiotoxicity.

THE STOMACH CANCER POOLING (STOP) PROJECT

Various consortia of epidemiological studies have been established during the last two decades, to pool and analyse data on risk factors for various cancers, but no such effort has yet been made for gastric cancer. A concerted strategy for the joint analysis of epidemiological investigations may allow new insights on gastric cancer aetiology. We have initiated a consortium of epidemiological investigations, named the “Stomach cancer Pooling (StoP) Project”. This is a pooled-analysis of worldwide studies (mainly of case-control design), using an individual-level data approach. Twenty-two studies from eleven countries have agreed to participate, for a total of about 9500 cases and over 22,000 controls. Subsequent development phases of the project will be: to complete data collection from participating studies; to harmonize the datasets using a standardized format; to conduct two-step statistical analyses to estimate the pooled odds ratios and 95% confidence intervals for several risk factors of interest; to conduct subgroup and other analyses to allow the interpretation of results; and to report the findings in a number of research papers. A project website will also be developed. Our aim is to examine in an uniquely large dataset the role of several risk factors for gastric cancer. Analyses of genetic factors will also be performed, by focusing on single nucleotide polymorphisms (SNP) identified in previous genome-wide association studies, and addressing potential gene-environment interactions. The large dataset will also allow to conduct separate analyses according to different histotypes (i.e., intestinal/diffuse type) and subsites (i.e., cardia/non-cardia) of gastric cancer, in order to identify potential different risk patterns and aetiological characteristics among subgroups of gastric cancer. Our Department at the IRCCS Istituto di Ricerche Farmacologiche Mario Negri has a central role in the project, as we are among the promoters of StoP consortium, and we will be responsible for data collection, harmonisation,
and validation, besides data analysis and publication of manuscripts on selected risk factors for gastric cancer.

**Laboratory of Epidemiology of Chronic Diseases**

**CASE-CONTROL STUDIES CONDUCTION**

Organization for the collection of information on patients’ selected characteristics and lifestyles, and of biological samples for case-control studies

Data collection of epidemiological data is going on and it includes: 1) interviews and interviewer management and training activity for new interviewers; 2) contacts with hospital department and ethical committee for study approval and conduction; 3) check for consistency and codification of patient questionnaires; 4) diagnosis and histological exam check; 5) organization and management of biological sample collection; 6) data input management.

Ongoing case-control studies include: adenocarcinoma of the esophagus-cardias, cancer of the bladder, and sarcomas. The overall updated dataset include about: 1250 cases of cancers of oral cavity and pharynx, 700 of the esophagus, 1100 of the stomach, 6500 of the colorectum, 600 of the liver, 120 of the biliary tract, 600 of the pancreas, 850 of the larynx, 500 cutaneous malignant melanoma, 7000 of the breast, 1000 of the cervix, 1000 of the endometrium, 200 of trophoblastic gestational disease, 200 of the vulva, 2000 of the ovary, 1300 of the prostate, 700 of the bladder, 800 of the kidney and renal pelvis, 600 of the thyroid, 200 of Hodgkin disease, 500 of non-Hodgkin disease, 450 of sarcomas, 300 of myelomas and about 18,000 controls.

Biological sample collection, aimed to study genetic polymorphisms, includes cancers of the oral cavity, pharynx, larynx, bladder, colorectum and sarcoma.

**SOFT TISSUE SARCOMAS: CASE-CONTROL STUDY OF RISK FACTORS AND A DESCRIPTIVE STUDY OF PRE-DIAGNOSTIC CLINICAL HISTORY**

Soft tissue sarcomas (STS) have low incidence resulting in a low statistical power in etiological studies and a limited experience of general practitioners in the clinical practice, often leading to diagnostic delays. Their dual classification by anatomical site and histology causes confusion in assessing their etiology. This project includes two integrated studies. The first study (case-control, coordinated by the Mario Negri Institute) is based on a validated questionnaire (with many covariates and based on a detailed food composition databases), the use of appropriate statistical analyses and measurements of toxic agent levels in biological tissues. The second (clinical study, coordinated with the collaboration of the University of Turin, Dipartimento di Medicina del Lavoro/CTO Maria Adelaide) is based on questionnaires reporting the history of medical visits and procedures before hospital admission, and detailed socioeconomic characteristics of cases. Cases are followed-up for 5 years. We collect blood samples and adipose tissue (in chirurgic patients) from cases and controls and neoplastic tissues from cases. The case-control study is aimed to identify and quantify risk factors and attributable risks in Italy for STS whose etiology is largely unknown. The clinical study is aimed to assess the clinical history of STS before hospital admission and its impact on the severity of the disease at correct diagnosis, and whether they can be influenced by patient socioeconomic characteristics and geographic area of residence. The major strengths of this project are: the large dataset due to the participation of most Italian reference hospitals for STS treatment; the detailed information on anatomical site and hystopathological type of STS; the interdisciplinary approach; the quantification of STS risk factors; the creation of a research biorepository for molecular genetic and for cytogenetic analyses; the preparation of guidelines contributing to early management of STS by general practitioners.
BLADDER CANCER STUDY
This project includes two parts: 1) the conduction of a case-control study of risk factors and genetic susceptibility of bladder cancer; 2) the collaboration in the International Consortium of Bladder Cancer (ICBC). Besides tobacco and exposure to aromatic amines, the main known risk factors for bladder cancer, several other factors have been considered, although their quantification and causal relation have not be assessed. Our case-control study of risk factors and genetic susceptibility of bladder cancer is designed to collect information and analyze the association with bladder cancer in relation to: family history, known risk factor whose quantification is still undetermined; coffee consumption, to establish whether, the moderate direct association observed in a few studies is real or due to confounding; fluid intake, as a low intake concentrate metabolites in urines and increases the contact of bladder epithelium with potential cancerogens; intake of selected drugs; diet, in terms of macro- and micronutrients, food groups and dietary patterns; professional and personal use of hair-dyes. The International Consortium of Bladder Cancer was formed in 2005 as an open scientific forum for epidemiologic research in bladder cancer. Investigators with bladder cancer studies, completed or ongoing, consider proposals for projects that pool data across studies or undertake coordinated research. The main aims of the bladder cancer consortium are: to have a forum for discussion in studying the molecular epidemiology of bladder cancer, and to facilitate the pooling of comparable data on environmental and genetic risk factors across studies in order to overcome the limited power of individual studies. Possible areas of collaboration include the evaluation of complex multigenic effects, interactions with cigarette smoking and other exposures, evaluation of sex-specific effects, evaluation of heterogeneity of genetic effects by cancer subgroups. We participate to three proposal evaluated by the Coordinating Committee: 1) to evaluate the association between hair dye use with bladder cancer, pooling data from case-control studies on bladder cancer with high quality information on hair-dye use. Moreover, genotyping data on metabolic pathways are also considered, mainly to evaluate the interaction of polymorphisms of genes involved in the metabolic pathways of hair-dyes (NAT1, NAT2, CYP2A1, GSTs and possibly other) on the risk of bladder cancer, and possibly to evaluate whether exposure to hair-dyes is associated with presence of p53 mutations; 2) to study the effect of the family history on the risk of bladder cancer, by investigating the risk associated with probands having first and second degree family members with bladder cancer and with cancers at other anatomical sites; 3) to investigate the effect of diet on the risk of bladder cancer, considering individual foods, macro- and micronutrients, groups of foods and dietary patterns.

COFFEE INTAKE AND THE RELATION WITH VARIOUS DISEASES
Coffee is the second most common beverage in the world after tea. Thus, any health effect of coffee is an important issue of public health. Besides caffeine, coffee contains many bioactive compounds with potential effects on health, including minerals and antioxidants, mainly phenolic compounds (such as chlorogenic, caffeic, ferulic and cumaric acids), melanoidsins and diterpenes (such as cafestol and kahweol), and coffee has been related with lower incidence of several diseases. In the last ten years we have studied the relation of coffee and decaffeinated coffee intake and cancer at several sites in our case-control studies, finding no relation with cancer of the esophagus, stomach, pancreas, larynx, melanoma, breast, ovary, prostate, kidney and non-Hodgkin disease, and finding an inverse relation of coffee with cancer of the oral-cavity and pharynx, colorectum, liver (including liver cirrhosis) and endometrium. Moreover we have conducted a series of meta-analysis on the relation of coffee and decaffeinated coffee with total and cause-specific mortality, cancers of the esophagus, pancreas, larynx and brain, confirming the absence of relation, and with cancers of the oral-cavity and pharynx (including a pooled analysis), colorectum, liver and endometrium confirming an inverse association.
Laboratory of Medical Informatics

Studies on the use of social media and medapps by health professionals in Italy
The Laboratory of Medical Informatics is involved in surveys which aim is to describe how physicians and health professionals are using social media tools (with particular interest on Facebook, Twitter and YouTube) and medical applications for smartphones and tablets. Such kind of surveys are ongoing in collaboration with ANMCO (Italian Society of Cardiologists), AIOM (Italian Society of Oncologists) and SIU (Italian Society of Urologists) to discover how these tools are used by Italian cardiologists, oncologists and urologists.

Studies on the use of social media by health organizations in Italy
The Laboratory of Medical Informatics is involved in a survey which aim is to describe how the 1,200 health organizations in Italy are using the web 2.0 and the social media tools (with particular interest on Facebook, Twitter and YouTube) as new media to communicate and share information with patients and citizens.

Training activities
In 2013, the Laboratory of Medical Informatics continued its training activity on issues related to the use of the Internet in medicine, and extended it to the use of the recent social media and web 2.0 technologies and tools in the medicine area. The members of the laboratory staff activated (or attended as invited teachers) a number of training courses, workshops, and master courses. Onsite CME courses for the Italian physicians have also been organized using the training/educational facilities and equipment available at the Mario Negri Institute.

Maintenance of the CARDIO.CARE, ONCO.CARE, GASTRO.CARE, NEURO.CARE, PNEUMO.CARE, BPCO.CARE, PAIN.CARE and DERMA.CARE websites
These indexes have been developed by the Laboratory of Medical Informatics in order to collect, classify, evaluate, and describe the most useful medical information on the web, and to provide Internet users with an easy means to surf the net. Several medical areas are covered including oncology (http://www.oncocare.it), neurology (http://www.neurocare.it), gastroenterology (http://www.gastrocare.it), cardiology (http://www.cardiocare.it), pulmonology (http://www.pneumocare.it, http://www.bpcocare.it), the pain care and management (http://www.paincare.it), and dermatology (http://www.dermacare.it). The project is in collaboration with intramural departments (Department of Oncology, Laboratory of Neurological Disorders and Department of Cardiovascular Research, Laboratory of General Practice Research, Laboratory of Translational and Outcome Research in Oncology) and extramural research groups (Italian Group for Epidemiologic Research in Dermatology, GISED).
DEPARTMENT OF PUBLIC HEALTH

STAFF

Head of Department
Maurizio BONATI, MD.

"Angelo & Angela Valenti" Centre for Health Economics (CESAV)
Head of Laboratory
Livio GARATTINI, Econ.D.

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Clinical Knowledge Engineering Unit
Head of Unit
Davide LUCIANI, MD.

Laboratory for medical research and consumers involvement
Head of Laboratory
Paola MOSCONI, Dr.Sci.Biol.

Laboratory for Mother and Child Health
Head of Laboratory
Maurizio BONATI, MD.

Pharmacoepidemiology Unit
Head of Unit
Antonio CLAVENNA, MD.
CURRICULA VITAE

Maurizio Bonati has a Medical School degree at the University of Milan. Areas of interest: Monitoring and epidemiological evaluation of drug utilisation and effects of drugs and vaccines in motherhood and childhood. Research methodology in general hospital and paediatric community practice. Transfer of information to the community. Epidemiology of paediatric and perinatal care.

Past and present roles both at the Mario Negri Institute and in other institutions: 1973-77 Research Fellow at the IRFMN, within the Neurochemistry Lab.; 1977-85 Research Assistant at the IRFMN, within the Clinical Pharmacology Lab.; 1986-93 Chief of the Perinatal Clinical Pharmacology Unit at the IRFMN; Advisor to WHO for the Drug Utilisation Research Group (pregnancy, paediatrics and breastfeeding); 1987-92 coordinator of the International Cooperative Study of Drug Use in Pregnancy, under the auspices of WHO and the support of EEC; 1992-93 co-editor of The Kangaroo; 2000-05 coordinator of the European Cooperative Study: “Development of the European register of clinical trials on medicines for children” (DEC-net), under the 5th Framework Programme’s Quality of life and Management of Living Resources; since 1989 he has been director of the Centre for Drug Information; since 1993 head of the Lab. for Mother and Child Health; since 1997 teacher for the Lombardy region’s professional training courses; since 2000 teacher for the Lombardy region’s professional training courses; since 2002 Editor of the Ricerca & Pratica scientific journal; since 2003 professor of the School of Specialisation in Paediatrics - University of Milan Bicocca; teacher at the annual European course “Evaluation of Medicinal Products in Children” (promoted by ESDPPP and Eudipharma); from May 2008 Head of Department Public Health at the "Mario Negri" Institute for Pharmacology Research; since 2010 coordinator of the European Cooperative Study “COHEMI-Coordination resources to Assess and Improve health status of migrants from Latin America”, under the 7th Framework Programme for Research and Technological Development (Programme Cooperation- Health).

Selected publications


Guido Bertolini got his Medical degree in 1989 at the University of Bologna, and the specialization in Pharmacological Research in 1993 at the “Mario Negri” Institute and in Gastroenterology in 1994 at the University of Pavia.

He founded and chaired from 1997 to 2000 the School of Clinical Methodology and Quality of Care Improvement at the Ospedali Riuniti di Bergamo and the Istituto di Ricerche Farmacologiche Mario Negri. From 1999 to 2003 he has been contract professor at the post-doctoral schools in Anaesthesiology and intensive Care, University of Brescia and Milano; from 2002 to 2005 he has been contract professor of Educational Science at the Faculty of Lettere e Filosofia, University of Bergamo.

Current research interests: Clinical Research Methodology, Continuous Quality of Care Assessment and Improvement, Health services research and outcome, Medical decision making, Medical Education. These interests are mainly developed within the fields of Intensive Care Medicine and Rare Diseases.
Since 1997 he chairs the GiViTI Coordinating Center for research in intensive care medicine. He has been Head of the Unit of Epidemiology and Education for Clinical Practice at the “Mario Negri” Institute and since 2001 he is the Head of the Laboratory of Clinical Epidemiology. From 2001 to 2005 he has been Vice-chairman of the Research Group on Cost-effectiveness, Section on Health Services Research and Outcomes – European Society of Intensive Care Medicine and, from 2001 to 2005, he has been President of the Scientific Committee of the “Ospedale maggiore” in Crema.

Selected publications:

Livio Garattini: got his degree in Economics in March 1983 at the Bocconi University in Milan.


Areas of interest: Health Economics and Health Policy Analysis.


Selected publications:

Paola Mosconi, Biol. Sci. D., graduated in 1982 (University of Milano), with a post-doctoral degree in Pharmacological Research (1984), is at present Head of the “Laboratory for medical research and consumer involvement” of the department of Public Health. Paola Mosconi is involved in several projects on issues pertaining the patient involvement in care aspects and outcome research. She published more than 300 articles in leading national and international journals, as well as books on issues related to her main areas of interest.
Significant experiences has been coordinated:
- development of research projects and strategies to involve patients or consumer associations in health debate, and clinical research, as consensus conference, jury of citizens;
- training for consumers on quality of information, and methodological aspects of clinical research;
- studies for estimate the type of information on diseases and treatments received by patients, mainly in cancer patients; set-up of websites targeted on consumers/patients [www.parcepicasalute.it, www.fondazionemattioli.it];
- studies for estimate the consumers’ level of satisfaction with the health services and cure;
- projects on the assessment of Quality of Life in randomised clinical trials or in epidemiological survey; translation and cultural adaptation of questionnaires for Quality of Life.

Paola Mosconi has participated as teacher, or coordinator, to the realization of training course on “Methodological aspects of clinical research” or “Evaluation of quality of life” for health care professionals and representatives of voluntary associations.

Paola Mosconi is the co-founder of the Italian Forum of EUROPA DONNA, i.e. a federation of European National groups created in 1991 with the aim of promoting ten programmatic points regarding prevention, information, lobbying actions, and quality of care for breast cancer patients. In Italy EUROPA DONNA activities involve more than 80 associations.

Paola Mosconi has been member of Ethics Committees, in one serve as president.

Selected publications:
- Donati S, Satolini R, Colombo C, Senatoro S, Cocolini R, Da Cas R , Spila Alegrani S, **Mosconi P**. Informing women on menopause and hormone therapy: *Know the menopause* a multidisciplinary project involving local healthcare system. PLOSOne 2013: 8 (12); http://dx.plos.org/10.1371/journal.pone.0085121

**Antonio Clavenna** graduated from the University of Milan with a degree in Medicine in 1994 and he is specialist in Clinical Pharmacology. He took his PhD at the Open University, London, in 2009. Since 2000 he has been working at the "Mario Negri" Research Institute of Milan as a Research Fellow in specialist in Clinical Pharmacology. He took his PhD at the Open University, London, in 2009.

Davide Luciani got his Medical Degree at the University of Bologna in 1995, and the post-doctoral certificate in "Tropical Medicine and Hygiene" at the University of Liverpool in 1997. In 2001, he spent one year at the Department of Statistical Science (University College London). Bayesian probabilistic applications, decision theory and the graphical approach to pathophysiological modelling represent his main interests. Within his research activity, these skills are meant as the main methodological ingredients in the formalization of clinical reasoning, in order to improve its effectiveness and to exploit its educational value. Since 2005 he is responsible of the Unit of Clinical Knowledge Engineering.

Selected publications

ACTIVITIES

The main aim of the Public Health Department is to understand which factors affect the health of individuals or entire populations and to define effective interventions for responding to their health needs. Special emphasis is therefore placed on prevention, so that the risks of contracting illness are lowered, and on the dissemination of independent, evidence-based information. The department’s effort cannot disregard the National Health System, however, which must guarantee access to, and quality of, care that is based on principles of equity and appropriateness and must guarantee it especially to the more vulnerable patient groups. It is in this context that the Public Health Department carries out its activities. In addition to its formal research activity, the department participates in, and organises, initiatives involving information dissemination, training, and debate aimed at healthcare professionals and social care workers, but also at the general population. These activities are also supported by the publication of the department’s two journals: *Ricerca&Pratica* and *Quaderni di Farmaco Economia*, and by the development of the @Partecipasalute website (www.partecipasalute.it)

"A. and A. Valenti" Centre for Health Economics (CESAV)

The "Angelo e Angela Valenti" Centre for Health Economics (CESAV) was established in 1992 at the "M. Negri Institute" and based at Villa Camozzi - Ranica (Bergamo) - Italy. CESAV is primarily a research centre, but also does educational work. The centre is involved in health economics and health policy research. The main areas of research are: Economic Evaluation of
Health Care Programs (i.e. assessment of costs and benefits of alternative health care treatments and services) and Comparative Health Policy Analysis (i.e. study of domestic and foreign health care systems, in particular aimed at identifying possible innovations for European countries).

**Laboratory of Clinical Epidemiology**

The general aim of the Laboratory of Clinical Epidemiology is to contribute to the improvement of health care in different medical fields. The guiding principles are mainly two: to help physicians in using the available knowledge and resources at their best, and to contribute to the growth of applied knowledge for clinical practice. The Laboratory operates in the field of Intensive Care Medicine. In the main area of activity the laboratory developed an electronic health record for the ICU which serves the dual purpose of simplifying and improving clinical documentation and provide accurate data to search for the improvement of quality of care. Within the Laboratory, the Unit of Clinical Knowledge Engineering aims to bring the value of clinical reasoning out, through the implementation of probabilistic models for its formalization, thus favouring the evaluation and the continuous improvement of complex clinical activities.

**Laboratory for medical research and consumers involvement**

The Laboratory promotes different research activities aimed at developing the participation of citizens and patients & their representatives to the decisions process regarding health. Among these consensus conferences, citizens' juries, *ad hoc* surveys. The Laboratory organizes a training course specifically dedicated to representatives of associations of citizens and patients that allows to deal effectively with the medical and scientific world. Other lines of laboratory research:

- projects for the assessment of the type of information provided on diseases and treatments;
- research on the best ways for the publication of health information and the results of scientific research, the development of internet portals on the issues of health information (www.partecipasalute.it, www.fondazionemattioli.it, http://indeep.istituto-besta.it/);
- projects involving groups of patients for the publication of information material;
- projects involving the assessment of quality of life and health, either through *ad hoc* studies on selected groups of patients, both through the development of questionnaires.

The second experience of deliberative democracy was completed through the method of juries of citizens on health issues, particularly on the PSA test for prostate cancer.

**Laboratory for Mother and Child Health**

The main objective of the Laboratory for Mother and Child Health is to ensure a better mother and child well-being by undertaking interdisciplinary and collaborative work in the field. Four broad areas, or spheres, of research have been selected:
- monitoring and epidemiological evaluation of utilisation and effects of drugs and vaccines;
- research methodology in general hospital and paediatric community practice;
- public health determinants of children’s well-being;
- transfer of health information to the community.

Special attention is given to activities involving countries in the north and south of the world. In addition to the formal research activities, the Laboratory promotes initiatives in the public health field, in particular those involving mother and child health care. The initiatives involve the participation in, and the organisation of, educational, training, and information-dissemination activities.
The critical and active transfer of scientific knowledge is a continuous, daily stimulus to the laboratory’s activity.

NATIONAL COLLABORATIONS

"A. and A. Valenti" Centre for Health Economics (CESAV)

Public and private institutions, other health care organizations (Ministry of Health, Regional and Local Health Authorities, Hospital Trusts).

Laboratory of Clinical Epidemiology

- Ospedale A. Manzoni, U.O. Anestesia e Rianimazione 1, Lecco.
- Ospedale San Giovanni Bosco, Servizio Anestesia e Rianimazione, Torino
- Università degli Studi di Brescia, Dipartimento di Specialità Chirurgiche, Scienze Radiologiche e Medico Forensi, Cattedra di Anestesia.
- A.O.Universitaria Careggi
- Università di Milano Bicocca, Dipartimento di Informatica Sistemistica e Comunicazione.
- Università degli Studi di Verona.
- CNT, Centro Nazionale Trapianti.

Laboratory for medical research and consumers involvement

- Age.Na.S. Agenzia Nazionale per i Servizi Sanitari Regionali, Roma
- Alleanza contro il Tumore Ovarico ACTO, Milano
- Associazione Alessandro Liberati – Network Italiano Cochrane
- Associazione Italiana Sclerosi Multipla AISM, Genova
- Azienda Ospedaliera, Arcispedale S. Maria Nuova, Reggio Emilia
- Centro Cochrane Italiano, Modena
- Fondazione Attilia Pofferi, Pistoia
- Fondazione per la ricerca sulla Fibrosi Cistica onlus FFC, Verona
- Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano
- Fondazione Nerina e Mario Mattioli Onlus, Milano
- Regione Toscana-GRC Gruppo Gestione Rischio Clinico, Firenze
- Zadig agenzia di editoria scientifica, Milano

Laboratory for Mother and Child Health

- Associazione Culturale Pedietri (ACP)
- A.O. Spedali Civili di Brescia
- Centro Antiveleni –Tossicologia Clinica – Ospedali Riuniti di Bergamo
- Centro per la Salute del Bambino (CSB)
- Fondazione Emanuela Zancan Onlus
- Il Pensiero Scientifico Editore
- Istituto di Istruzione Superiore Giuseppe Lagrange, IPSEOA Gianni Brera
– Istituto Superiore di Sanità (ISS)
– Istituto Don Calabria CTD Negrar
– Osservatorio Italiano Salute Globale (OISG)
– Unità Operativa di Neuropsichiatria dell'Infanzia e dell'Adolescenza, Fondazione IRCCS Ca’ Granda – Ospedale Maggiore Policlinico di Milano
– Università degli Studi di Firenze – Dipartimento Area Critica Medico Chirurgica Clinica Malattie Infettive, S.O.D. Malattie Infettive e Tropicali
– Università degli Studi di Milano, Bicocca – Facoltà di Medicina – Clinica Pediatrica
– Università degli Studi di Pavia – Scuola di Specializzazione in Neuropsichiatria Infantile

INTERNATIONAL COLLABORATIONS

"A. and A. Valenti" Centre for Health Economics (CESAV)

– CES (Collège des Economistes de la Santé) of Paris
– Corvinus University of Budapest
– Global Fund of Geneva
– Servicio Canario de la Salud, S/C de Tenerife
– University of Birmingham
– University of Hannover
– University of York
– University Pompeu Fabra of Barcelona
– University Erasmus of Rotterdam
– WidO of Bonn

Laboratory of Clinical Epidemiology

– Clinica di Anestesiologia e Terapia Intensiva, Jena, Germania
– Dipartimento di Anestesiologia e Cure Intensive, Università di Varsavia, Polonia
– Dipartimento di Cure Intensive, Ospedale Generale di Novo Mesto, Slovenia
– Dipartimento di Pneumologia e Cure Intensive, Ospedale Generale di Nicosia, Cipro
– Istituto Bloomsbury di Cure Intensive, Istituto di Ricerca Biomedica, University College Londra, Regno Unito
– Istituto di Anestesia e Cure Intensive, Università di Semmelweis, Budapest, Ungheria
– Machine Intelligence Group, Università di Aalborg, Danimarca
– Terapia Intensiva Pediatrica, Soroka University Medical Center, Beer-Sheva, Israele

Laboratory for medical research and consumers involvement

– Centre for Health Communication and Participation, Australian Institute for Primary Care and Ageing, La Trobe University, Melbourne, Australia
– Cochrane Consumer network, United Kingdom
– European AIDS Treatment Group, Belgium
- German Network of the Coordinating Centres for Clinical Trials U Koeln, Germany
- Institute National de la Santé et de la Recherche Médicale, France
- Oxford University Hospitals, United Kingdom
- Rigshospitalet, Copenhagen University Hospital Copenhagen, Trial Unit, Denmark
- University Medical Center Freiburg (Universitätsklinikum Freiburg), Germany

**Laboratory for Mother and Child Health**

- Agenzia Europea per i Medicinali (EMA)
- Centro di Epidemiologia Comunitaria e Medicina Tropicale (CECOMET), Ecuador
- Clinica Infantile Colsubsidio, Colombia
- Coletivo de Estudios Aplicado y Desarrollo Social Juan XXIII, Bolivia
- European Society for Developmental Perinatal & Paediatric Pharmacology (ESDPPP)
- Fundació Privada Clinic per la Ricerca Biomedica, Spagna
- Fundacion Salud Ambiente y Desarrollo, Ecuador
- International Society of Drug Bulletins (ISDB)
- Ospedale Robert Debré, Francia
- Organizzazione Mondiale della Sanità (OMS)
- Taller de Educacion y Comunicacion Guarani Asociacion, Bolivia
- Unione Europea (UE)
- Università di Amsterdam – Universiteit Van Amsterdam, Olanda
- Università College London Hospital NHS Foundation Trust, UK
- Università di Nottingham - Derbyshire Children's Hospital, UK
- Universidad Peruana Cayetano Heredia, Perú

**EDITORIAL BOARD MEMBERSHIP**

"A. and A. Valenti" Centre for Health Economics (CESAV)

**INTERNATIONAL:**
Acta Bio Medica; Applied Health Economics and Health Policy; Biomedical Statistics and Clinical Epidemiology; BMC-Health Services Research; Health Policy; The European Journal of Health Economics; Generics and Biosimilars Initiative Journal; Journal of Medical Economics.

**NATIONAL:**
FarmacoEconomia News; Quaderni di FarmacoEconomia.

**Laboratory of Clinical Epidemiology**

**NATIONAL:**
Laboratory for medical research and consumers involvement

INTERNATIONAL:
Health and Quality of Life Outcomes

NATIONAL:
www.partecipasalute.it
www.fondazionemattioli.it

Laboratory for Mother and Child Health

INTERNATIONAL:
European Journal Clinical Pharmacology; Journal of Clinical Pharmacology & Pharmacoepidemiology; Saludarte.

NATIONAL:
Quaderni di Farmacoeconomia; Ricerca & Pratica.

PEER REVIEW ACTIVITIES

"A. and A. Valenti" Centre for Health Economics (CESAV)

Applied Health Economics and Health Policy; BMC-Health Services Research; British Medical Journal Health Policy; PharmacoEconomics; Epilepsia; The European Journal of Health Economics; Expert Review of Pharmacoeconomics & Outcomes Research; Generics and Biosimilars Initiative Journal; Journal of Medical Economics.

Laboratory of Clinical Epidemiology

INTERNATIONAL:
Intensive Care Medicine; Critical Care Medicine; American Journal of Respiratory and Critical Care Medicine; BMJ Open.

NATIONAL:
Ricerca & Pratica

Laboratory for medical research and consumers involvement

INTERNATIONAL:
Health Expectations, The Breast, Health and Quality of Life Outcomes; Cochrane Collaboration; Journal of Biological Markers.

NATIONAL:
Ricerca & Pratica

Ricerca & Pratica;
Dedalo. Gestire i sistemi complessi in sanità.
Laboratory for Mother and Child Health

INTERNATIONAL:

NATIONAL:
Annuali Istituto Superiore di Sanità; Medico e Bambino.

NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP

Laboratory of Clinical Epidemiology

- National Health Plan Research Commission, Emilia Romagna Region

Laboratory for medical research and consumers involvement

- Comitato AIOM, Linee Guida psicosociali
- Comitato Direttivo Onlus Attilia Pofferi
- Comitato Direttivo Fondazione Nerina e Mario Mattioli onlus
- Comitato Etico Arcispedale Santa Maria Nuova, Reggio Emilia
- Comitato Etico Azienda USL Bologna
- Comitato Guida Slow Medicine
- Comitato Tecnico Scientifico Associazione ACTO

Laboratory for Mother and Child Health

- Ethical committee A.O. "Ospedale Maggiore" of Crema
- Therapeutic Formulary, Valle d'Aosta Autonomous Region
- Independent Expert Group for the assessment of "Impacts of framework programmes in the area of Public Health Research" European Commission, DG Research & Innovation

EVENT ORGANIZATION

"A. and A. Valenti" Centre for Health Economics (CESAV)

May
Laboratory for medical research and consumers involvement

June
Citizens’ juries for health: il caso modello dello screening per il carcinoma della prostata, 14-15 June 2013, Modena.

October
Congress Fondazione Nerina e Mario Mattioli onlus. Il tumore dell’ovaio: una visione a 360°, 29 October 2013, Milan.

November

December
Congress Associazione Alessandro Liberati, Network Italiano Cochrane. Open, dalla condivisione dei risultati ad una ricerca trasparente per il bene comune, 13 December 2013, Napoli.

Laboratory of Clinical Epidemiology

January
Workshop, CREACTIVE Meeting, January 22-23, Ranica (BG).
Workshop, PROSAFE, core pediatrico meeting, January 24, Ranica (BG).

February
Workshop, DOMUS meeting, February 19, Ranica (BG).

May
Workshop, Kick-off meeting COMPACT-2, May 28, Ranica (BG).

June
Workshop, Meeting annuale MargheritaTre, June 03-04, Ranica (BG).

September
Workshop, Meeting TI Cardiochirurgiche, September 12, Ranica (BG).

November
Congresso, Meeting annuale GiViTI/ CREACTIVE kickoff meeting, November 13-15, Pesaro.

Laboratory for Mother and Child Health

May
1\textsuperscript{th} edition IO PEDALO PER LA RICERCA “Tutti in bicicletta. A spasso tra i luoghi della ricerca”. Laboratorio per la Salute Materno Infantile dell’IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.
Congresso “ADHD per una condivisione dei percorsi diagnostico terapeutici”. Laboratorio per la Salute Materno Infantile dell’IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.

October
Congresso “Bisogni comunicativi complessi e partecipazione nei contesti di vita, verso una conoscenza diffusa?”. Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Laboratorio per la Salute Materno Infantile dell’IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.

December
Congress “Progetto Migranti: 5 anni di dialogo tra il bisogno e la cura”. Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Laboratorio per la Salute Materno Infantile dell’IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.

CONFERENCE AND WORKSHOP CONTRIBUTIONS

"A. and A. Valenti" Centre for Health Economics (CESAV)

April
Optional copayments on anti-cancer drugs, is it an option? Regional tenders in Italy on biosimilars, potentially competitive? Congress “Third Croatian and third adriatic congress on pharmacoeconomics and outcomes research”. Section for Pharmacoeconomics and Outcomes Research, Croatian Society of Clinical Pharmacology and Therapeutics, Croatian Medical Association; Brjuni.

Dicembre
HTA in Italy promising or confusing? 5th Annual Market Access Day “The Place of Uncertainty within Public Decision”. The European Market Access University Diploma (EMAUD) for Life Sciences; Parigi.

Laboratory of Clinical Epidemiology

January

February

March
Utilizzo della cartella clinica elettronica Margherita Tre. Course: “Utilizzo del software Margherita Tre” – Firenze.

May

ANNUAL REPORT 2013

**June**

**September**
Congress: “World sepsis day” – Milano.

**October**
Other infectious disease research networks: models, lessons learnt and collaboration with GLOPID-R. Challenges, experiences, solutions, lessons learnt. Contributions to and collaboration with GLOPID-R. Congress: “Global Research Collaboration for Infectious Disease Preparedness” – Annecy (Francia)
Course: “I gruppi collaborativi nella ricerca Biomedica” – Bergamo.

**November**
Congress “GiViTI Veneto” – Treviso.

**December**
Utilizzo della cartella clinica elettronica Margherita Tre. Course: “Utilizzo del software Margherita Tre” – Asti.

**Laboratorio di ricerca sul coinvolgimento dei cittadini in sanità**

**February**
Congress La roadmap di CAMbrella. “Una strategia pan-europea per la ricerca clinica in Medicina non Convenzionale (CAM)” ; Bologna.

**March**
Congress “Se una notte d’inverno un decisore... Come DECIDE, dalle evidenze alle decisioni nel SSN”; Roma.
Workshop Qualità di vita in RSA: “proposta per la realizzazione di un’indagine empirica. Misure della qualità della vita”. LIUC Università Cattaneo; Castellanza (Va).
Congress Psoriasi e vitiligine: “i pazienti con i medici, i medici per i pazienti: teoria, pratica, certezze e prospettive. Il coinvolgimento dei cittadini e dei pazienti per una nuova sanità”; Milano.
Congress Istituto Nazionale dei Tumori “C’era una volta il Comitato Etico. Sintesi e alcune proposte”; Milano.
Congress Cosa devo sapere per decidere. “I diritti del paziente: libertà di cura, corretta informazione, coinvolgimento dei familiari e ruolo del volontariato”; Modena.

April
Meeting AIOM Linee Guida “Assistenza psicosociale dei malati oncologici”; Milano.
Lesson Liceo Classico A. Volta, Como. “La qualità della comunicazione scientifica con particolare riferimento all’area della medicina”; Como.
Symposium Giornata mondiale dell’emofilia: “i bisogni semplici nel confronto con i sistemi complessi. Prospettive per un sistema sanitario in evoluzione”; Milano.

May
Congress Salute e partecipazione: “dal progetto PartecipaSalute al progetto ECRAN”; Pisa.

June
FISM Advisory Board “Migliorare la sintesi dei risultati della ricerca”; Bologna.

October
Congress AVAPO. “Oncologia e qualità della vita”; Venezia.
Seminary Democrazia deliberativa e giurie dei cittadini. “Presentazione e condivisione dei risultati”; Roma.
Course L’Accademia del cittadino II Edizione. “Il metodo della ricerca clinica, fase degli studi e parole chiave: controllo, randomizzazione, mascheramento e indipendenza”; Montecatini Terme (PT).
Training Course L’Accademia del cittadino II Edizione. “Internet e informazioni sulla ricerca clinica, come e dove: l’esempio In-Deep”; Montecatini Terme (PT).
Training Course L’Accademia del cittadino II Edizione. “Registri e data base per la ricerca clinica”; Montecatini Terme (PT).
Congress Il tumore dell’ovaio: una visione a 360 gradi. “Conoscere e condividere i temi della ricerca”; Milano.

November
XXII GiViTI Meeting “Creative Kick-off meeting”; Pesaro.
Start-Up Meeting “Validazione psicometrica e clinica di uno strumento breve per la valutazione del paziente onco-ematologico anziano Mini CGA-MDA”; Reggio Emilia.
Congress Acili Treviso L’uso corretto dei farmaci. “I farmaci: non se ne parla mai abbastanza!”; Treviso.
XI Italian Convention of Investigators in cystic fibrosis. “Citizens’ jury and decision making on cystic fibrosis carrier screening: to screen or not to screen? FFC project #9/2013”; Verona.
2° National Congress Slow Medicine Scegliere con saggezza. “Conclusioni degli Ordini e dei Collegi professionali, dei cittadini, della stampa”; Torino.

**Laboratory for Mother and Child Health**

**January**
Il Registro della Regione Lombardia: potenzialità delle reti regionali. Meeting of the “Centri di Riferimento del Registro Nazionale dell’ADHD”. Istituto Superiore di Sanità; Rome.

**March**
Le molte facce della povertà. La nascita e l’infanzia, indicatori di diritto. I farmaci contraccettivi. Course della Società Internazionale Supeiro di Studi Avanzati (SISSA); Trieste.
Farmaci generici: valutazione della bioequivalenza e analisi dei profili prescrittivi. Course “Generici o di marca ....? I pediatri incontrano gli esperti”; Associazione Culturale Pediatri del Friuli Venezia Giulia; Monfalcone (GO).
I farmaci contraccettivi. IX Congresso nazionale Pediatria On Line “Congresso POL 2013”;
Pediatria On Line (POL); Lazise (VR).
Peculiarità dell’uso dei farmaci nei bambini. Course “5° modulo didattico: Salute Materno-Infantile”. Università degli Studi di Brescia; Brescia.

**April**
Health determinants of migrants. COordinating resources to assess and improve HEalth status of MIGRANTS from Latin America: The COHEMI project. International Symposium on Latin American Migrant Health (ISLAMH) “Salud de Migrantes Latinoamericanos”. International ISLAMH; Lima, Peru.

**May**
L’uso razionale degli psicofarmaci. Congress “ADHD per una condivisione dei percorsi diagnostico terapeutici”. Laboratorio per la Salute Materno Infantile dell’IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.

**June**
Efficacy of Nebulised Beclometasone in Viral Wheezing Prophylaxis (ENBe Study). 14° Congress ESDPPP “Novel Targets and Future Challenges for Drug Therapy in Neonates & Children”, European Society for Developmental Perinatal and Pediatric Pharmacology (ESDP); Salisburgo, Austria.

**July**
Matching vulnerable children needs and health services: strategies and contents. Congress “Transatlantic forum on inclusive early years. Investing in the development of young children from migrant and low-income families”. The King Baudouin Foundation; NY, USA.
L’uso dei farmaci per i bambini. Course “MarioNegri Summer Student”. IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.

September
Dove quando e per chi investire nella prossima sanità. Seminary “Club delle 2”. IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.
Cosa c’è di nuovo in farmacologia? Course. Università degli Studi di Milano-Bicocca, Facoltà di Medicina e Chirurgia, Scuola di Specializzazione in Pediatria; Monza (MB).
Terapia psicofarmacologica nell’età evolutiva: alcuni principi pratici. Seminary. Università degli Studi di Pavia, Pavia.
Farmaci essenziali in Pediatria: utilizzo razionale nelle patologie infettive, respiratorie e gastroenteriche. 3° National Congress SiMPeF “Qualità delle cure in età pediatrica un progetto da sostenere e difendere”. Sindacato Medici Pediatri di Famiglia; Milan.

October
Effects of the systematic introduction of a low-cost bubble nasal continuous positive airway pressure in a large neonatal intensive care unit in Nicaragua. 54° Annual Meeting European Society for Pediatric Research “ESPR PORTO”. European Society for Pediatric Research; Port, Portugal.
Psicofarmacologica dell’età evolutiva: quali novità. Seminary. Università degli Studi di Pavia; Pavia.
Research and activities in mother and child health. European Master “Sustainable Regional Health System: Erasmus Mundus”. IRCCS Istituto di Ricerche Farmacologiche Mario Negri (IRFMN); Milan.
Efficacia del beclometasone versus placebo nella profilassi del wheezing virale in età prescolare. Lo studio ENBe La domanda, il contesto, la strategia di ricerca e i risultati. XXV National Congress ACP “Ognuno al suo lavoro per il bambino e la sua famiglia”. Associazione Culturale Pediatri; Monza (MB).

November
III sezione: Simposio COHEMI-tubercolosi: una sfida per il nord e il sud del mondo. 7° National Congress SIMET “SIMET 2013 Torino”. Società Italiana di Medicina Tropicale, A.S.L. TO2; Turin.
Farmaci e bambini. Corso di Clinica Pediatria. Università degli Studi di Milano-Bicocca, Facoltà di Medicina e Chirurgia, Scuola di Specializzazione in Pediatria; Monza (MB).
Principi di appropriatezza per l'uso dei farmaci durante la gravidanza. Master “Discipline Regolatorie del Farmaco”. Università degli Studi di Catania; Catania.
Il beclometasone è efficace nei bambini che «fischiano»? I risultati dello studio ENBe. Seminary “Club delle 2”. IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.
Determinanti della prescrizione (geografici e socioeconomici). Seminary “AIE Farmacoepidemiologia”. Istituto Superiore di Sanità (ISS), Dipartimento di Epidemiologia del Servizio Sanitario Regionale Regione Lazio (D/EP/Lazio), Associazione Italiana di Epidemiologia (AIE); Rome.

December
I Bisogni. Ma diamo i numeri? Congress “Progetto Migranti: 5 anni di dialogo tra il bisogno e la cura”. Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico; Milan.
Lo screening della depressione post partum nel setting della pediatria di famiglia. Uno studio osservazionale con i PLS della ASL Milano 1. Meeting, UONPIA Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico; Milan. 

GRANTS AND CONTRACTS

"A. and A. Valenti" Centre for Health Economics (CESAV)

- Abbott
- AIFA
- Grunenthal-Prodotti Formenti
- Merck Serono
- Sanofi Aventis
- Sanofi Pasteur MSD
- Schering Plough
- Vivisol

Laboratory of Clinical Epidemiology

- A.O. Como
- A.O. Legnano
- A.O. Lecco
- A. O. Reggio Emilia
- A.O. Sant’Andrea di Roma
- ASL 1 Sassari
- ASL 2 Olbia
- ASL 3 Genovese
- ASL AL
- ASL TO2
- ASL TO4
- Azienda USL9 Grosseto
- Azienda Sanitaria di Firenze
- Bellco SpA
- Brahms
- Commissione Europea DG Research & Innovation
- CNT
- IRCCS Policlinico S.Matteo di Pavia
- Ospedale Evangelico Internazionale di Genova
- Regione Emilia Romagna
- Regione Toscana

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Laboratory for medical research and consumers involvement

- Age.Na.S. Agenzia Nazionale per i Servizi Sanitari Regionali, Roma
- Arcispedale Santa Maria Nuova, Reggio Emilia
- European Commission, Brussels
- Fondazione per la ricerca sulla Fibrosi Cistica FFC onlus, Verona
- IRCCS Istituto Neurologico Carlo Besta, Milano
- Università degli Studi di Verona

Laboratory for Mother and Child Health

- AIFA, Agenzia Italiana del Farmaco
- A.O. Spedali Civili di Brescia
- IRCCS Burlo Garofolo, Trieste
- IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano
- Provincia di Milano
- Regione Lombardia – Assessorato alla Sanità
- Regione Valle d'Aosta – Assessorato alla Sanità
- Unione Europea

SCIENTIFIC PUBLICATIONS (2013)

"A. and A. Valenti" Centre for Health Economics (CESAV)


**Letter**
Curto A, van de Vooren K, Bonati M, Garattini L. The model to calculate the economic burden of HPV-related diseases in Italy lacks transparency and contains flaws. Plos ONE 2013; e-pub: http://www.plosone.org/annotation/listThread.action;jsessionid=7D534DB49B1BC5DB180349F5676E9CA?root=60089


**Laboratory of Clinical Epidemiology**


**Laboratory for medical research and consumers involvement**


**Laboratory for Mother and Child Health**


**Review**


**Letter**

Bonati M. Re: Prescribing methylphenidate for moderate ADHD. *BMJ* 2013; http://www.bmj.com/content/347/bmj.f6216/rr/670296.

Bonati M, Reale L. Re: Attention-deficit/hyperactivity disorder: are we helping or harming? *BMJ* 2013; e-pub: http://www.bmj.com/content/347/bmj.f6172/rr/674372.


**LAY PRESS SELECTION (2013)**

"A. and A. Valenti" Centre for Health Economics (CESAV)

Casadei G. Biosimilari e sostituibilità: a che punto siamo? *Giornale Italiano di Farmacoeconomia e


**Laboratory of Clinical Epidemiology**


**Laboratory for medical research and consumers involvement**


Laboratory for Mother and Child Health


Clavenna A, Bonati M. Differenze geografiche nel profilo prescrittivo quantitativo e qualitativo degli antibiotici in età pediatrica. GIFF 2013;5:5-10.


Clavenna A, Piovani D, Fortinguerra F. Generici e bambini ... Yes,we can!. Quaderni ACP 2013;20(4):186.


Confalonieri V. Vivere con la musica. R&P 2013;170:82-83.


OTHER PUBLICATIONS (2013)

Laboratory of Clinical Epidemiology


Laboratory for Mother and Child Health


RESEARCH ACTIVITIES

"A. and A. Valenti" Centre for Health Economics (CESAV)

Educational activities
Educational activities are developed only if related to research studies, in order to offer original contributions which naturally reinforce the research aims.

Economic Evaluation of Health Care Programmes
The aim of this research area is to assess the costs of pathologies and the cost-effectiveness ratios of the diagnostic/therapeutic existing alternatives. In general, analyses can be classified into two groups: partial economic evaluations (e.g. cost of illness analysis) and full economic evaluations (e.g. cost-effectiveness analyses).

Comparative Health Policy Analysis
The aim of this research area is to study the organization of health care systems, in order to draw lessons from international comparisons. This is particularly important in a "market" like health care where economic competition lacks by definition and therefore public regulation plays a crucial role.

Quaderni di FarmacoEconomia
QdF is a quarterly journal of pharmacoeconomics published by CESAV. It is designed as a tool to favour a critical approach to the economic aspects of the pharmaceutical sector among NHS professionals, with particular reference to economic evaluations and drug policies at the national and international levels. It was first published in 2006 with the aim to keep the "voice" of independent research alive and to improve the critical skills of Italy’s health workers. The editors of QdF believe in the importance of offering the chance to receive updates and critical inputs on pharmacoeconomy to health system operators without a strong background on the subject. The ultimate goal is a context in which those working in this field won’t have the illusion of finding a "magic solution" and won’t accept for gold everything that is published. There is a critical risk, however, of disappointment in the long run and a loss of credibility in the pharmacoeconomy field. This magazine represents an opportunity to read the more debated economic and drug policy issues with a critical mind and adequate tools.
Laboratory of Clinical Epidemiology

Quality of care in the Intensive Care Units

The main purpose of these research projects is the assessment and improvement of the quality of care in Italian Intensive Care Units (ICUs). It is a multi-annual project promoted on behalf of GiViTI, a collaborative network composed by more than half of the Italian ICUs and coordinated by the Laboratory. The main focus is the Project Margherita. Its aim is the continuous evaluation of the quality of care and it is based on a free software developed by the Laboratory and distributed to all the ICUs adhering to the GiViTI group. The software has been realized on a modular structure, which enables to easily integrate the basic data collection (the “core” of Margherita) with the data collection of specific research projects (the “petals” of Margherita).

Since January 2011, Margherita became an international project. Thanks to funding from the European Union and other contracts of the laboratory have in fact been able to develop new software and to distribute the project to eight countries: Slovenia, Hungary, Poland, Cyprus, Israel, Afghanistan, Sudan and Switzerland.

Appropriateness of the Intensive Care Units

ICU is a high technology environment, that requires a high number of high-level personnel. Hence, the cost of these units is extremely important and a special attention not to waste resources is mandatory. In this field, the Laboratory launched a study to assess the level of appropriateness of the use of ICU beds, in an Italian regions: Lombardia. Such an evaluation is based on the understanding that the level of care provided by an ICU should correspond to the level of care it can theoretically provide, given the available resources. In this framework, patients are classified as requiring high-, low-, or ordinary-care, and beds are independently classified are high- or low-level. The appropriateness evaluation protocol adopted verify the concordance between these two separate classifications.

The reconstruction of clinical reasoning in the medical practice and education

This area represents the main concern of the Unit of Clinical Knowledge Engineering, whose objective is the valorization of clinical reasoning in solving complex clinical problems. The diagnosis of pulmonary embolism still represents a relevant clinical challenge, due to the complexity of the patient's clinical presentation and the variability of diagnostic resources among Centres. In this regards, we are conducting an Italian multicenter study, involving mainly Emergency Units, with the aim of prospectively validating the diagnostic software BayPAD (Bayes Pulmonary embolism Assisted Diagnosis). Such a tool, relying on a probabilistic model covering 72 clinical variables and doing without the need to input all the contemplated observations, would overcome the main reasons which prevented ordinary clinical guidelines to be largely accepted. Moreover, the results of the retrospective validation of the system have been obtained.

The Unit started a project for the realization of a software assisting the physician in tracing back the basis of his clinical decisions before the description provided by clinical reports, among those that are typical of particular medical specialty. The software has the double target to create specific applications based on probabilistic models representing complex clinical decision problems, and to involve physicians in their construction. The last target is achievable given the strong analogy between the causal structure of the exploited models (bayesian networks) and the pathophysiological structure of medical knowledge. By this, it will be given the chance to adopt this system within medical training projects, with a special attention to e-learning programs.
An electronic health record to promote research in Intensive Care Medicine

The main aim of this project is the continued development of an electronic health record (EHR) that allows the assessment of indicators of the process of care in the ICU. A multidisciplinary team of intensivists, ICU nurses, epidemiologists, statisticians, and IT specialists, had the responsibility of planning the HER, that is now already shared by 30 Italian ICUs. This made it possible to launch the first analysis of the process that has as its goal the improvement of the practice of weaning from the ventilator.

Home artificial nutrition in Italy

The SINPE (Italian Society of Artificial Nutrition and Metabolism) with the support of the Laboratory of Clinical Epidemiology promotes the project "DOMUS, the new register of home artificial nutrition".

DOMUS project created with the aim to describe three types of patients who are subjected to artificial nutrition at home:
- cancer patients
- patients with benign severe chronic intestinal
- patients undergoing enteral nutrition at home

and to reveal the activity, efficacy and safety of programs of NAD (Artificial Nutrition at Home), on base of SINPE indicators.

Laboratory for medical research and consumers involvement

ECRAN project

During 2013 most of activities of the project has been carried out. The ECRAN, European Communication on Research Awareness Needs project, has been designed to develop a portfolio of open educational resources, including a film, for the general population about the challenges raised by independent clinical research. These messages are focused on:

i) the importance of public understanding of the need for and basic principles of clinical trials, fostering active involvement of patients in trials and of their representatives in trial design;

ii) the need for independent clinical trials driven by healthcare issues, to optimise treatment strategies through comparison of benefits and harms of multiple therapeutic options, supporting evidence-based clinical practice and reduction in healthcare inequalities;

iii) the need for transparency and optimal use of data, to promote the cost-effectiveness of treatments and to reduce the economic burden of diseases;

iv) the need for multinational cooperation, taking advantage of Europe’s population size and diversity, and of its medical expertise.

One of the tools developed is an animated film about clinical trials, dubbed in all the 23 official European languages (http://ecranproject.eu/node/4). The film has been developed thanks to the collaboration among the ECRAN partners, the RAI-SuperQuark and the Studio Bozzetto &Co. Its modular structure allows to display the whole film or its 8 different modules about: A clinical trial, Ethics committees, Randomization, Double blinding, Analyzing the data, One trial is not enough..., Outcomes have to be important to patients, Some pitfalls of trials.

Other simple and easy communication material and tools developed by the ECRAN project are:

- a website (http://ecranproject.eu) in 6 languages, with an inventory of resources available in 23 languages researchable for example by topic, author, and media type;
- a serious/educational game developed in 6 languages to capture the interest of young European citizens and students;
- a Media section dedicated to journalists to disseminate the contents of the ECRAN project uniformly across countries.
• an interactive sibling web page, started in English language under the title Testing Treatments.

All the material and tools are developed under creative common license and are freely available at the website of the project.

The ECRAN project involves 9 partners, included group of patients and citizen representatives.

The IN-DEEP project
Integrating and deriving evidence, experiences and preferences: developing research-based health information applicable to decision making and self-management by people with MS.

In-DEEP is a collaboration between project teams in Australia and Italy. In Italy IN-DEEP is promoted by Fondazione IRCCS Istituto Neurologico Carlo Besta, IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, in collaboration with Associazione Italiana Sclerosi Multipla (AISM). It is supported by a grant of the Federazione Italiana Sclerosi Multipla (FISM).

The aim of the project is to explore how people with MS integrate health information with their needs, experiences, preferences and values and how these factors can be integrated into an online resource of evidence-based health information provision for people with MS and their families.

In 2013 the template on interferon developed during the first phase of the project has been implemented into a website (http://indeep.istituto-besta) and an online survey has been carried out to evaluate the website. The survey was closed in January 2013 with the participation of more than 500 subjects. The results demonstrate a good impact both in terms contents and usability of information. During the year a second model, a symptom the fatigue, has been developed.

Jury of citizen
A jury of citizens gathered in Modena resolved that the National Health Service should advise against the individual PSA as a screening test for prostate cancer in men of 55-69 years. This is a summary of the deliberations of the jury: a research project developed by asking a group of citizens to decide on behalf of the community. The basic idea is that decisions about medical interventions that have a collective nature and impact on the community, as well as on the individual, should be shared with the public, put in a position to decide thanks to transparent and complete information. The project was promoted by PartecipaSalute - coordinated by IRCCS-Mario Negri Institute, the Italian Cochrane Centre and Zadig agency of scientific publishing - and the National Agency for Regional Health Services (Agenas). Thanks to the collaboration with AUSL Modena the jury was held in Modena. The project involved the collaboration of representatives of the Scientific Medical Societies, AUSL companies, journalists, representatives of the Regions, Health Economists, representatives of voluntary organizations, Agenas, National Institute of Health and Research Institutes. The project has been supported by the National Agency for Regional Health Services. More information about http://www.partecipasalute.it/cms_2/giurie-cittadini/prostata/1972.

PartecipaSalute: a strategic alliance between patient groups, citizens and scientific medical communities
This project is carried out in collaboration with the Italian Cochrane Centre and Zadig agency of scientific publishing, began in September 2003. This project experiments initiatives with the aim of directing:
- patients’ associations and citizens to increased participation and discussion on health care issues and choices in medicine;
- professional and scientific organizations in a constructive relationship with patients and citizens and their associations to accept and satisfy their demands and their
expectations about the production (clinical research) and dissemination of scientific information.

In the course of 2013 were organized:
- the VIII training course "Orientarsi in salute e sanità", in collaboration with Regione Toscana;
- the Italian translation of the Press Release of The Cochrane Collaboration;
- the meeting “Da PartecipaSalute alle Giurie dei cittadini: cosa sta cambiando nel coinvolgimento dei cittadini in sanità” where the 10 years of the project have been celebrated

A strong point of the project is the development and the site of PartecipaSalute. The site is updated with new articles and insights every week, while every two weeks the newsletter is sent to a mailing list of more than 2,500 people.

MDS-GA Project: Development and validation of the short version of an instrument for multidimensional evaluation of elderly patients with acute myeloid leukemia or advanced myelo-dysplastic syndrome.

The aim of the project is to evaluate, in the Italian setting, the quality (content/face validity) and impact (feasibility and effects on management indicators of elderly patients) of a "short" instrument. The validation process in the Italian context was organized in five sequential phases of activity, which contributed to the overall assessment of the goodness/quality of the questionnaire.

During 2013, the tool has been consolidated and we proceeded to the finalization of the interventional study protocol for Phase 3: the evaluation of the internal validity of the instrument in its final version. This phase will require the collection of data from a sample of cases, estimated at 100-120 individuals. The study was launched at the end of the year through a cooperative group of a dozen centers, group leader with the Careggi Hospital in Florence.

Where is the evidence? Translation of the book Testing Treatments

The book "Testing Treatments", wrote among others by Sir Iain Chalmers - one of the fathers of evidence-based medicine - has seen the release of a second edition, updated and expanded. This version has been the subject of translation by a group of collaborative work – IRCCS-Istituto Mario Negri and Italian Cochrane Centre. The book translated into Italian under the title "Where is the evidence? Better research for better healthcare" is free download and its contents are, and will be, the subject of the developments on the site http://it.testingtreatments.org/, whose translation into Italian was supported by the ECRAN project.

Gynecological cancers and Mattioli Foundation

Since 1995, the Nerina and Mario Mattioli Foundation Onlus, in close collaboration with the Department of Oncology, IRCCS-Istituto Mario Negri, has helped to develop and catalyze interdisciplinary cooperation and facilitate the rapid transfer of knowledge between laboratory and clinic, in hope to obtain benefits for all patients with tumors of the female genital tract, especially ovarian cancer. The ovarian cancer is the sixth most common type of cancer in European women and the leading cause of cancer death in the female genital. The activities carried out in 2013 are two: first the development of the site www.fondazionemattioli.it in which methods used in both preclinical research are presented in a simple way - stem cells, angiogenesis, Genomics -, and in clinical multicenter studies. The second is an analysis of the database ClinicalTrial.Gov with the keyword "ovarian cancer" investigating the availability of the results of the studies recorded in the special section of the same database. Finally, the available data were compared with the possible publication on the Medline database.
Follow-up in oncology setting
Two studies on follow-up have been designed and carried out in collaboration with the Laboratory of Giovanni Apolone.
The first in collaboration with the Network Oncologica Piemontese regards the follow-up of patients with endometrial cancer organization for which the evidence available is not sufficient to draw a path of sure effectiveness. TOTEM study, that has the characteristics of an open randomized multicenter study, comparing two different modulations of visits and examinations. The second study that takes place in the context of the 6th Integrated Project Oncology (Health Ministry) provides for the comparative assessment of two follow-up for women at moderate-low risk with a diagnosis of breast cancer and lead to a randomization minimalist follow-up coordinated by the oncologist or by general practitioner. The study started the randomization in September 2010.

Quality of life projects
No specific research projects have been carried out on quality of the life evaluation. However we have been supporting and coordinating other groups using the instruments of quality of life translated and validated by our research group, SF-36, SF-12, PGWBI. During the year the website http://crc.marionegri.it/qol has been periodically updated.

Laboratory for Mother and Child Health
Efficacy of Nebulised Beclometasone versus placebo in preventing viral wheezing in pre-school children. (ENBe)
The results of the study “Efficacy Of Nebulised Beclometasone Versus Placebo In Preventing Viral Wheezing In Pre-School Children” (ENBe) were presented on the 11 October 2013 during the 25th National Congress of the Associazione Culturale Pediatri (Cultural Paediatric Association, ACP). The study, the first ever performed in Italy involving family paediatricians as investigators, started in October 2010 and ended in October 2012.

Beclometasone, an inhaled corticosteroid, is one of the drugs most commonly prescribed to children. Every year nearly 2 million Italian children and adolescents received at least one prescription. Despite it should be use as treatment for asthma, beclometasone is commonly prescribed for common cold, cough or sore throat, and the likelihood for Italian children to receive an inhaled steroid is 3 fold greater than their peers living in other European countries. An independent randomized controlled trials was therefore performed with the aim to evaluate the effectiveness of beclometasone in preventing wheezing in viral upper respiratory tract infection (URTI)
The study “Efficacy Of Nebulised Beclometasone Versus Placebo In Preventing Viral Wheezing In Pre-School Children (ENBe)”, coordinated by the IRCCS -Istituto di Ricerche Farmacologiche "Mario Negri" with the Associazione Culturale Pediatri, was funded by the Italian Medicines Agency (Agenzia Italiana del Farmaco, AIFA) with a grant for independent research on drugs. ENBe was the first double blind randomized controlled trial performed in the Italian paediatric primary care setting.
In all, 1371 children 1-5 years of age with upper respiratory tract infection and at least one viral wheezing episode in the 12 months preceding the visit were visited by 40 Italian FPs. A total of 525 children were enrolled: 264 children were randomized to beclometasone (400 mcg) and 261 to placebo. Both drug and placebo were administered
twice daily through a nebuliser. The therapy lasted 10 days. 521 children were visited at
the end of the treatment period and 507 completed the 6 month observational follow up
period.

The percentage of children with wheezing diagnosed by the paediatrician during the
URTI episode (primary outcome measure) was 7% in the beclometasone versus 11% in
placebo arm, with an absolute risk of -4.3% (95%CI -9.1 to 0.6).

In all, 63% of parents rated the treatment as useful, with no differences between
beclometasone (64%) and placebo (61%).

At the end of the treatment period parents reported URTI symptoms for 46% of
children, with no differences between the two groups.

The findings from the ENBe study confirm that the effectiveness of inhaled steroids in
preventing viral wheezing is scant. Moreover, no benefits were proved in reducing
URTI symptoms. A more rational use of this drugs by physicians and parents is
therefore needed.

ENBe Newsletter. As part of the ENBe study the Laboratory set up a newsletter aimed at
providing investigators and interested health operators with a periodic bibliographic update of
the recent scientific literature via email. A total of 13 issues have been sent to 105 health
professionals (mainly family paediatricians). The newsletter has identified a total of 205
scientific articles. (enbe@marionegri.it)

Pharmacoepidemiology in the Lombardy Region
The Laboratory for Mother and Child Health is involved in the analysis of the drug prescription
profile in children and adolescents through the EPIFARM (Epidemiologia del farmaco) project
funded by the Lombardy Region.

During 2013 the activities concerned, in particular:

1) prescription profile in the pediatric population; 2) Reduction of antibiotic prescription costs.

1) Prescription profile in the pediatric population
- The analysis was focused on the comparison of drug prescription profile in three
Italian regions: Lombardia, Lazio and Puglia. In 2008 1,861,425 children and
adolescents received 6,844,307 reimbursable prescriptions corresponding to 878
active substances. Despite this fact only 175 of them were used in all the Local
Health Units (LHUs).

- Significant differences in the prevalence of drugs in children and adolescents
were observed. The average prevalence was 56.4% ranging from 43.1% to
70.0%. The prevalence was higher in southern regions. Amoxicillin clavulanate
was the most used drug in all the regions and in 31 out of 33 LHUs analysed.
Amoxicillin was the drug that had the widest variability in use among the LHUs
(ranging from 9.1% to 52.1% of treated children).

- The prevalence of the two most used drug classes was evaluated also at the
district level: the average antibiotic prevalence rate was 47.9% (ranging from
34.0 to 67.9% among districts), while the antiasthmatic prevalence rate was
21.4% (11.7-35.6%). Both at the LHU and district level an inverse correlation
was found between drug prevalence, latitude and per-capita average annual
income. In particular children/adolescents living in districts in the lower quintile
of average income were more exposed to antibiotic (OR=1.75; 1.74-1.77) and
anti-asthmatic (OR 1.56, 1.55-1.57) prescriptions.

2) Reduction of antibiotic prescription costs:

...
- The prescription profile of a group of paediatricians involved from years in initiatives concerning care operating in the northern part of the Lombardy region was compared to the prescription profile of the other regional colleagues.
- The prevalence rate of antibiotic was not different in the two group of paediatricians. Concerning amoxicillin, first choice antibiotic for the most common community paediatric infections, the prescription rate was twofold in the first group when compared to the group of colleagues not involved in educational initiatives (70 vs 35 packages/100 resident children). The rate of hospital admission for respiratory tract infections and for their major complications did not differ significantly between the two groups (0.8 vs 1% and 25 vs 28/100.000 children, respectively).
- The expenditure for antibiotic in outpatient children under 15 in the Lombardy Region in 2008 was 18.5 million euros. By applying the same prescriptive pattern observed in the group of paediatricians involved in educational initiatives to the rest of the regional colleagues we hypothesized a cost reduction of about 3.6 million euros, equal to one fifth of the expenditure.

Prevalence of post-partum depression in mothers and fathers
The Laboratory for Mother and Child Health participated in the study “Valutazione dell’incidenza della depressione post-partum nelle madri e nei padri”. (Evaluation of post-partum depression in mothers and fathers). The project was funded by the Local Health Unit “Milan 1”, and involved family paediatricians and mental health services. The aim of the study was to estimate the prevalence of post-partum depression in mothers and fathers, through a screening with the Edinburgh Postnatal Depression Scale performed in the paediatric primary care setting. The screening was proposed by paediatricians to parents of children born between 1 February and 31 July 2012. Parents with depressive symptoms were advised to contact a mental health service.
Mothers and or fathers of 2727 (74%) newborns agreed to participate to the study. In all, 126 out of 2706 (4.7%, 95%CI 3.9-5.5%) mothers and 24 out of 1420 (1.7%, 95% CI 1.0-2.4%) fathers resulted positive to the EPDS test. Of the 126 mothers resulted positive to the EPDS screening, only 11 (8.7%) attended the psychiatric services of the LHU: for 8 of them the diagnosis of postpartum depression has been confirmed.
The presence of mood and/or anxiety disorder during pregnancy was the main risk factor for postpartum depression.

FP7 Projects
1) TINN – Treat Infections in NeoNates
The TINN project, Treat Infections in Neonates, is part of the European Union’s Seventh Framework Project (GA-223614 and is aimed at gathering the experience of numerous centres across Europe in the neonatal research field in order to produce detailed evidence on the safety and efficacy of ciprofloxacin and fluconazole use in neonatal sepsis. The project began in 2008 and has, as one of its goals, the obtainment of a Paediatric Use Marketing Authorization (PUMA).
A survey on the use of ciprofloxacin and fluconazole by neonatal intensive care units (NICU) in Europe was conducted in the first phase of the project (2009/2010). In all, 200 NICUs participated, representing 32 countries, mainly Italy, the UK, and France. The survey found great variability in therapeutic schemes and indications for use of the two drugs, both between and within countries. Significant doubts on the part of clinicians concerning safety and efficacy issues were also revealed, highlighting a need for additional evaluation and information on the optimal use of the drugs. The TINN study was recently granted a two-year extension in order to conclude the ongoing clinical trials and will end in 2015.
http://tinn-project.org/
2) TINN 2
The TINN2 (Treat Infections in Neonates 2) project began in January 2011 and is a complementary part of the first TINN project. It is also part of the European Union’s 7th Framework Programme (GA-260908). TINN2’s aim is to study azithromycin, an antibiotic effective against Ureaplasma, for the prevention of broncopulmonary dysplasia (BPD) in neonates. One of the goals of the project is to obtain the PUMA (Pediatric Use Marketing Authorization) for the drug. Several studies show a relationship between Ureaplasma colonization and BPD development in neonates. Azithromycin is an antibiotic effective against this mycoplasma and studies conducted up to the present show interesting results.

BPD is one of the European Medicines Agency’s selected therapeutic areas that need specific pharmacological assessments specific to neonates.

As for the first TINN, the initial stage of the project included a European survey intended to define the use of this antibiotic in the neonatal intensive care units (NICU), and to collect the opinion of senior neonatologists about its use in the treatment of Ureaplasma infections. Over 800 TIN, located in 28 different countries, were selected and contacted and about 200 TIN completed the entire questionnaire. The results show that there is still much uncertainty about the actual involvement of Ureaplasma in the development of BPD, that azithromycin is not a drug of first choice for the treatment of BPD, and that there are still doubts about its safety and efficacy in neonates.

The project is ongoing and is expected to end in 2015. http://tinn2-project.org/

3) COHEMI
Coordinating resources to assess and improve health status of migrants from Latin America - COHEMI Project- HEALTH.2010.3.4-5
European health systems are committed to meeting the challenge of understanding the needs of migrant populations and adapting their services to meet these needs. The difficulties inextricably linked to this challenge are caused by the complexity of migration patterns and the differences between migrant population across EU countries. Currently, the limited available data show that attempts to incorporate migrants’ health needs, in particular those of migrants from non-EU countries, into EU health systems have remained scattered and uncoordinated.

In January 2011 a project called COHEMI-Coordination resources to Assess and Improve health status of migrants from Latin America, began. It is a three-year project, funded under the 7th Framework Programme for Research and Technological Development (Programme Cooperation-Health, GA-261495) that sees the IRCCS-Istituto di Ricerche Farmacologiche Mario Negri as coordinator of a major consortium of 10 partners located in Europe (Italy, Spain, The Netherlands and UK) and Latin America (Bolivia, Ecuador and Peru).

COHEMI’s general objective is to coordinate referral centres dealing with endemic Latin American (LA) diseases in order to:
- provide an analysis of health systems and legislation on migrants and assess the determinants of health;
- evaluate the care strategy of 3 diseases typical of LA countries (Chagas, Cisticercosis/Epilepsy, Strongyloidiasis);
- produce, as a product of the first two steps, new procedures and suggestions shared at the migration policies level, that take into account the specific cultural needs of migrants.

Many bibliographic reviews have been carried out in the different specific sections of the project (health care organization and legislation, Chagas disease, Cisticercosis/Epilepsy, Strongyloidiasis, tuberculosis, hypertension and cardiovascular diseases, anthropological and socio-cultural aspects) and the study design for evaluating specific diseases has been defined.
The work done has been shared among all the partners and presented at public scientific meetings in Italy and abroad. Particularly, in 2013, the last year of the project, next to the workshop focused on single diseases and socio-anthropological aspects organized in Europe and Latin America, two international symposium were organised, the first one in Lima, Peru, on April 12ve and 13th, and the second one, the COHEMI Final Symposium, in Milan, Italy, on December, 2nd and 3rd, at the IRCCS – Istituto di Ricerche Farmacologiche Mario Negri. During the Final Symposium in Milan, the results of the project and the draft of the Policy briefs addressed to the health authorities, the socio-anthropological Agenda on access to health care for Latin American migrants, and the COHEMI recommendations addressed to health professionals, were presented and discussed with national and international experts.

http://www.cohemi-project.eu

COHEMI Newsletter. Publication Publication of the of the COHEMI project’s newsletter began in 2011. The newsletter, which is one of the project’s official products, represents an information and updating tool for two types of users: health staff and members of the general public interested in migrant health, and COHEMI partners working on the project alongside the IRCCS - Istituto di Ricerche Farmacologiche Mario Negri. The version addressed to the general public includes a bibliographic update from the recent scientific literature on migrant health and diseases covered by the COHEMI project: Chagas disease, Strongyloidiasis, Cysticercosis, Tuberculosis, and Cardiovascular diseases. The newsletter can be downloaded from the COHEMI website and those who wish to receive it may insert their email address on the related section of the project webpage http://www.cohemi-project.eu/Pages/Newsletter/Newsletter.aspx In addition to a bibliographical update, the version designed for the project’s partners includes news and updates on the work performed and information on scheduled meetings and events. In 2013, Newsletter N. 5 and N. 6 were published. Two newsletters were published in 2011, three in 2012 and two in 2013. The COHEMI newsletter is available on the Mario Negri’s website in the section: “L’Istituto Mario Negri per il Medico”:

http://www.marionegri.it/mn/it/index.html

The Lombardy Region’s ADHD Register

The Lombardy Region’s ADHD Register was launched in June 2011 within the project called “Sharing diagnostic-therapeutic approaches for ADHD in Lombardy” with the funding of the Lombardy Region. The project involves 18 referral centres and the coordinator is the UONPIA (Child and Adolescent Neuropsychiatric Unit) of the A.O. Spedali Civili of Brescia. The project includes training initiatives for health care workers who provide assistance to ADHD patients and their families, initiatives to increase information on ADHD, and a regional register of the ADHD cases. The register was designed as a disease register and therefore collected information not only on the patients diagnosed with ADHD under pharmacological treatment (as foreseen by the national register), but also on all patients who visited the referral centres for a suspected ADHD. The register then permits the:

- monitoring of diagnostic paths;
- defining of the prevalence of the disorder;
- monitoring of non pharmacological treatment programs as well;
- continuation of pharmacovigilance activity by extending the monitoring on the use of the drugs other than atomoxetine and methylphenidate;
- quantifying the workload for the referral centres.

At the end of 2013, 1494 patients have been included in the register, 840 of whom had a confirmed ADHD diagnosis, 437 were not diagnosed for ADHD, and 262 were still under diagnostic evaluation. In the most cases patients were referred to the centers by the school
(33%) or the parents (28%). In all, 79% of the 840 patients with a confirmed ADHD diagnosis received a non pharmacological prescription, 3% only a pharmacological one, 12% both the prescriptions, and the remaining patients are still awaiting for any therapy. The most frequent comorbidities were learning disabilities (36%), sleep disorders (13%) and oppositional/defiant disorder (13%).

**ADHD Newsletter.** The publication of ADHDNEWS continues. It is a laboratory initiative aimed mainly at providing a monthly bibliographic update of the recent scientific literature to those interested. By December 2012, 73 issues of the newsletter had been published and sent to the 471 people registered (138 psychiatrists and neuro-psychiatrists, 83 psychologists, 66 medical doctors, 37 paediatricians, 51 members of school staff, and 96 “others”) and 3549 scientific studies had been reported. The newsletter is available on the Mario Negri’s website in the section “L’Istituto Mario Negri per il Medico”: [http://www.marionegri.it/mn/it/index.html](http://www.marionegri.it/mn/it/index.html)

**The activities of the Italian NGO Group for the CRC**
The laboratory is part of the Working Group for the "Convention on the Rights of the Child" (CRC) in Italy.

The laboratory is part of the Working Group for the "Convention on the Rights of the Child" (CRC) in Italy. On June 6, 2013 was launched on the 6th Report Update on the monitoring of the Convention on the Rights of the Child in Italy from 2012 to 2013, at the presence of the Minister of Labour and Social Policy, Enrico Giovannini, the Deputy Minister of employment and social policies, Maria Cecilia Guerra and the Guarantor for childhood and adolescence, Vincenzo Spadafora.

They worked in the drafting of the 51 paragraphs of the report, 113 third sector, representatives of 82 associations that are part of the network, active since 2001. With the publication of the 6th Report of renovation, the Group continues to monitor the implementation of the CRC, in our country, the UN Convention on the Rights of the Child (CRC) and its Optional Protocols. The CRC report, through the recommendations placed at the end of each paragraph, the competent institutions provide specific and actionable for primarily promoting a change. The recommendations continue to be numerous, some reiterate criticisms reported by the CRC group in 2012, to point out that little has been done in the past year to ensure effective and consistent implementation of the CRC in Italy.

The report is available at: [http://www.gruppocrc.net](http://www.gruppocrc.net).

**Co-operation with countries with limited resources**
As an expression, test, and original method of manifesting the choice to make the laboratory’s research transferable and accessible to all populations, the laboratory promoted and provided assistance to projects in, and for, the South of the world, in collaboration with Non-Governmental Organizations (NGOs) and the World Health Organization. The technical and organisational support for carrying out socio-sanitary projects in countries with limited resources continues.

**The Surveys**
**Migrants and Health.** As part of the activities performed by the Laboratory for the COHEMI project, a series of interviews to policy makers working in the field of migration was carried out in Italy on priority issues for the implementation and development of policies on the health of migrants.

**Evaluation of the assistance provided in the transition to adult ADHD services for patients in the National Register.** The literature review highlights the fact that 40% of adolescents with ADHD may need to maintain therapy into adulthood, especially those who have one or more psychiatric comorbidities. Up to now, the prevalence of patients who have continued to access
the territorial child neuropsychiatric services once they turned 18 years old, as well as the transition passages to adult psychiatric services, have not been formally assessed. For these reasons the project foresees the involvement of both the ADHD referral centres and the Lombardy Region’s territorial child neuropsychiatric services. The main objective is to evaluate the current provision of assistance and the transition passage to adult ADHD services in order to:

- perform a quantitative and qualitative analysis of ADHD subjects who have reached the adult age and of those who are almost adults (16-17 years);
- analyse the procedures currently employed in the transition from child and adolescent neuropsychiatric services to adult psychiatry services and the difficulties in this passage;
- set out shared procedures that can guarantee continued assistance while taking into consideration the needs of ADHD patients and their families.

Hey mom, did you know?

Lo sai Mamma

The laboratory, along with the Associazione Culturale Pediatri (Paediatricians’ Cultural Association) and the Federfarma Lombarda participates in the initiative “Lo sai mamma?” (“Mom, did you know?”). The initiative is aimed at providing mothers with information on their children’s health through the creation of informational pamphlets distributed in pharmacies throughout the Lombardy Region.

Ricerca & Pratica

Ricerca & Pratica was born in January, 1985, as a manifestation of the “Mario Negri” Institute for Pharmacological Research. Today, the journal is part of the International Society of Drug Bulletins (ISDB), which represents independent journals.

For more than twenty years, the journal has represented an arena for all those professionals who collect data and carry out studies in general practice with the aim to increase their knowledge and to improve their practice. R&P is also appreciated for its ability to go beyond the merely clinical aspect of medicine, without, however, forgetting that it is to this aspect that the readers dedicate most of their time and effort.

Through its activity, R&P can therefore represent an exclusive, independent observation point. It is also an area that promotes contemplation, evaluation, and information by applying of tools such as data trustworthiness and importance, the balance between benefits and risks and between benefits and costs, independence from conflicts of interest, and the realistic objective to contribute to a progressive, equally distributed improvement in the population’s health.
# LABORATORY OF REGULATORY POLICIES

## STAFF

<table>
<thead>
<tr>
<th>Position</th>
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<tbody>
<tr>
<td>Head</td>
<td>Vittorio BERTELE', M.D.</td>
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<tr>
<td>Senior scientist</td>
<td>Rita BANZI, ChemPharm. D, PhD</td>
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<tr>
<td>Senior scientist</td>
<td>Pasquale Lorenzo MOJA, Dr.Med.</td>
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<td>Chir</td>
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<td>Ricercatore junior</td>
<td>Valentina PECORARO, Dr. Biol.</td>
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<tr>
<td>Visiting scientist</td>
<td>Roberta JOPPI, ChemPharm. D</td>
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<tr>
<td>Visiting scientist</td>
<td>Stefanos BONOVAS, Dr. Med. Chir</td>
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CURRICULUM VITAE

Vittorio Bertele' is a clinical pharmacologist. He got his MD degree in 1977 and the specialization in Internal Medicine in 1982, both at the Milan University Medical School. He was research fellow at the Harvard Medical School and then worked at the Milan University and the “Mario Negri” Institute. His main areas of interest have been clinical pharmacology of drugs active on the haemostatic and vascular system, epidemiology of interventions in the cardiovascular area, and clinical trials and drug utilization studies in the cardiovascular area.

He was CPMP expert at the EMEA, member of the Committee for Drug Price Negotiation at the Italian Ministry of Health, and member of the Technical-Scientific Committee at the Italian Drug Agency. At present he is head of the Regulatory Policies Laboratory at the “Mario Negri” Institute, secretariat of the ECRIN Scientific Board, member of the Italian Horizon Scanning Center, member of the ethics committee of Reggio Emilia, member of the HTA group of the Lombardy region.

Selected recent publications of the previous decade

5. Garattini S, Bertele’ V. Non-inferiority trials are unethical because they disregard patients’ interests. Lancet 2007; 370 : 1875-1877
12. Garattini S, Bertele’ V. The scientific community should lobby to be able to apply for drug licences. BMJ 2012 344 : c3553

ACTIVITIES

Critical appraisal of clinical methodology.

Critical evaluation of the benefit-risk profile of drugs.

Assessment of emerging technologies.

Optimisation of drug use and healthcare fund stewardship including potential reforms and initiatives to achieve this for both new and existing drugs.

Critical appraisal and recommendations for European Pricing and Reimbursement systems including generics, interchangeable products within a class and new innovative medicines.
Cooperation to the design and conduct of pharmacovigilance and pharmacoepidemiology studies in Europe.

Evaluation of the appropriateness of drug legislation, institutions, and regulatory procedures with respect to public health needs.

Cooperation to the development and functioning of the pan-European Infrastructure for clinical trials (ECRIN, European Clinical Research Infrastructure Network) provided as Secretariat of the ECRIN Scientific Board and European Correspondent for Italy.

Support to the activities of the ECRIN-IA (European Clinical Research Infrastructure Network-Integrating Activities) Scientific Board which is in charge of assessing projects requiring the services of ECRIN. Coordination of the evaluation process conducted by the Board and external peer-reviewers.

Set up and management of the ECRIN-IA WP7 competitive Call aimed to facilitate the conduction of multinational studies by providing free services for their multinational implementation. Support to the ECRIN-IA WP7 Call Scientific Board evaluation of the candidate proposals.

Support to the conduction of multinational clinical trials in Italy (local project management).

Coordination of the Task “Identification of key steps on monitoring activities” within the WP8 of the ECRIN, European Clinical Research Infrastructure Network.

Support to the development of systematic reviews and meta-analysis on the efficacy and safety of drugs, and clinical guidelines using the GRADE approach.

Support to the development of decision tools applied to the adoption of new vaccine in the Lombardy region.

MAIN FINDINGS

Finalisation of the ECRIN –IA WP7 competitive call to support multinational clinical trials in Europe. Critical appraisal of 47 letter of intents of European trials; first study session of the ECRIN-IA Scientific Board to select the projects admitted to the second stage of the evaluation; critical appraisal of 14 full proposals (study protocol, budget plan, feasibility of the trial extension at the European level); selection and contact with a panel of external referees; second study session of the ECRIN-IA Scientific Board to select the projects finally supported by ECRIN (eight projects).

Critical appraisal of seven transnational clinical research projects to be conducted with the methodological and operating support of ECRIN.

Support to the development of the National ECRIN network (Ita-CRIN).

Coordination of the ECRIN activity in Italy.

Support to the local management of an European clinical trial on the use of nilvadipine for the treatment of Alzheimer disease (Nilvad trial).
Critical appraisal of clinical research methodological aspects as the adoption of placebo as a control or non-inferiority design in clinical trials.

Critical appraisal of animal research methodology and reporting as the lack of proper study protocols, sample size calculation, unclear reporting, use of endpoints difficult to be translated in clinical settings.

Development of Pan-European strategies for the rational use of new and existing drugs including policies to enhance the managed entry of new drugs as well as reduce prescribing of more expensive interchangeable single sourced products in a class once generics are available: establishment of the Piperska Group.

Development of new models and strategies to optimise the managed entry of new drugs including suggestions for risk sharing arrangements given current concerns. This co-ordinated via the Piperska group leading to changes such as the recent changes in the German Health Insurance system for pricing new drugs.

Recommendations for Pan-European pricing policies for generics as well as interchangeable brands in a class once generics are available; with countries increasingly learning from each other. Alongside this, potential additional demand side measures that countries can introduce to further enhance their prescribing efficiency, with countries continuing to learn from each other.

Assessment of emerging technologies in the frame of the Italian Horizon Scanning Project which provides decision makers with timely information on the potential clinical impact and cost-effectiveness of new health technologies.

Critical review of the criteria to assess pharmaceutical innovation and include new drugs in the national reimbursement schemes.

Network meta-analysis about biotech drugs in the treatment of patients affected by ulcerative colitis

Systematic reviews with meta-analysis about selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) for migraine and tension-type headache prophylaxis in adults (Cochrane methodology).

Participation in the ENCePP (European Network of Centres of Pharmacovigilance and Pharmacoepidemiology) and the PROTECT consortium which under the coordination of the European Medicines Agency (EMA) and the support of IMI aims at improving design and conduct of pharmacovigilance activities and pharmacoepidemiological studies in Europe. The PROTECT consortium is also developing and testing innovative methods to integrate and present information on drug-related benefits and risks.

Raising awareness among interested parties about the deficiencies of the present EU pharmaceutical legislation and about our proposals to improve it in the public health interest.

Participation in discussion on clinical research transparency and ethics at the European and international levels.
NATIONAL COLLABORATIONS

Istituto Superiore di Sanità
Department of Health Lombardy Region
Italian Horizon Scanning Project
Italian Cochrane Network
University of Milan

INTERNATIONAL COLLABORATIONS

European Medicine Agency (EMA)
European Clinical Research Infrastructure Network (ECRIN)
European Network of Centres in Pharmacoepidemiology and Pharmacovigilance (ENCePP)
International Society for Pharmacoepidemiology (ISPE) – European chapter - EuroDURG
Piperska network involving health authority and health insurance personnel from across Europe to enhance the rational use of new and existing drugs
Karolinska Institutet, Division of Clinical Pharmacology, Department of Laboratory Medicine, and Centre for Pharmacoepidemiology; Department of Drug Management and Informatics, SE
University of Liverpool Management School, Prescribing Research Group, UK
Cochrane Collaboration
International Information Network on New and Emerging Health Technologies (EuroScan)
World Health Organisation (Department of Essential Drugs and Medicines Policy)

EDITORIAL BOARD MEMBERSHIP

Ricerca & Pratica

NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP

European Clinical Research Infrastructure Network (ECRIN) Scientific Board, Secretariat
EuroDURG - Treasurer
Scientific Committee of the Italian Horizon Scanning Project  
Reggio Emilia Ethics Committee  
TTRAM Lombardy Region  

**SCIENTIFIC PUBLICATIONS (2013)**  


**RESEARCH ACTIVITIES**

Critical appraisal of clinical methodology and animal research methodology  
Raising awareness about potential biases in clinical research, confounders in animal research and the importance of appropriate reporting in primary and secondary studies.  

Development of a Pan-European Infrastructure for clinical trials  
Support to the development and functioning of an European infrastructure to support the planning and conduct of clinical trials by connecting National clinical trial units and centers.
This infrastructure (ECRIN, European Clinical Research Infrastructure Network) offers support in terms of clinical trial services to clinical investigators and sponsor of multinational clinical trials in Europe.
Support to the development of a clinical trial infrastructure in Italy (ItaCRIN).

Production of systematic reviews to evaluate health-care interventions
Development and update of systematic reviews on drugs or other type of interventions (organisational, educational, diagnostic). Dissemination of their results to the scientific community through publications on the Cochrane Library or other medical journals) and general public. Systematic reviews may also be used as the basis for the development of clinical guidelines.

Assessment of emerging technologies
Collecting information on emerging medicines with respect to their potential clinical impact and their cost effectiveness and ranking the new products according to their possible marketing authorization date, their potential innovation grade, therapeutic and economic impact, possible price and NHS sustainability with the aim to provide decision makers with timely information on the potential clinical impact and cost effectiveness of new health technologies.

Critical evaluation of the EU pharmaceutical legislation
Raising awareness among interested parties about the deficiencies of the present EU pharmaceutical legislation and about our proposals to improve it in the public health interest.

Critical appraisal of ongoing reforms including pricing reforms in major European countries
Evaluation of ongoing reforms across Europe to drive down generic prices and corresponding originator brands, as well as potential prices of interchangeable brands once standards become available as generics, and the potential for cross cultural learning, to release valuable resources to fund increased volumes and new innovative drugs without prohibitive increases in general taxation or health insurances to continue to provide equitable and comprehensive healthcare in Europe.

Development of Pan-European strategies to enhance the rational use of existing drugs
Enhancing the rational use of drugs including increased prescribing of generics with an approach that has become known as the ‘four Es’, namely: economics; enforcement; education and engineering. The objective again being to help fund increased volumes and new valuable innovative drugs within finite budgets. In addition, development of new models to optimize the managed entry of new drugs including horizon scanning and critical drug evaluation pre-launch (below) and post launch activities.

Development of strategies to optimize the managed entry of new drugs
This includes the development of new models to optimize the managed entry of new drugs incorporating horizon scanning, budget impact and critical drug evaluation activities pre- and peri-launch, as well as post launch activities including evaluation of risk sharing arrangements and patient registries.

Development of Pan-European strategies for pharmacovigilance
Developing and testing innovative methods to integrate and present information on benefits and risks in order to provide all stakeholders (patients, prescribers, regulators and pharmaceutical companies) with accurate and useful information on drug-related risks and benefits.
CENTRE OF COMPUTER SCIENCE ENGINEERING

STAFF

Research and Communication Informatics

Head of Division  ROSSI Lorenzo Marco

Division of I.C.T. Services and Management

Head of Division  BAZZI Davide
CURRICULA VITAE

**Lorenzo Marco Rossi** graduated in Biomedical Engineering at Politecnico of Milan. He has been working with the Institute Mario Negri since 1998.

Main areas of interest are:
1. Planning and realization of software system for in-plant automatization
2. Planning and realization of products for multimedia divulgation

**Davide Bazzi** graduated in Informatics with ABACUS specialization at IstitutoTecnicoIndustrialeStatale of Corsico. He has been working with the Institute of Mario Negri since 1997.

Main areas of interest are:
1. Planning, realization and management of communication Network and Data Center
2. Definition and management of technological innovation for ICT systems
3. Planning and realization of technological innovation for ICT systems
4. Definition and application of organization’s methodologies and processes for the Informatics Security Management

ACTIVITIES

In order to fulfill even more specialization needs in informatics development, the Centre of Computer Science Engineering is organized, considering the acquired skills, in three distinct division bound each other by a strong collaborative relationship.

The Centre of Computer Science Engineering gathering informatics multidisciplinary aspects promotes and propose itself to coordinate and harmonize the development of the tools for the management information, improving the integration between informative procedures making more efficacious communication process and management of scientific and administrative data, in order to support and fasten decisional, management, clinical trials and scientific processes.

RESEARCH ACTIVITIES

**Implementation of Clinical Trials’ gathering forms (E-CRF)**

- Lab. Translational and Outcome Research in Oncology (Dep. Oncology)
  - Trial CERP
Maintenance and management of data gathering forms for the following clinical trials

- Lab. Neurological Disorders (Dip. Neuroscience):
  - RegistroEuropeo SLA
  - Trial L-ACETYLCARNITINE
  - Trial ANTIEPILETTICI
  - Trial EPILESSIA E STROKE
  - Trial EPO VS MP IN SPINAL SHOCK
  - Trial VALPROATO
  - Trial THEOREM
  - Trial ANTIEPILETTICI
  - Trial ADONE
  - Trial EDU-COM

- Lab. Clinical Trials (Dip. Oncology)
  - Trial FOLFOX
  - Trial TOP
  - Trial COMETS
  - Trial TAILOR
  - Trial HEAD & NECK
  - Trial GLAUCOMA PEDIATRICO
  - Amendment to Trial TAILOR
  - Trial ITACAS 2

- Lab. New Drug Development Strategies (Dip. Oncology)
  - Trial MAPS
  - Trial STARSPAN
  - Trial TRIAC

- Dip. Epidemiology
  - Trial CADASIL

- Lab. Quality Assessment of Geriatric Therapies and Services
  - Trial GISAS
  - Patients registry for Polipathologies and Politherapies – SIMI web

Web based applications connected to the projects
- Design Internal Proposal Management System
- Development of the Order Management System
- Development of the Human Resources Management System
- Management of the Database hosting data about recovers, prescriptions and examinations provided from RegioneLombardia for covenant data analysis.
- Support to data processing in recipes analysis for RegioneLombardia
One of the buildings on the Mario Negri Institute campus is The Catullo and Daniela Borgomainerio Center built in 1987 thanks to a donation from Mrs. Angela Marchegiano Borgomainerio. This is a Center for the study of rare childhood diseases and even today some of the laboratories housed in the building still conduct this research. For example, the study of new therapies used to treat a very rare form of acute myeloid leukemia, known as acute promyelocytic leukemia. A number of new studies are being done to identify new drugs having different mechanisms able to synergize with trans retinoic acid.

Research on epidemiological childhood leukemia is also done at the Borgomainerio and a similar line of research involves testicular cancer in adolescents and young adults. We also do research aimed at finding evidence based therapies for children.

Paediatric research activities done at the Borgomainerio Center are also performed in collaboration with groups located at other Institute locations including, The Aldo and Cele Daccò Center for Clinical Research on Rare Diseases at Ranica in Bergamo, the Regional Centre for Drug Information (CRIF) and the Laboratory for Mother and Child Health (Department of Public Health) which are both located in Milan.
THE LIBRARY

STAFF

Head Librarian         Vanna Pistotti

The Library, specialized in pharmacology and clinical epidemiology, was founded in 1963 thanks to a generous donation from the Gustavus and Louise Pfeiffer Research Foundation, in Denville, New Jersey, USA.

Numerous public and private organizations help keep it operative, through donations in money or books, and subscriptions to periodicals.

STAFF

One Head and two Assistants

WHAT THE LIBRARY OFFERS

The library has a collection of about 5000 textbooks, monographs and congressional proceedings, and 100 periodicals of which a major part are in an electronic format. The books are classified according to the US National Library of Medicine Classification and the Medical Subject headings of Medline (MeSH). Besides the internal collection, the Library has access to other Library consortia (SBBL, GIDIF-RBM).

DATABASES AND ELECTRONIC JOURNALS

From every computer in the Institute it is now possible to have access to more than 8000 electronic journals and to three of the most important databases, PubMed, the Cochrane Library and Embase.
SPECIAL PROJECTS
The Library cooperates to the realization of the Italian Information Specialists’ (GIDIF, RBM) journal catalog which is updated annually and to the catalog of the Lombardy Biomedical Library Consortium, a network that serves, through Internet, the scientific community in this District.

It collaborates to the Institute web site, particularly taking care of the Publications section, both scientific and lay press.

TRAINING
Every year courses on the use of the database and electronic journals are organized. These courses are designed for use by those working at the Institute but outsiders who are interested may attend.

CONTRACTS
Since 1994 the library has been part of the Lombard Biomedical Library System. 14 university and research organisation libraries in Lombardy take part in this project, which allows easy, free access to scientific information to over 140 centres and institutions the Lombardy Region.

EVENTS AND COURSES
Anna Maria Astori Center
Bergamo

ANNUAL REPORT 2013
departments and laboratories
DEPARTMENT OF MOLECULAR MEDICINE

STAFF

Head Ariela BENIGNI, Biol.Sci.D., Ph.D.

Laboratory of Cell Biology and Regenerative Medicine
Head Marina MORIGI, Biol.Sci.D., Ph.D.

Unit of Platelet-Endothelial Cell Interaction
Head Miriam GALBUSERA, Biol.Sci.D.

Unit of Developmental Biology
Head Barbara IMBERTI, Biol.Sci.D., Ph. D.

Laboratory of Immunology and Genetics of Organ Transplantation and Rare Diseases
Head Marina NORIS, Chem.Farm.D., Ph.D.

Unit of Cellular Biology of Autoimmunity and Transplant Rejection
Head Sistiana AIELLO, Biol.Sci.D

Unit of Cellular and Molecular Biology of Transplantation Tolerance
Head Federica CASIRAGHI, Chemist

Unit of Genetics and Molecular Basis of Renal Diseases
Head Roberta DONADELLI, Biol.Sci.D.

Laboratory of Pathophysiology of Experimental Renal Disease and Interaction with other Organ systems
Head Carla ZOJA, Biol.Sci.D., Ph.D.

Unit of Pathology and Immunopathology
Head Mauro ABBATE, M.D.

Unit of Experimental Models of Kidney Diseases
Head Daniela CORNA, Chemist

Laboratory of Gene Therapy and Cellular Reprogramming
Head Susanna TOMASONI, Biol.Sci.D., Ph.D.

Unit of Advanced Microscopy
Head Elena GAGLIARDINI, Biol.Sci.D., h.D.
CURRICULUM VITAE

Ariela Benigni got the Biol.Sci. degree in 1979 at the University of Milano, Italy, and the Ph.D. at Maastricht University, Netherlands, in 2001.

Educational training: in 1979 Post Doctoral Fellow, Istituto di Ricerche Farmacologiche Mario Negri (IRFMN), Laboratory of Cancer Chemotherapy, Milan, Italy; in 1980-1981 Post Doctoral Fellow, Associazione Bergamasca per lo Studio delle Malattie Renali, Laboratory of the Division of Nephropathy and Dialysis, Ospedali Riuniti di Bergamo, Italy; in 1982 Post Doctoral Fellow, Centre Regional de Transfusion Sanguine de Strasbourg, France; in 1989 intership at Brigham and Women’s Hospital, Laboratory of Prof. Barry Brenner, Boston.

Areas of interest: vasoactive and inflammatory mediators of progressive renal injury with a particular emphasis on endothelin-1; combined treatment of antipertensive and renoprotective drugs to halt progressive chronic renal injury; use of stem cells for tissue regeneration in acute and chronic renal failure; study of the renal regeneration mechanisms; in vivo e in vitro gene transfer; prevention of acute and chronic graft rejection through gene therapy; induction of kidney transplant tolerance by gene therapy; correction of genetic deficiency in rare diseases.

Employment: in 1983 Scientist, IRFMN, Laboratory of Kidney Disease, Bergamo, Italy; in 1990-1994 Head Laboratory of Prostaglandin and Leukotriene Metabolism, IRFMN, Bergamo, Italy; from January 1991 Scientific Secretary, IRFMN, Bergamo, Italy; in 1994-1999 Head Laboratory of Vasoactive and Inflammatory Mediators of Tissue damage, IRFMN, Bergamo, Italy; from January 2000 Head, Department of Molecular Medicine, IRFMN, Bergamo, Italy; 1996-1998: Associate Editor, Journal of Nephrology; 2003-2005: Associate Editor, Kidney International. 2010-2011: Associated Editor International Journal of Artificial Organs. 2013: Academic Editor of PeerJ and of Plos One; member of the Editorial Board of Expert Opinion on Therapeutic Patents. 2007-2012: Consultant World Health Organization (WHO) for the multicentre observational study “Screening for Pre-eclampsia: evaluation of the predictive ability of angiogenic factors for Pre-eclampsia”; during 2007 Senior Fellow at the University of Oxford, Nuffield Department of Obstetrics & Gynaecology. From 2013 member of the Visiting Committee di AERES – Agence d’Évaluation de la Recherche et de l’Enseignement Supérieur, Necker Enfants Malades Institute (INEM), Université Paris Descartes, Inserm and CNRS, Parigi. She trained 6 Ph.D students from Open University, London.

Selected publications:


• S. Conti, P. Cassis, A. Benigni. Aging and the renin-angiotensin system. Hypertension 2012;60:878-883


Marina Morigi got her Biol.Sci. degree in 1987 at the University of Milano, Milano, Italy and the Ph.D. at Maastricht University, Netherlands, in 2005.

Educational training: in 1984-1987 Research training, IRFMN, Bergamo, Italy; in 1987-1995 Post Doctoral Fellow, IRFMN, Bergamo, Italy; in 1991 Stage at Brigham and Women’s Hospital, Laboratory of Dr. P. Marsden, Boston, USA.

Selected publications:


• S. Conti, P. Cassis, A. Benigni. Aging and the renin-angiotensin system. Hypertension 2012;60:878-883


**Employment:** since 1995 Scientist, IRFMN, Bergamo, Italy; in 1996-1999 Head, Unit of Renal and Endothelial Cell Biology; since 2000 Head, Laboratory of Cell Biology and Xenotransplantation. Since 2010, Lab denomination changed to Laboratory of Cell Biology and Regenerative Medicine.

**Areas of interest:** Stem cell therapy and tissue regeneration: the potential of adult stem cells of different origin, and renal progenitor cells to differentiate and to regenerate renal tissue in acute and chronic experimental models of renal disease. Stem cell therapy with embryonic stem cells and iPS differentiated toward renal precursors to cure acute and chronic renal diseases. Kidney Organogenesis. Isolation of renal progenitors from renal tissue and urine. Role of Shigatoxin in the pathogenesis of endothelial dysfunction and microvascular thrombosis in Hemolytic Uremic Syndrome. Role of complement activation on renal cell disfunction and thrombosis. Renal toxicity of the proteins filtered through the capillary barrier: in vitro model to study intracellular signals, gene expression and production of inflammatory mediators in cultured proximal tubular cells and glomerular epithelial cells.

**Selected publications**
- Morigli M, Introna M, Immunology and Genetics of Rare Diseases and Organ Transplantation, Bergamo, Italy. Areas of interest: immunology of transplantation, tolerance induction; genetics of hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, focal segmental glomerulosclerosis, diabetic nephropathy, role of nitric oxide and arginine dysfunctions in uremia and in pre-eclampsia.

**Employment:** in 1994-1996 Head, Unit of Endothelial Cell Pathophysiology, IRFMN, Bergamo, Italy; 1996-1999 Head, Laboratory of Cellular and Molecular Biology of the Immune Response and Autoimmunity, IRFMN, Italy; from January 2000: Head, Laboratory of Immunology and Genetics of Rare Diseases and Organ Transplantation, Department of Molecular Medicine, IRFMN, Bergamo, Italy.

**Selected publications**

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**Marina Noris** got her degree in Pharmaceutical Chemistry and Technologies in 1986 at the University of Rome “La Sapienza” and the Ph.D. at Maastricht University, Netherlands, in 2005.

**Educational training:** in 1984-1986 Fellow, Istituto di Chimica Farmaceutica e Tossicologica, University of Rome, Italy; in 1986-1987 Post Doctoral Fellow, Istituto di Chimica Farmaceutica e Tossicologica, University of Rome, Italy; in September 1987-March 1994 Post Doctoral Fellow, IRFMN, Unit of Mediators of Inflammation and Tissue Damage, Laboratory of Kidney Disease, Bergamo, Italy.

**Areas of interest:** immunology of transplantation, tolerance induction; genetics of hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, focal segmental glomerulosclerosis, diabetic nephropathy, role of nitric oxide and arginine dysfunctions in uremia and in pre-eclampsia.

**Employment:** in 1994-1996 Head, Unit of Endothelial Cell Pathophysiology, IRFMN, Bergamo, Italy; 1996-1999 Head, Laboratory of Cellular and Molecular Biology of the immune response and autoimmunity, IRFMN, Italy; from January 2000: Head, Laboratory of Immunology and Genetics of Rare Diseases and Organ Transplantation, Department of Molecular Medicine, IRFMN, Bergamo, Italy.


Susanna Tomasoni got her Biological Science degree in 1991 at the University of Milan and the Ph.D in Physiology at the University of Milan in 1995.


Areas of interest: construction of adeno viral vectors for gene therapy; in vitro and in vivo gene transfer techniques; use of adeno viral and adeno-associated viral vectors to prevent acute and chronic allograft rejection; induction of kidney transplant tolerance by cell and gene therapy; correction of genetic deficiency in rare diseases by gene therapy; involvement of microRNAs in the progression of renal disease; generation of induced pluripotent stem cells from adult somatic cells; mesenchymal stem cell-derived exosomes as mediators of cell-to-cell communication.

Employment: in 1998-2000 Scientist, IRFMN, Bergamo, Italy; from 2000 Head, Unit of Gene Therapy, IRFMN, Bergamo, Italy; from 2010 Head, Laboratory of Gene Therapy and Cellular Reprogramming, IRFMN, Bergamo, Italy.

Selected publications:


Carlamaria Zoja got her Biol.Sci. degree at the University of Milano, Italy, in 1979 and the Ph.D. at the University of Maastricht, The Netherlands in 2001.

Educational Training: in 1979-1981 Post Doctoral Fellow, ‘Associazione Bergamasca per lo studio delle Malattie Renali’, Laboratory of the Division of Nephrology and Dialysis, Ospedali Riuniti di Bergamo, Italy; in 1981-1983 Post Doctoral Fellow, Center for Thrombosis and Vascular Research, Department of Research Katholieke Universiteit, Leuven, Belgium; in 1983-1985: Post Doctoral Fellow, IRFMN, Laboratory of Kidney Disease, Bergamo, Italy; in 1988 stage at Case Western Reserve University, Cleveland, Ohio, USA; in 1989 stage at Brigham and Women’s Hospital, Boston, USA.

Areas of interest: experimental models of kidney diseases of immunological and non immunological origin; vasoactive and inflammatory mediators of renal disease progression; role of proteinuria in progressive kidney damage; protection of renal disease progression by a multidrug approach; novel immunosuppressive and anti-inflammatory strategies for the treatment of lupus nephritis; role of Shigatoxin in the pathogenesis of endothelial dysfunction in Hemolytic Uremic Syndrome.

Employment: since 1985 Scientist, IRFMN, Bergamo, Italy; in 1990-1994: Head, Unit of Experimental Modelling for Human Renal Diseases, Laboratory of Kidney Diseases, IRFMN, Bergamo, Italy; since 1995: Head, Laboratory of Experimental Models of Kidney Diseases, IRFMN, Bergamo, Italy. In November 2010 Lab denomination changed to ‘Laboratory of Physiopathology of Experimental Renal Disease and Interaction with other Organ Systems’. In 2004-2007 member Editorial Board, Journal of the American Society of Nephrology. Since January 2010 Leader WP5.2, SysKid collaborative project (FP7).

Selected publications:

Mauro Abbate obtained his M.D. degree in 1988 at the University of Brescia, Italy.

Educational training: in 1984-1988 Graduate Student, IRFMN, Bergamo, Italy; in 1988-1992 Post Doctoral Fellow, IRFMN, Bergamo, Italy; in 1992-1994 Research Fellow, The Renal Unit, Massachusetts General Hospital, HMS, Boston, USA.
**Areas of interest:** renal disease progression: the role of proteinuria, complement, and mediators of injury in progressive kidney damage; mechanisms of glomerular injury; anti-GBM glomerulonephritis; mechanisms of tubular injury; kidney fibrosis; the renal biopsy; membranous nephropathy.

**Employment:** in 1996 - 2000: Scientist, IRFMN, Bergamo, Italy; from 2000 Head, Unit of Renal Pathology and Immunopathology, IRFMN, Bergamo, Italy.

**Selected publications:**


**Sistiana Aiello** got the Biol.Sci. degree in 1993 at the University of Milano, Italy, and the Specialization in Pharmacology Research in 1996, at IRFMN, Bergamo, Italy.

**Educational training:** in 1990-1993 research training, IRFMN, Bergamo; in 1993-2000 post doctoral fellow, IRFMN, Bergamo.

**Areas of interest:** transplant immunology with a particular interest on dendritic cell biology and mechanisms by which T Regulatory cells arise and work; in vitro and in vivo studies on new compounds with immunosuppressive capacity or capable to prevent ischemia/reperfusion tissue injury; vasoactive and inflammatory mediators of progressive renal injury with a particular emphasis on platelet activating factor (PAF) and nitric oxide (NO).

**Employment:** since 2000 Scientist within Laboratory of Immunology and Genetics of Rare disease and Organ Transplantation; IRFMN, Bergamo; since 2006 Head, Unit of Cellular Biology of Autoimmunity and Transplant Rejection, IRFMN, Transplant Research Center “Chiara Cucchi de Alessandri e Gilberto Crespi”, Ranica.

**Selected publications:**

Federica Casiraghi has obtained his degree in Industrial Chemistry in 1988, and the degree in Clinical Monitoring and in Biochemical Research in 1993-1994 at IRFMN, Bergamo, Italy. 


Areas of interest: Transplant immunology with particular focus on pharmacological and cellular therapies for induction and maintenance of transplantation tolerance. Characterization of regulatory T cells in renal transplant patients and in experimental models of allograft tolerance. Impact of different immunosuppressive drugs on T cell function in renal transplant patients. Vasoactive and inflammatory mediators of progressive renal injury with a particular emphasis on arachidonic acid metabolites.

Employment: since 1994 Scientist within Laboratory of Immunology and Genetics of Rare Disease and Organ Transplantation, IRFM, Bergamo; since 2006 Head, Unit of Cellular and Transplantation Tolerance, Transplant Research Center “Chiara Cucchi de Alessandri e Gilberto Crespi”, Ranica.

Selected Publications:

Daniela Corna obtained her degree in Industrial Chemistry in 1985, and the degree in Biochemical Research Technician in 1988-1989 at IRFMN, Bergamo, Italy.


Areas of interest: experimental models of kidney disease in transgenic animals and non; mediators of injury and the role of proteinuria in the progression of kidney disease; new therapies to slow the progression of kidney disease.

Employment: 1986-2010 Researcher in the Department of Molecular Medicine, IRFMN, Bergamo; since 2010 Head, Unit of Experimental Models of Kidney Disease, IRFMN, Bergamo.

Selected publications:


Roberta Donadelli got the Biol.Sci. degree in 1992 at the University of Milano, Italy, and the Specialization in Pharmacology Research in 1995, at IRFMN, Bergamo, Italy. 

Educational training: in 1990-1992 research training, IRFMN, Bergamo; in 1992-1999 post doctoral fellow, IRFMN, Bergamo; 1996 stage at the Medical Policlinic, Ludwig-Maximilians University, Munich, Germany; 2002-2003 guest scientist at the Department of Molecular and Experimental Medicine, Division of Hemostasis and Thrombosis, The Scripps Research Institute, San Diego, USA.

Areas of interest: genetics of atypical HUS, TTP, FSG and MPGN; expression and functional studies of mutants codifying for complement proteins and ADAMTS13; expression and functional studies of mutations in the gene identified in patients with glomerulopathy with fibronectin deposits; generation of knock-in mice as a murine model of aHUS; molecular mechanisms involved in the renal disease progression; shear-stress induced genes.

Employment: since 1999 Scientist within Laboratory of Experimental Models and Renal Diseases; IRFMN, Bergamo; since 2010 Head, Unit of Genetics and Molecular Basis of Renal Diseases, IRFMN, Transplant Research Center “Chiara Cucchi de Alessandri e Gilberto Crespi”, Ranica.

Selected publications:


Elena Gagliardini got her Biological Science degree in 1998 at the University of Milan and the Ph.D. at the Open University of London, UK, in 2007.

Educational training: in 1996-1998 graduate student, IRFMN, Bergamo, Italy; in 1998-2006 Research Fellow, IRFMN, Bergamo, Italy.

Areas of interest: mechanisms of progression of acute and chronic experimental renal diseases; vasoactive and inflammatory mediators of progressive renal injury; pathogenesis of the idiopathic and secondary membranous nephropathy; combined treatment of antipertensive and renoprotective drugs to halt and also regress progressive renal injury; mechanisms underlying
tissue regeneration; ultrastructure and function of glomerular filter in physiological or pathological conditions.

Employment: from 1996 Scientist, IRFMN, Bergamo, Italy; from 2010 Head, Unit of Advanced Microscopy, IRFMN, Bergamo, Italy

Selected publications:

Miriam Galbusera got her Biol.Sci. degree in 1981 at the Università degli Studi di Milano.


Areas of interest: ADAMTS-13 and VWF in thrombotic microangiopathies, VWF biochemistry, xenotransplantation, platelet-endothelial cell interaction under flow condition, platelet pathophysiology in uremia, receptor studies in kidney and platelets.

Employment: 1995 - 1999: Scientist, IRFMN, Bergamo, Italy; from 2000 Head, Unit of Platelet-Endothelial Cell Interaction, IRFMN, Bergamo, Italy.

Selected publications:

Barbara Imberti got her Biol.Sci. degree in 1994 at the University of Pavia, Pavia, Italy and the Ph.D. with the Open University Research School London, UK in 2007.
Educational training: 1995-1997 Post-Graduate professional qualification, Specialist in pharmacological Research, IRFMN, Bergamo, Italy; 1999-2000 Research training at Georgia Institute of Technology, Petit Institute for Bioengineering and Bioscience, Atlanta, GA, USA; Areas of interest: Embryonic stem cells and induced pluripotent stem cells (iPSC) for renal lineage differentiation and cell therapy in acute or chronic kidney damage; renal organogenesis and regenerative pathways.

Employment: 2001-2007 Scientist IRFMN, Bergamo; 2007-2011 Senior Scientist, Molecular Medicine Department, IRFMN Bergamo, since 2010 Head, Unit of Developmental Biology, IRFMN, Bergamo, Italy.

Selected publications:


INTRODUCTION TO THE DEPARTMENT’S ACTIVITIES

The Department of Molecular Medicine was established in 1999 at the Negri Bergamo laboratories to coordinate the work of four laboratories and seven units. The activities of the Department of Molecular Medicine are strictly interrelated with those of the Department of Renal Medicine of the Clinical Research Center for Rare Diseases Aldo e Cele Daccò.

The following major objectives have been pursued:
1) identification of mediators and mechanisms responsible for the relentless decline of renal function in kidney diseases and development of therapeutic interventions to slow or even halt the disease progression to end-stage renal failure;
2) understanding the mechanisms underlying endothelial cell dysfunction in thrombotic microangiopathies and hyperacute rejection of xenograft
3) finding new strategies for modulating the immune response and preventing acute and chronic rejection of kidney allograft as well as exploration of immunological pathways leading to donor specific unresponsiveness and tolerance of the graft;
4) investigation of the molecular and genetic basis of rare diseases such as hemolytic uremic syndrome/thrombotic thrombocytopenic purpura and pre-eclampsia and search for disease-susceptibility genes or gene polymorphisms predicting the patient's response to drug therapy in more common and complex polygenic disorders.

Such goals have been pursued using various approaches: 1) experimental models of kidney diseases of immunological and non-immunological origin mimicking human renal diseases to study vasoactive and inflammatory mediators and to test novel antiproteinuric and renoprotective drugs; 2) in vitro cultures of renal cells to address the toxicity of protein overload reproducing the condition of exaggerated protein traffic of proteinuric progressive
nephropathies; 3) in vitro models to assess the interaction of vascular endothelial cells with leukocytes and platelets under controlled flow conditions; 4) in vivo maturation of functional renal organoids by tissue engineering; 5) experimental models of kidney allotransplant to study immunological processes responsible for acute and chronic rejection, the nephrotoxicity of immunosuppressor drugs as well as to explore pathways responsible for accommodation; 6) gene transfer of viral constructs carrying genes encoding immunomodulatory molecules to overcome acute rejection of allotransplantation avoiding immunosuppression; 7) identification of candidate genes with linkage analysis and search for mutations as well as assessment of gene polymorphisms.

**FINDINGS/MAIN RESULTS**

Endothelin-1 by binding to its ETA receptor promotes β-arrestin activation in glomerular podocytes and alters their phenotype and sustains renal injury.

C3a identified as a key determinant of podocyte dysfunction and loss in Shigatoxin-associated HUS.

A cannabinoid receptor type 2 agonist ameliorates renal disease in a mouse model of type 2 diabetes.

The kidney is a site of FGF23 production during renal disease progression in experimental type 2 diabetes.

Identified a new mechanism of action of angiotensin II that, via the alteration of the Notch1/Snail/nephrin axis, perpetuates glomerular damage in diabetic nephropathy.

Discovered new mechanisms of angiotensinII-induced damage in advanced glomerular proliferative disorders through AT1/SDF-1/CXCR4 activation.

Pre-transplant infusion of Mesenchymal Stromal Cells in kidney transplant patients promotes regulation of immune response toward transplanted kidney.

Exosomes released by mesenchymal stem cells contain genetic information that are transferred to and contribute to the repair of damaged tubular cells.

Identification of inductive protocol for induced pluripotent stem cell differentiation towards renal progenitors.

A new technique to create in vivo functional renal organoids with filtration structures generated by single cell suspensions of embryonic renal cells.

Established a new method of gene therapy to the kidney by adeno-associated vectors to prevent chronic rejection of the graft.

Peri-transplant B cell depletion in kidney transplant recipients is associated with the development of anti-donor antibodies.
ADAMTS13 predicts renal and cardiovascular events in type 2 diabetic patients and response to therapy.

Combined complement gene mutations in atypical hemolytic uremic syndrome influence clinical phenotype.

**NATIONAL COLLABORATIONS**

Centro Dislipidemie "Enrica Grossi Paoletti", Ospedale Niguarda Cà Grande, Milano
Consorzio per la Ricerca sul Trapianto di Organi, Tessuti, Cellule e Medicina Rigenerativa CORIT, Padova
Clinica di Pediatria Oncoematologica, Università di Padova, Padova, Italia.
Fondazione I.R.C.C.S. Policlinico San Matteo, Pavia
Laboratorio di Biologia dello Sviluppo, Dipartimento di Biologia Animale, Università degli Studi di Pavia, Pavia
Laboratorio di Terapia genica e cellulare, G. Lanzani, Divisione di Ematologia, Ospedale Papa Giovanni XXIII di Bergamo
Laboratorio di Tecnologie della Riproduzione, AVANTEA Srl, Cremona
Laboratorio di Virologia, Istituto Nazionale per le Malattie Infettive L. Spallanzani, Roma
Dipartimento di Istologia Microbiologia e Biotecnologie Mediche, Università di Padova
Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale, Università di Bologna
Dipartimento di Patologia Clinica, Centro Regionale per Biomarcatori, Fondazione ABO, Venezia, Italia.
Dipartimento di Patofisiologia Clinica, Sezione di Nefrologia, Università di Firenze
Dipartimento di Scienze Farmacologiche, Università di Milano
International Centre for Genetic Engineering and Biotechnology, Molecular Medicine Group, Trieste
Istituto di Medicina Interna e Geriatria e Centro di Ricerca Emotasi, Università Cattolica, Roma
Istituto Nazionale dei Tumori Regina Elena, Roma, Italia
U.O. di Ostetricia e Ginecologia, Azienda Ospedaliera Spedali Civili di Brescia
Stem Cell Processing Laboratory, Clinic of Paediatric Oncohematology, University of Padova

**INTERNATIONAL COLLABORATIONS**

Assistance Publique-Hopitaux de Paris, Hôpital Europeen Georges-Pompidou, Service d’Immunologie Biologique, Paris, France
Academisch Ziekenhuis Maastricht, Interne Geneeskunde, Maastricht, The Netherlands
Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA
Biogazelle NV, Zwijnaarde, Belgium
Centro de Investigaciones Biológicas and Centro de Investigacion Biomedica en Enfermedades Raras, Madrid, Spain
Charité Universitätsmedizin Berlin, Germany
Children's Hospital and Regional Medical Center, University of Washington, Seattle, USA
Department of Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, NY, USA
Departments of Pediatrics and Human Genetics, University of Michigan, Ann Arbor, USA
Department of Medicine, Division of Rheumatology, Washington University School of Medicine, St. Louis, USA
Duke University Medical Center and Durham Veterans Affairs Medical Center, Durham, North Carolina, USA
Emergentec Biodevelopment GmbH, Vienna, Austria
Hans-Knoll Institute for Natural Products Research, Jena, Germany
Hospital of Bellvitge, Barcelona, Spain
INSERM (Institut National de la Santé et de la Recherche Médicale), Nephrology and Dialysis Department, Unit UMR S 702, Paris, France
Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Palo Alto, USA
Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK
Klinikum der Ludwig Maximillians Universitat Munchen, Germany
Max-Plank Gesellschaft zur Forderung der Wissenschaften, Hpi of experimental endocrinology, Hannover, Germany
Medical University of Innsbruck, Austria
MISOT (Mesenchymal Stem Cells in Solid Organ Transplantation) study group
Mosaiques Diagnostics GmbH, Hannover, Germany
New York Medical College, Valhalla, NY, USA
Otto-von-Guericke-University Magdeburg, Germany
Pediatric Nephrology Division, Center for Pediatrics and Adolescence Medicine, Heidelberg, Germany
Rosalind Franklin University of Medicine and Science, Chicago, USA
Saarland University Hospital, Homburg/Saar, Germany
The imperial college of science, technology and medicine, Londra, UK
UCD Conway Institute, University College Dublin, Ireland
University of British Columbia, Vancouver, Canada
University of Colorado Cardiovascular Institute, Denver, USA
University of Colorado Denver School of Medicine, Division of Nephrology and Hypertension, Colorado, USA
University of Groningen, the Netherlands
University of Pittsburgh School of Medicine, Pittsburgh, USA
Wake Forest Institute of Regenerative Medicine, Wake Forest University of School of Medicine, Winston- Salem, NC, USA
Weizmann Institute of Science, Rehovot, Israel
The Jikei University School of Medicine, Institute of DNA Medicine, Tokyo, Japan

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PEER REVIEW ACTIVITIES
American Journal of Kidney Disease
American Journal of Hypertension
American Journal of Pathology
American Journal of Pathology-Renal Physiology
American Journal of Transplantation
PARTICIPATION IN EVENTS IN WHICH THE DEPARTMENT WAS INVOLVED

Third Annual Meeting SysKid, FP7, Vienna, Austria, January 31 –February 2, 2013


50th ERA-EDTA Congress, Istanbul, May 17-21, 2013
Tissue Engineering and Regenerative Medicine International Society EU-meeting? Istanbul, Turkey  June 17-20, 2013

24th Congress of the International Society of Thrombosis and Haemostasis, Amsterdam, Olanda, June 29 - July 4 2013

Meeting STELLAR Project. Leiden, July 2, 2013

Convegno “CELLULE STAMINALI E CUORE: CANTIERI DAL FUTURO” Pisa, July 9 -10, 2013

14th European meeting of Complement, Jena, Germany, August 17-21, 2013

15th International Congress of Immunology, Milano, August 22-27, 2013

The International Conferences on Endothelin, Tokyo, Japan, September 8-11, 2013

ISN Forefronts Symposium on “Stem Cells and kidney regeneration” Firenze, September 12-15, 2013

First Meeting on OMICS toward the systems biology approach in renal disease and kidney transplantation, Valenzano (Bari), September 13-14, 2013

54° Congresso Nazionale Società Italiana Nefrologia Firenze September 25-28, 2013

Tavola Rotonda “Malattie e cure: quale ruolo hanno i nostri geni?”, Mario Negri Institute Alumni Association (MNIAA), Milano, November 18, 2013


Corso Nazionale SISET, Roma, November 28-29, 2013

55th Congress of the American Society of Hematology, New Orleans, December 7-10, 2013

Master di II livello in Anestesia, Terapia Intensiva Neonatale e Pediatrica, Ospedale Papa Giovanni XXIII, Bergamo, Dicember 17, 2013

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**GRANTS AND CONTRACTS**

Comitato Telethon Fondazione ONLUS
Commissione Europea
European Foundation for the Study of Diabetes
Fondazione Aiuti per la Ricerca sulle Malattie Rare (ARMR)
Fondazione ART per la Ricerca sui Trapianti ONLUS
Fondazione Cariplo
F. Hoffman – La Roche Ltd
Ministero della Salute
Regione Lombardia
AbbVie Inc.


**RESEARCH ACTIVITIES**

**Laboratory of Cell Biology and Regenerative Medicine**

Identification of inductive protocol for induced pluripotent stem cell differentiation towards renal progenitors

In collaboration with the Laboratory of Gene Therapy and Cellular Reprogramming

Kidney diseases are key determinant of the poor health outcomes of major noncommunicable diseases and contribute substantially to the global burden. Acute kidney injury (AKI) represents one of the most relevant public health issues leading to millions of deaths. The dimension of the
phenomenon remarks the urgency for innovative and effective therapeutic approaches. Renal stem and progenitor cells have been proposed as candidates for cell-based therapy. Induced pluripotent stem cells (iPSCs) represent an attractive source of renal progenitor cells (RPCs) for a patient-specific treatment. In this context, our laboratory focused on the set up of a specific inductive protocol for the derivation of renal progenitors. To this aim we have employed iPSC commercially available and iPSC derived by the Laboratory of Gene Therapy by means of human fibroblasts transfection with lentivirus containing transcription factors OCT4, SOX2, KLF4 e cMyc. The inductive protocol is divided in two stages involving the exposure of the cells to specific molecules (small molecules), retinoic acid and activin A followed by the treatment with nephrogenic factors. By this protocol, we obtained the loss of pluripotency markers and the acquisition of markers specific for mesoderm (brachyury), intermediate mesoderm (Osr1, Pax8, Pax2, WT1) and metanephric mesenchyme (Six2, Sall1). Results have been confirmed by gene expression and immunofluorescence. In a second stage, we have obtained the expression of molecules typically expressed by renal progenitors (Six2, CD24, NCAM). At the moment we are evaluating the nephrogenic and regenerative potential of the renal progenitors in animal models of acute and chronic kidney failure.

**b-arrestin-1 drives endothelin-1-mediated podocyte activation and sustains renal injury**

*In collaboration with the Unit of Advanced Microscopy of the Laboratory of Gene Therapy and Cellular Reprogramming and the Laboratory of Pathophysiology of Experimental Renal Disease and Interaction with other Organ Systems*

Activation of endothelin-A receptor (ETAR) by endothelin-1 (ET-1) drives epithelial-to-mesenchymal transition (EMT) in ovarian tumor cells through b-arrestin signaling. Here, we investigated whether this pathogenetic pathway could affect podocyte phenotype in proliferative glomerular disorders. In cultured mouse podocytes, ET-1 caused loss of the podocyte differentiation marker synaptopodin and acquisition of mesenchymal marker-smooth muscle actin (SMA). ET-1 promoted podocyte migration via ETAR and increased b-arrestin-1 expression. Activated ETAR recruited b-arrestin-1 to form a trimeric complex with Src leading to epithelial growth factor receptor (EGFR) transactivation and b-catenin phosphorylation, which promoted gene transcription of Snail. Increased Snail expression fostered ET-1-induced migration as confirmed by Snail knockdown experiments. Silencing of b-arrestin-1 prevented podocyte phenotypic changes and motility and inhibited ETAR-driven signaling. In vitro findings were confirmed in adriamycin (ADR)-induced nephropathy. Mice receiving ADR developed renal injury with loss of podocytes and hyperplastic lesion formation. b-arrestin-1 expression increased in visceral podocytes as well as in podocytes entrapped in pseudo-crescents. Selective ETAR antagonist sitaxsentan prevented podocyte loss, formation of the hyperplastic lesions, and normalized glomerular b-arrestin-1 expression and Snail expression. Increased b-arrestin-1 levels in podocytes retrieved in crescents of patients with proliferative glomerulopathies confirmed the translational relevance of these findings and suggested the therapeutic potential of ETAR antagonist for a group of diseases still in need of a specific treatment.

**Shigatoxin promotes podocyte injury in experimental Hemolytic Uremic Syndrome via activation of the alternative pathway of complement**

*In collaboration with the Laboratory of Gene Therapy and Cellular Reprogramming and the Laboratory of Pathophysiology of Experimental Renal Disease and Interaction with other Organ Systems*

Shiga toxin (Stx)-producing E.coli O157:H7 is the offending agent of post diarrhea-associated hemolytic uremic syndrome (D+HUS), a disorder of glomerular ischemic changes and widespread microvascular thrombosis. We previously documented that Stx induces glomerular
complement activation, generating C3a responsible for microvascular thrombosis in experimental HUS. In this study, we have shown that the presence of C3 deposits on podocytes is associated with podocyte damage and loss in HUS mice generated by the co-injection of Stx2 and LPS. Because podocyte adhesion to glomerular basement membrane is mediated by integrins, the relevance of integrin-linked kinase (ILK) signals on podocyte dysfunction was evaluated. Podocyte expression of ILK increased after Stx2/LPS injection and preceded the upregulation of transcription factor Snail and the downregulation of nephrin and ?-actinin-4. Factor B deficiency as well as an inhibitory antibody to factor B protected HUS mice against Stx2/LPS-induced podocyte dysregulation. In search for intracellular mechanisms underlying these disorders, we evaluated in vitro the effect of C3a, the anaphylatoxin generated as a result of complement activation, on podocyte dysfunction. In cultured podocytes, C3a promoted upregulation and redistribution of ILK together with a reduction of ?-actinin-4 expression. These effects were associated with an activation and subsequent nuclear translocation of Snail via ILK. Treatment with C3a also promoted podocyte motility, which was inhibited by ILK silencing. These results identify a new mechanism of glomerular damage for Stx-HUS, based on Stx-induced complement activation and C3a generation as key activators of ILK signaling pathway leading to podocyte dysfunction and loss.

Creating filtration structure and power from single cell suspensions

In collaboration with the Unit of Advanced Microscopy of the Laboratory of Gene Therapy and Cellular Reprogramming and the Unit of Pathology of the the Laboratory of Pathophysiology of Experimental Renal Disease and Interaction with other Organ Systems

Chronic kidney disease (CKD) is a life-threatening condition affecting hundreds of million people in the world, and the number of patients is rapidly increasing worldwide. The shortage of transplantable organs creates an imperative need for tissue-engineered alternatives by multiple approaches. We have shown that organoids constructed in vitro from suspensions of embryonic kidney cells can integrate into living recipients. The present studies were aimed at establishing whether organoids constructed from murine embryonic kidney cells and implanted into a rat host could i) develop fully mature glomerular slit diaphragms typical of the adult-like epithelial barrier, as required to serve normal ultrafiltration, ii) display additional features of selective filtering function that may translate into differential tubular reabsorption of multiple fluorescent tracers.

Renal organoids from E11.5 mouse kidney cells were implanted beneath the renal capsule of a rat host and allowed to mature for 2 weeks. Electron microscopy analysis showed glomeruli in various developmental stages, with glomerular capillary walls covered by podocytes. Filtration slits between foot processes reached full maturation, with formation of typical slit diaphragms. We tested the ultrafiltration function of intragraft nephrons by detection of injected fluorescent dextrans of increasingly high molecular weights into host blood system. Proximal tubule cells selectively concentrated low MW dextrans, and the apical colocalization with megalin indicated physiological reabsorption of probes that gained access to the tubular lumen by glomerular ultrafiltration. No 155 kDa dextran was found in tubular lumen, indicating efficient restriction exerted by the glomerular barrier against filtration of high MW macromolecule. These results provide evidence that organoids from suspensions of renal progenitor cells may offer a valuable approach to the task of constructing anatomically mature kidney tissue capable of complex functions. Hopefully, investigating further this system will be useful for regenerative medicine applications and, in the meantime, for developmental, drug-screening, or disease-modeling studies.

Laboratory of Immunology and Genetic of Rare Diseases and Organ Transplantation
Mesenchymal stromal cells and kidney transplantation: pretransplant infusion protects from graft dysfunction while fostering immunoregulation

Bone marrow-derived mesenchymal stromal cells (MSC) have emerged as useful cell population for immunomodulation therapy in transplantation. Moving this concept towards clinical application, however, should be critically assessed by a tailor-made step-wise approach. Here, we report results of the second step of the multistep MSC-based clinical protocol in kidney transplantation. We examined in two living-related kidney transplant recipients whether: (i) pre-transplant (DAY-1) infusion of autologous MSC protected from the development of acute graft dysfunction previously reported in patients given MSC post-transplant, (ii) avoiding basiliximab in the induction regimen improved the MSC-induced Treg expansion previously reported with therapy including this anti-CD25-antibody. In patient 3, MSC treatment was uneventful and graft function remained normal during 1 year follow-up. In patient 4, acute cellular rejection occurred 2 weeks post-transplant. Both patients had excellent graft function at the last observation. Circulating memory CD8(+) T cells and donor-specific CD8(+) T-cell cytolytic response were reduced in MSC-treated patients, not in transplant controls not given MSC. CD4(+) FoxP3(+) Treg expansion was comparable in MSC-treated patients with or without basiliximab induction. Thus, pre-transplant MSC no longer negatively affect kidney graft at least to the point of impairing graft function, and maintained MSC-immunomodulatory properties. Induction therapy without basiliximab does not offer any advantage on CD4(+) FoxP3(+) Treg expansion (ClinicalTrials.gov number: NCT 00752479).

In kidney transplant patients, alemtuzumab but not basiliximab/low-dose rabbit anti-thymocyte globulin induces B cell depletion and regeneration, which associates with a high incidence of de novo donor-specific anti-HLA antibody development.

In this single-center matched-cohort study, we evaluated the phenotype of repopulating B cells and its correlation with donor-specific anti-HLA Ab development and long-term graft function in 16 renal transplant recipients and 32 age- and gender-matched controls induced with alemtuzumab or basiliximab (Bas)/low-dose rabbit anti-thymocyte globulin (rATG), respectively. Alemtuzumab, but not Bas/rATG, profoundly depleted peripheral B cells in the first 2 mo posttransplantation. Early posttransplant, naive B cells were significantly depleted, whereas Ag-experienced and memory B cells were partially spared. Transitional B cells transiently increased 2 mo posttransplant. At month 6 posttransplant, pregerminal center B cells emerged, a process promoted by increased BAFF serum levels. Thereafter, B cell counts increased progressively, mainly due to expansion of naive B cells. Conversely, Bas/rATG did not modify the B cell phenotype throughout the follow-up period. Alemtuzumab was associated with a higher incidence of de novo DSA compared with Bas/rATG. DSA development was predicted by changes in the B cell compartment and correlated with worse long-term graft function. Thus, alemtuzumab-induced B cell depletion/reconstitution may promote chronic humoral responses against the graft.

ADAMTS13 predicts renal and cardiovascular events in type 2 diabetic patients and response to therapy.

In patients with diabetes, impaired ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13) proteolysis of highly thrombogenic von Willebrand factor (VWF) multimers may accelerate renal and cardiovascular complications. Restoring physiological VWF handling might contribute to ACE inhibitors' (ACEi) reno- and cardioprotective effects. To assess how Pro618Ala ADAMTS13 variants and related proteolytic activity interact with ACEi therapy in predicting renal and cardiovascular complications, we genotyped 1,163 normoalbuminuric type 2 diabetic patients from BErgamo NEphrologic Diabetes Complications Trial (BENEDICT). Interaction between Pro618Ala and ACEi was
significant in predicting both renal and combined renal and cardiovascular events. The risk for renal or combined events versus reference Ala carriers on ACEi progressively increased from Pro/Pro homozygotes on ACEi (hazard ratio 2.80 [95% CI 0.849-9.216] and 1.58 [0.737-3.379], respectively) to Pro/Pro homozygotes on non-ACEi (4.77 [1.484-15.357] and 1.99 [0.944-4.187]) to Ala carriers on non-ACEi (8.50 [2.416-29.962] and 4.00 [1.739-9.207]). In a substudy, serum ADAMTS13 activity was significantly lower in Ala carriers than in Pro/Pro homozygotes and in case subjects with renal, cardiovascular, or combined events than in diabetic control subjects without events. ADAMTS13 activity significantly and negatively correlated with all outcomes. In patients with diabetes, ADAMTS13 618Ala variant associated with less proteolytic activity, higher risk of chronic complications, and better response to ACEi therapy. Screening for Pro618Ala polymorphism may help identify patients with diabetes at highest risk who may benefit the most from early reno- and cardioprotective therapy.

Combined complement gene mutations in atypical hemolytic uremic syndrome influence clinical phenotype.

Several abnormalities in complement genes reportedly contribute to atypical hemolytic uremic syndrome (aHUS), but incomplete penetrance suggests that additional factors are necessary for the disease to manifest. Here, we sought to describe genotype-phenotype correlations among patients with combined mutations, defined as mutations in more than one complement gene. We screened 795 patients with aHUS and identified single mutations in 41% and combined mutations in 3%. Only 8%-10% of patients with mutations in CFH, C3, or CFB had combined mutations, whereas approximately 25% of patients with mutations in MCP or CFI had combined mutations. The concomitant presence of CFH and MCP risk haplotypes significantly increased disease penetrance in combined mutated carriers, with 73% penetrance among carriers with two risk haplotypes compared with 36% penetrance among carriers with zero or one risk haplotype. Among patients with CFH or CFI mutations, the presence of mutations in other genes did not modify prognosis; in contrast, 50% of patients with combined MCP mutation developed end stage renal failure within 3 years from onset compared with 19% of patients with an isolated MCP mutation. Patients with combined mutations achieved remission with plasma treatment similar to patients with single mutations. Kidney transplant outcomes were worse, however, for patients with combined MCP mutation compared with an isolated MCP mutation. In summary, these data suggest that genotyping for the risk haplotypes in CFH and MCP may help predict the risk of developing aHUS in unaffected carriers of mutations. Furthermore, screening patients with aHUS for all known disease-associated genes may inform decisions about kidney transplantation.

Two patients with history of STEC-HUS, post-transplant recurrence and complement gene mutations.

Hemolytic uremic syndrome (HUS) is a disease of microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. About 90% of cases are secondary to infections by Escherichia coli strains producing Shiga-like toxins (STEC-HUS), while 10% are associated with mutations in genes encoding proteins of complement system (aHUS). We describe two patients with a clinical history of STEC-HUS, who developed end-stage renal disease (ESRD) soon after disease onset. They received a kidney transplant but lost the graft for HUS recurrence, a complication more commonly observed in aHUS. Before planning a second renal transplantation, the two patients underwent genetic screening for aHUS-associated mutations that revealed the presence of a heterozygous CFI mutation in patient #1 and a heterozygous MCP mutation in patient #2, and also in her mother who donated the kidney. This finding argues that the two cases originally diagnosed as STEC-HUS had indeed aHUS triggered by STEC infection on a genetic background of impaired complement regulation. Complement gene sequencing should be performed before kidney transplantation in patients who developed ESRD...
following STEC-HUS since they may be undiagnosed cases of aHUS, at risk of post-transplant recurrence. Furthermore, genetic analysis of donors is mandatory before living-related transplantation to exclude carriers of HUS-predisposing mutations.

**Laboratory of Pathophysiology of Experimental Renal Disease and Interaction with other Organ systems**

**Effects of cannabinoid receptor type 2 (CB2) agonist in a mouse model of type 2 diabetic nephropathy**

Type 2 diabetes is the major cause of progressive CKD in the world. Diabetic kidney disease is associated with a dramatic excess of cardiovascular (CV) morbidity. Clinical trials have shown that inhibitors of renin angiotensin system can slow the progression of diabetic nephropathy (DN). However, in an advanced phase of the disease ACE inhibitors or Angiotensin II receptor blockers provide imperfect renoprotection, and CV risk remains elevated. Novel and multimodal strategies targeting pathogenetic pathways other than Angiotensin II are therefore worth exploring for diabetic patients who remain at risk of poor renal and CV outcomes. Experimental evidence suggests that impaired regulation of the cannabinoid receptor type 2 (CB2) may have a role in DN. CB2 is expressed by podocytes both in murine and human kidney. CB2 expression was downregulated in kidney biopsies from patients with DN. Renal levels of the CB2 ligand 2-arachidonylglycerol were reduced in mice with type 1 diabetes and early treatment with a selective CB2 agonist ameliorated albuminuria, podocyte protein loss and infiltration of monocytes. No data are available as to experimental type 2 DN. In the present study we investigated the effects of a CB2 agonist versus standard therapy with ACE inhibitor on renal disease progression in a murine model of type 2 DN, starting at a phase of overt disease. We used the BTBR ob/ob leptin-deficient mice, a recently characterized model of type 2 DN, which better mirrors human DN than previous murine model. BTBR ob/ob mice received from 10 to 21 weeks of age vehicle, CB2 agonist or lisinopril as standard therapy for comparison. BTBR wild-type mice served as controls. Results showed that diabetic mice given vehicle developed progressive increase of albuminuria levels as compared with controls. CB2 agonist reduced albuminuria already after 2 weeks of treatment (34% inhibition versus vehicle) and the effect was maintained during time (at 21 weeks, 36% reduction). Lisinopril reduced urinary albumin excretion by 41% and 55% versus vehicle at 12 and 21 weeks, respectively. The antiproteinuric effect of both drugs was associated with amelioration of the defective nephrin expression of diabetic mice. CB2 agonist limited mesangial matrix increase and glomerulosclerosis to a similar extent as ACE inhibitor. Interstitial inflammation was significantly lowered by 36% and 60% after CB2 agonist and lisinopril, respectively. Treatments did not affect hyperglycemia and dyslipidemia. These data indicate that CB2 is an important target of therapy in type 2 DN. Should future experiments demonstrate a superior renoprotective effects of CB2 agonist added on top of ACE inhibitor with respect to each drug alone, this might have therapeutic implications for type 2 DN.

**Renal expression of FGF23 in progressive renal disease of diabetes and the effect of ACE inhibitor**

*In collaboration with the Laboratory of Gene Therapy and Cellular Reprogramming*

Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone produced in response to an increase in phosphorus load or high levels of calcitriol or parathyroid hormone. FGF23 acts by inducing renal phosphate excretion by kidney proximal tubular cells through reduction of the expression of sodium phosphate co-transporters. FGF23 also suppresses the production of vitamin D active form in the kidney. FGF23 exerts its intrarenal biological function by binding to cognate FGF receptors requiring the presence of Klotho as a co-receptor. The site of synthesis...
of FGF23 is primarily the bone, although FGF23 is also expressed by brain, thymus, liver, spleen and heart. Since the kidney is an important target of FGF23, and the circulating levels of FGF23 have been found to increase in association with disease progression and cardiovascular events in chronic kidney disease and diabetic nephropathy, we wondered whether the kidney could be a source of FGF23 during the development of renal disease. We took advantage of the Zucker diabetic fatty (ZDF) rat model of human type 2 diabetic nephropathy characterized by obesity, hyperlipidemia, insulin resistance, progressive renal injury and cardiac abnormalities. Renal expression of FGF23 and Klotho was assessed in ZDF and control lean rats at 2, 4, 6, 8 months of age. We also investigated whether renoprotective effects of angiotensin converting enzyme (ACE) inhibitor in this model were associated with modulation of renal FGF23 and Klotho expression. To this aim ZDF rats were treated with ramipril from 4, when proteinuric, to 8 months of age. Results showed that FGF23 mRNA was not detectable in the kidney of lean rats, nor of ZDF rats at 2 months of age. FGF23 became measurable in the kidney of diabetic rats at 4 months and significantly increased thereafter. FGF23 protein localized in proximal and distal tubules. Renal Klotho mRNA and protein decreased during time in ZDF rats. As renal disease progressed, serum phosphate levels increased in parallel with decline of fractional phosphorus excretion. Ramipril limited proteinuria and renal injury, attenuated renal FGF23 upregulation and ameliorated Klotho expression. Ramipril normalized serum phosphate levels and tended to increase fractional phosphorus excretion. These data indicate that during progressive renal disease the kidney is a site of FGF23 production which is limited by ACE inhibition. Interfering pharmacologically with the delicate balance of FGF23 and phosphorus in diabetes may have implications in clinics.

**Laboratory of Gene Therapy and Cellular Reprogramming**

**Mechanism of cell-to-cell communication between MSC and damaged tubular cells**

*In collaboration with the Laboratory of Cell Biology and Xenotransplantation*

Bone marrow-mesenchymal stem cells (BM-MSC) ameliorate renal dysfunction and repair tubular damage of acute kidney injury (AKI) by locally releasing growth factors including the insulin-like growth factor-1 (IGF-1). The restricted homing of BM-MSC at the site of injury led us to investigate a possible gene-based communication mechanism between BM-MSC and tubular cells. Human BM-MSC (hBM-MSC) released microparticles and exosomes enriched in mRNAs. We demonstrated that hBM-MSC-derived exosomes contain the mRNA specific for IGF-1 receptor (IGF-1R) and that the transfer of this specific mRNA in damaged tubular cells is able to increase their proliferation. These findings suggest that the horizontal transfer of the mRNA for IGF-1R to tubular cells through exosomes potentiates tubular cell sensitivity to locally produced IGF-1 providing a new mechanism underlying the powerful renoprotection of few BM-MSC observed in vivo. We are now evaluating the renoprotective effects exerted by cells different from MSC and the possible role played by the released exosome as therapeutically active component of cells.

**Induction of pluripotent stem cells from somatic cells**

*In collaboration with the Laboratory of Cell Biology and Xenotransplantation*

We generated induced pluripotent stem cells (iPS) by reprogramming human neonatal fibroblasts. Using a lentiviral vector containing a unique reprogramming cassette including the four transcription factors OCT4, KLF4, SOX2 and cMyc we obtained four clones of iPS that have been fully characterized for their pluripotency. We evaluated the expression of the pluripotency markers such as Oct4, Nanog, Tra-1-81, Tra-1-60, SSEA3 e SSA4 by real-time PCR and immunohistochemistry. They showed a normal karyotype and they were able to
generate embryoid bodies and spontaneously differentiate into the three germ layers. Injected into NOD-SCID mice these cells induced the formation of teratomas confirming their pluripotency. In collaboration with the Laboratory of Cell Biology and Xenotransplantation, we are now studying a protocol to differentiate iPS into renal progenitor cells.

**Gene therapy to prevent chronic rejection of a solid organ**

Short-term outcome of organ transplantation has improved remarkably in the past 20 years, with a rate of one-year graft survival of about 90%. However, similar improvements in long-term outcome – 10 to 15 years survival – have not been achieved. Acute rejection is well controlled by the actual anti-rejection drugs that however do not prevent the development of chronic rejection, the leading cause of end-stage renal disease. In a previous study we demonstrated that gene transfer into the rat donor kidney of a gene encoding for CTLA4Ig prevented the activation of the immune system of the recipient. CTLA4Ig is a fusion protein able to prevent the full activation of alloreactive T cells by blocking the costimulatory pathway CD28-B7. Gene delivery was mediated by the AAV vector, a non-pathogenic virus able to sustain a prolonged expression of the recombinant protein. The engineered kidney transplanted in a fully incompatible animal had prolonged graft survival without the need of systemic immunosuppression. These findings may have important implication in the transplant medicine. However, before moving to the clinic, additional pre-clinical studies are mandatory to demonstrate that the same procedure is valid in a species closer to human. This was the aim of a project performed in collaboration with two other groups, the International Centre for Genetic Engineering and Biotechnology (ICGEB) in Trieste and the Consortium for Research in Organ, Tissue and Cell Transplantation and Regenerative Medicine (CORIT) in Padova. In this study we evaluated whether gene delivery of AAV-LEA29Y (CTLA4Ig with two aminoacid substitutions that increase its biological potency) into the kidney of non-human primates was able to efficiently infect the organ and to induce the production of the LEA29Y protein. The AAV-LEA29Y vector was produced in large quantity by the group of prof. Mauro Giacca to the ICGEB. Four autotranplantation experiments have been performed in Padova following a protocol approved by the Ministero della Salute. These experiments allowed us to demonstrate that the AAV vector was able to infect the non-human primate kidney, and induced the production of the recombinant protein locally into the kidney. The next step will be the identification of the mechanisms underlying the process of immunomodulation induced by LEA29Y.

**Angiotensin II contributes to the pathogenesis of diabetic renal dysfunction in rodents and humans via notch1/snail pathway**

*In collaboration with Laboratory Cell Biology and Regenerative Medicine*

Over the past 25 years the burden of diabetes mellitus has almost doubled worldwide, and projection for the future are alarming. Mortality in diabetes is essentially driven by the concomitant kidney disease that may progress to end-stage renal disease in 30 to 40% of patients. One of the major factors involved in the progression of kidney disease is the vasoconstrictor angiotensin II (Ang II). The Ang II alters the structure and function of the glomerular filter through its hemodynamic effects and its ability to reduce the expression of nephrin, an essential protein of the glomerular slit diaphragm. In this study we investigated the role of Ang II and the molecular mechanism activated in diabetic nephropathy.

In the ex vivo model of isolated rat kidney the perfusion of Ang II induced a reduction of nephrin expression with concomitant loss of the functionality of the glomerular filter as demonstrated by increased urinary protein excretion. In cultured human podocytes, Ang II reduced the expression of nephrin via the activation of Notch1, the transmembrane receptor mediating the transcription of different genes, and the nuclear translocation of Snail, known repressor of nephrin expression. The abnormalities of the Notch1/Snail/nephrin axis observed in vitro were similar to those observed in renal biopsies of diabetic rats. The pharmacological
treatment with the inhibitor of the renin-angiotensin system (RAS) reduced proteinuria and increased the levels of nephrin through regulation of Notch1 and Snail. To examine the clinical relevance of what observed in animals, we examined biopsies of patients with advanced diabetic nephropathy. Even in biopsies of diabetic patients we confirmed the increase of synthesis/activity of Notch1, translocation of Snail and reduced expression of nephrin, changes that were improved by treatment with a RAS inhibitor.

The present study demonstrated that Ang II plays an important role in perpetuating the glomerular damage in both experimental and human diabetic nephropathy via a persistent activation of Snail and Notch1 signal in podocytes and that eventually results in a reduction in the expression of nephrin, the whose integrity is crucial to the glomerular filtration barrier.

**Nature and mediators of parietal epithelial cell activation in glomerulonephritides of human and rat**

*In collaboration with Laboratory Drug Development*

In several human proliferative glomerulonephritides, a common pathogenetic mechanism is represented by the activation of the Bowman’s capsule parietal epithelial cells, which proliferate and migrate in response to podocyte injury. Recently, a population of CD133+CD24+ progenitor cells has been proposed to be the major constituent of the resulting crescentic lesions, glomerular abnormalities constituted by multilayers of cells accumulating between the Bowman’s capsule and the glomerular capillary tuft. However, the cellular composition of crescentic lesions is controversial, and mediators involved in progenitor cell proliferation and migration into the Bowman’s space have been poorly explored to date.

Here, by analyzing renal biopsies of patients with proliferative and non proliferative glomerulopathies, we demonstrated that dysregulated CD133+CD24+ progenitor cells of the Bowman’s capsule invade the glomerular tuft exclusively in proliferative disorders. The abnormal proliferation and migration of renal progenitors may be explained by the inflammatory nature of these glomerular disorders. Accordingly, we showed that podocytes, possibly activated by the inflammatory microenvironment, strongly expressed the chemokine stromal-derived factor-1 (SDF-1), providing the ligand for CXCR4 receptor up-regulated on parietal progenitor cells, ultimately allowing their migration and proliferation. Beside chemokines, cell proliferation resulting in crescentic lesion formation, might be also promoted by increased expression of the angiotensin II type1 (AT1) receptor on parietal progenitor cells.

Data obtained in human biopsies were validated in a rat experimental model by analyzing renal tissues of Munich Wistar Fromter rats with proliferative glomerulonephritis. In this latter model, similar changes of CXCR4, SDF-1 and AT1 receptor expression were observed in respect to normal control rats. Coming back to humans, we found that in a patient with crescentic glomerulonephritis, ACE inhibitor normalized CXCR4 and AT1 receptor expression on progenitors concomitant with regression of glomerular lesions.

These results suggest that crescentic lesions derive from the abnormal proliferation and migration of renal progenitors in response to injured podocytes. Targeting the angiotensin II/AT1 receptor/SDF-1/CXCR4 pathway may be beneficial in severe forms of glomerular proliferative disorders.
DEPARTMENT OF BIOMEDICAL ENGINEERING

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CURRICULA VITAE

**Andrea Remuzzi** got his degree in Mechanical (Biomedical) Engineering in 1979, Politecnico di Milano.

**Research experience:** 1980 Politecnico di Milano, Dipartimento di Ingegneria Biomedica; 1981 Istituto Mario Negri (Milano), Laboratorio di Farmacologia Cardiovascolare; 1982-83 Massachusetts Institute of Technology, Mechanical Engineering Department, Cambridge, USA.

**Areas of interest:** biological transport phenomena, mathematical models, renal pathophysiology, cellular response to mechanical stimulation, tissue engineering, pancreatic islet transplantation, clinical databases, computational fluid dynamics.


**Selected publications**


**Research experience:** 1977-81 CNR Institute of Neurosciences - Cell Mol Pharmacology - and Department of Medical Pharmacology, University of Milan, Milan, Italy; 1982-83 Laboratory of the Division of Nephrology and Dialysis, Ospedali Riuniti di Bergamo, Bergamo, Italy; 1984-85 University of Michigan, Medical School, Department of Pathology, Medical Science I, Ann Arbor Michigan, USA; 1985-89 Mario Negri Institute for Pharmacological Research, Laboratory of Kidney Disease, Bergamo, Italy.

**Areas of interest:** glomerular permeability, renal disease progression, podocytes, angiotensin II, reactive oxygen species, intracellular molecular signaling.

**Chronology of appointment:** From 2000 Head Laboratory of Renal Biophysics, Department of Biomedical Engineering, IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Bergamo; 1994-2000 Head, Unit of Inflammatory Mediator of Leukocyte Origin; 1989- 94 Scientist, 1985-89 post-doctoral fellow Mario Negri Institute for Pharmacological Research, Laboratory of Kidney Disease, Bergamo, Italy.

**Selected publications**


**Bogdan Ene-Iordache** got an MS in Mechanical Engineering in 1990 at the Petroleum & Gas University in Ploiesti (Romania).

**Education:** He completed a training program in biomedical engineering at the Mario Negri Institute in Bergamo and in the Clinical Research Center for Rare Diseases “Aldo e Cele Daccò” in Ranica, Bergamo.

**Main interests:** Renal research (hemodynamics and modeling of the arteriovenous fistula for vascular access, morphometry of renal glomeruli) and controlled clinical trials (data management and data analysis). Other research interests include clinical research informatics, applied clinical informatics and development of electronic health record (EHR) systems.

**Roles:** From 1992 to 1996 he was visiting scientist, and since 1996 to 1999 researcher in the Bioengineering Lab at Negri BERGAMO Laboratories, Bergamo. Since January 2000 is the Head of the Biomedical Technologies Laboratory, Department of Biomedical Engineering. He is coordinating the IT activities in the Clinical Research Center for Rare Diseases “Aldo e Cele Daccò”.

**Selected publications**


Research experience: 1991-94 Mario Negri Institute for Pharmacological Research, Bergamo, Italy.

Areas of interest: techniques of kidney decellularization, isolation of pancreatic islets from human, bovine, pig and rat pancreas, cell culture, immunosolisation devices for pancreatic islets, differentiation of progenitor pancreatic cells in insulin containing cells, immunohistochemistry.

Chronology of appointment: From 2000 Head Unit of Tissue Engineering, Department of Biomedical Engineering; 1991-2000 fellow laboratory of Renal research, Mario Negri Institute for Pharmacological Research, Bergamo, Italy.

Selected publications


Anna Caroli graduated in Mathematics in 2003 at Milan University, and obtained her PhD in 2010 at Maastricht University.

Training activities: February - June 2005: Neuroimaging training at Brain Imaging Centre, Montreal Neurological Institute, Montreal - Canada

Areas of interest: medical and preclinical image acquisition and processing in the renal and cerebral (Alzheimer’s Disease) fields

Roles: since 2004: Researcher at the Laboratory of Epidemiology and Neuroimaging, IRCCS Fatebenefratelli, in Brescia, Italy; since 2008 to 2012: Researcher at the Medical Imaging Unit (Biomedical Technology Laboratory, Bioengineering Department), IRCCS Istituto di Ricerche Farmacologiche Mario Negri, in Bergamo; since July 2012 to date: head of the Medical Imaging Unit, Bioengineering Department

Selected publications


Sergio Carminati has obtained his Accounting Diploma in 2000, at Istituto Tecnico Commerciale Camanghè of Zogno. He completed his training at “Clinical Research Center for Rare Diseases Aldo e Cele Daccò”.

Educational Training: in 2001 he got his postgraduate qualification in Computer Programmer at ENAIP Lombardia di Bergamo.

Areas of interest: Data management for Clinical Trial, Web-based Applications for Epidemiology and Outcome Research.

Support activities: web sites development and administration, Local Area Network (LAN) – System administration, Videoconferences / Meetings assistance.

Roles: from 2001 to 2002 he did the civil service at Laboratory of Biomedical Technologies. From 2002 to 2011 he worked at Laboratory of Biomedical Technologies, Department of Bioengineering. Since February 2010, Head Unit of Clinical Research Informatics, Department of Bioengineering

Selected publications


2. Genovesi S. Hypertension and kidney function in an adult population of West Bengal, India: Role of body weight, waist circumference, proteinuria and rural area living, Nephrology (Carlton) 2013.


INTRODUCTION TO THE DEPARTMENT’S ACTIVITIES

The Department of Bioengineering conducts research at experimental and clinical level. Department to investigate pathophysiological processes through the use of engineering techniques and to develop innovative treatment strategies. The main tools used for this research consists of theoretical models, diagnostic imaging, histological measures, physical and chemical parameters and cell cultures. Ongoing studies involve four main areas: 1) the study of the mechanisms responsible for the progression of chronic kidney disease; 2) the study of the role of hemodynamics in the development of vascular damage; 3) the development of laboratory techniques for tissue engineering; 4) the development of information systems for the management of clinical data and images generated in the context of controlled clinical trials and in clinical practice.

FINDINGS/MAIN RESULTS

We produced evidence demonstrating a possible relationship between haemodynamic conditions in the vascular access for hemodialysis and the development of neointima formation which is responsible for the failure of access.

We developed and implemented, in a distributed computing system, a tool for computer aided planning of vascular surgery used to make the vascular access in patients on hemodialysis.

By quantifying the volumes of the renal tissue performed on CT images of patients with polycystic kidney disease we have shown a beneficial effect of the administration of somatostatin in reducing the increase in renal volume.

We activated and supported a network of specialists in Nephrology for the collection of clinical data aimed at monitoring the quality of the pharmacological treatment of progressive chronic kidney disease in current clinical practice.

We developed a support system for evaluating the appropriateness of drug treatments and possible drug interactions. The system is currently in use as part of a clinical research project involving primary care physicians and medical specialists.

We have developed methodologies to regenerate a new organ in the laboratory starting from a kidney decellularized and subsequently recellularized with stem cells.

NATIONAL COLLABORATIONS

Dipartimento di Bioingegneria, Politecnico di Milano, Milano
Unità di Diabetologia, Ospedali Riuniti, Bergamo
Unità di Nefrologia, Ospedali Riuniti di Bergamo
STMicroelectronics, Agrate Brianza, Milano
Dipartimento di Ingegneria Industriale e Dipartimento di Ingegneria dell'Informazione e Metodi
Matematici, Università di Bergamo
Facoltà di Medicina e Chirurgia, Università degli Studi di Milano
Brembo S.p.A. Stezzano, Bergamo – Italia

INTERNATIONAL COLLABORATIONS

Massachusetts Institute of Technology, Cambridge MA, USA.
Department of Mathematics and Computer Science, Emory University, Atlanta, Georgia, USA
Simula Laboratories, Oslo, Norway
Academisch Medisch Centrum, Amsterdam, the Netherlands
University of Toronto, Ontario, Canada.
Ghent University, Ghent, Belgium.
Technical University, Eindhoven, The Netherlands.
University Hospital, Maastricht, The Netherlands.
The University of Sheffield, Sheffield, United Kingdom.
ESAOITE, Maastricht, The Netherlands.
Beta O2, Tel Aviv, Israel.

EDITORIAL BOARD MEMBERSHIP

International Journal of Artificial Organs (Andrea Remuzzi)
ISRN Nephrology (Daniela Macconi)

PEER REVIEW ACTIVITIES

Acta Diabetologica
African Journal of Pharmacy and Pharmacology
American Journal of Kidney Diseases
American Journal of Pathology
American Journal of Physiology
Annals of Biomedical Engineering
ASME Journal of Biomechanical Engineering
Artificial Organs
Biomaterials
Cell Transplantation
Cells & Materials Journal
Contemporary Clinical Trials
Hemodialysis International
IEEE Transactions on Biomedical Engineering
IEEE Transactions on Image Processing
EVENT ORGANIZATION

Seminar: "Vascular Endothelium, Biomechanical Forces and the Pathogenesis of Atherosclerosis", Michael Gimbrone, Center for Excellence in Vascular Biology, Brigham & Women's Hospital, Centro Anna Maria Astori – Bergamo.

Club delle 2: "Endothelial response to shear stress and intimal hyperplasia in vascular access for hemodialysis", Marco Franzoni, Istituto Mario Negri, Sala Conferenze Villa Camozzi – Bergamo.

Club delle 2: "Placing a primary arteriovenous fistula that works: more or less known aspects, new ideas" Bogdan Ene-Iordache, Istituto Mario Negri, Sala Conferenze Villa Camozzi – Bergamo.

Club delle 2: "Angiotensina II, target terapeutico per la remissione/regressione delle nefropatie progressive", Daniela Macconi, Istituto Mario Negri, Centro Anna Maria Astori – Bergamo.

Seminar: "Validation of a patient-specific hemodynamic computational model for planning vascular access surgery in hemodialysis patients", Simone Manini, Istituto Mario Negri Sala Conferenze - Villa Camozzi.

PARTICIPATION IN EVENTS IN WHICH THE DEPARTMENT WAS INVOLVED

Corso Teorico-Pratico avanzato di eco color Doppler carotideo e vertebrale, Milano 21-23 Febbraio 2013.


V International Conference on Computational Bioengineering, 11-13 Settembre 2013, Leuven, Belgio.


Invitation to the ERA-EDTA CME Course: “Clinical and scientific advances in management of patients with ADPKD”, 12-13 Settembre 2013, Oxford, UK.

Tissue Engineering and Regenerative Medicine International Society – Asia Pacific Chapter (TERMIS-AP) 2013 annual conference, 23-26 October 2013, Shanghai and Wuzhen, PR China.

American Society of Nephrology Kidney Week 2013 (ASN2013), 5-10 Novembre 2013, Georgia World Congress Center, Atlanta, GA.

GRANTS AND CONTRACTS

Research grants AIFA - studi clinici controllati (VARIETY, VALID, ATHENA, ARCADIA, ANSWER, COSTANT, CRESO2, PROCEED, PREDICTION).

Research grant PKD foundation - ALADIN trial “Effect of long-acting somatostatin on disease progression in ADPKD: a long-term three year follow-up study”.

Research grant Baxter – ASAP trial “Acute Start Access Programme”.

Research grant SigmaTau – DIABASI trial

Research grant ABBOT – PROCEED trial

Research grant ISN per il Kidney Disease Data Center (KDDC – COMGAN).
Contributo Regione Lombardia per data management del Centro di Coordinamento della Rete Regionale per le Malattie Rare.


Progetto di ricerca - FP7 UE - RESET "Dreaming of no more renal dialysis: how self-derived tissue and cells can replace renal function". FP7- 268632 - Project Coordination.


Progetto di ricerca in collaborazione con Roche: Polycystic Kidney Disease (PKD) and the “Intermediate volume”.

Progetto di ricerca in collaborazione con Brembo S.p.A.: “Valutazione del rischio potenziale legato alla produzione di pastiglie per freni e al loro utilizzo”.

Progetto di ricerca in collaborazione con Fresenius S.p.A. “Studio pilota per valutare l’impatto del modello matematico predittivo (AVF.SIM) nella pratica della clinica convenzionale”.

**SELECTION OF SCIENTIFIC PUBLICATIONS FROM 2013**


**RESEARCH ACTIVITIES**

**Laboratory of Renal Biophysics**

**Investigation of renal vascular changes by microCT**

The microCT is a diagnostic imaging tool that allows to generate high-resolution images of anatomical structures and tridimensional reconstruction for the morphological analysis of skeletal tissues and organ vasculature. In order to reconstruct the tridimensional kidney vasculature, the kidney is perfused with Microfil, a radiopaque silicone polymer, excised and scanned with MicroCT. After acquisition, images are processed by specific softwares. In particular, thresholding techniques are used to generate binary images of the entire kidney and kidney vasculature and to estimate kidney and vasculature volume. In addition, if diameter and branching level values of each vessel are computed, it is possible to reconstruct and analyze kidney vasculature network and its geometry. The application of this technique to nephropathies allows to identify structural changes of renal vascular architecture during disease progression and to study how these changes correlate with functional parameters. In this context, the 3D reconstruction by microCT of the vascular network in kidneys from rats with progressive nephropathy at advanced stage of the disease has documented a simplification of the renal vasculature and microvascular rarefaction. We have also applied the microCT to assess the integrity and patency of vascular segments as well as the entire vasculature integrity of the renal vessels during a rat kidney decellularization procedure. Real time acquisition of X-Ray projection of decellularized kidney during infusion of Microfil allowed to assess a uniform distribution of the contrast agent through the vascular network without extravasation into the surrounding tissue. These findings document integrity and patency of the renal vascular architecture that was well preserved as further confirmed by 3D digital reconstruction.

**Molecular mechanisms underlying insulin resistance.**

_In collaboration with the Laboratory of Cellular Biology and Regenerative Medicine (Department of Molecular Medicine)_

Angiotensin II (Ang II) promotes insulin resistance that has an important impact on the type 2 diabetes and its related renal and cardiovascular complications. Increased mitochondrial reactive oxygen species (ROS) are emerging as intracellular mediators of multiple form of insulin resistance although their functional role in Ang II-induced insulin resistance and the underlying mechanism(s) involved have not been explored. We have recently documented that Ang II down-regulates Sirt3 gene expression in cultured cells via Ang II type 1 receptor (AT1R) and disruption of such receptor promotes organ protection from age-induced oxidative stress via Sirt3 upregulation. Sirt3, a member of the sirtuin family of NAD+-dependent deacetylases, localizes in the mitochondria where it functions to maintain basal ATP levels and regulates the activity of proteins involved in metabolic pathways and antioxidant defense. Our research was
aimed at assessing whether Ang II induces insulin resistance in skeletal muscle cells through mitochondrial oxidative stress and Sirt3 dysregulation. In this setting, we also investigated whether acetyl-L-carnitine - that ameliorates impaired glucose tolerance and insulin resistance in subjects with a clustering of risk factors for diabetes mellitus and cardiovascular disease - affects insulin sensitivity through the Sirt3 modulation. Finally, the implication of Sirt3 as a potential target of acetyl-L-carnitine protective effect was explored in vivo in high fat diet-fed insulin resistant mice.

**Laboratory of Biomedical Technologies**

**Remission Clinic Network**

Many forms of chronic kidney disease progress with a constant rate of renal function loss towards the end stage renal disease (ESRD). These forms of kidney disease are often associated with arterial hypertension and urine proteins, known as aggravating factors in the progression of the disease. Controlled clinical trials have demonstrated that specific treatments of hypertension with drugs that decrease the urinary excretion of proteins (ACE-inhibitors) are effective in reducing the rate of decline of GFR and even in reaching stabilization or recovery of renal function allowing to delay the start of dialysis or the need of kidney transplant in subjects with chronic kidney disease.

In collaboration with the Renal Medicine Department, we started to monitor patients affected by proteinuric nephropathies (Remission Clinic protocol) with the aim at verifying whether GFR improvement might be obtained in routine clinical practice, as well. Our laboratory developed a web-based application and established a network of specialists involved in the treatment of chronic progressive nephropathies distributed nationally ([http://clinicalweb.marionegri.it/remission](http://clinicalweb.marionegri.it/remission)). Our tool offers computer support to medical specialists from all participating centres to gather, extract and analyse real time clinical data for patients with chronic kidney diseases treated according to the guidelines of Remission Clinic protocol. In addition, our tool allows real time analyses and quality controls of this clinical activity to assess to what extent adherence to the protocol may itself slow the progression of nephropathy in time.

**KDDC – a centre for data collection and surveillance of prevention programs on non-communicable chronic diseases in emerging countries**

Chronic kidney diseases are emerging as a global threat to human health. Prevalence and incidence of renal diseases in developing countries are not known, and this is an obstacle to the adoption of preventive measures. Prevention is the only hope for these countries where treatment options for end stage renal failure are simply not available to the vast majority of the population because of their costs.

The International Society of Nephrology (ISN), through the Global Outreach (GO) initiatives, has established a research committee to face the problem of prevention of kidney diseases in developing countries. The coordination of the team and intervention programs was committed to the Mario Negri Institute for Pharmacological Research at the Clinical Research Centre “Aldo e Cele Daccò”. The general aim of the project is to define programs in developing countries to identify those subjects who are at risk of developing a renal disease later in life, in order to design a prevention strategy on national basis by means of interventions of the local ministries of health to governmental and financial level. The Kidney Disease Data Centre (KDDC) established in our Laboratory, is dedicated to data management for the prevention programs underway in emerging countries. We have set up an a tool to collect clinical data from different centres located world-wide ([http://comgan.marionegri.it](http://comgan.marionegri.it)). Data are stored in a dedicated server hosted in the Institute’s server farm in Milan. Results of our epidemiological analyses, shared also with medical staff of the various centres, allow us to have a general overview on the health
of population under study. The prevention programs started in 2006 and today KDDC owns more than 100,000 records of subjects from 16 countries located all over the world. We have published the results of screening programs in Moldova and in India, a cross-sectional study in poor countries (Bangladesh, Nepal, Georgia, Bolivia) respect to the USA, and a screening on over 11,000 subjects from Nepal, India and Mongolia. All these studies have shown the burden of chronic diseases in these countries and demonstrated the feasibility of the ISN prevention programs. Through the activity of KDDC it is possible to monitor the course of the actual screening projects, to tailor them to the specific needs of each participating country, and even more important to commence follow-up programs in low income countries.

Development of computerized systems for controlled clinical trials
Numerous clinical trials are conducted in the Clinical Research Center for Rare Diseases “Aldo e Cele Daccò”. These studies must be carried out in accordance with all regulatory requirements (GCP, EMEA, FDA). Every clinical study requires a paper case report form (CRF) for the collection of patients’ clinical data. These clinical information must be verified for inconsistency by dedicated monitoring staff, and then recorded electronically.

In our Laboratory, we have developed applications tailored for data management of clinical studies using relational databases systems (RDBMS) and specific programs aimed to data elaboration, validation and extraction for subsequent statistic analyses. For the DEMAND study we have developed an innovative electronic CRF based on laptop computers. During the year 2009, we have developed and published a web-based portal for electronic data capture and clinical data management for the clinical trials (http://clintrials.marionegri.it) conducted at the Clinical Research Center “Aldo e Cele Daccò”. The platform consists of a framework for developing web-based e-CRF, a platform for clinical monitoring and a randomization system for controlled trials.

Data Management for the Regional Network for Rare Diseases of Lombardy
Our Laboratory contributes to the management of the Regional Network for Rare Diseases of Lombardy. We are directly involved for the development and maintenance of the web site of the centre and management of a regional Registry for Rare Diseases. We have set-up the databases and developed related web-pages for centres, rare diseases archive, patient associations and congenital rare diseases. These are published on the homepage of the web-site (http://malattierare.marionegri.it/).

The Registry for Rare Diseases was born in 2007 as a collaboration between Mario Negri Institute, Lombardia Informatica and Regione Lombardia. The aim was to create a regional registry for rare diseases where all medical staff from Lombardy could register information regarding rare diseases. The application (Sistema Malattie Rare - SMR) is actually in use in almost all centres dedicated for rare diseases in Lombardy and can be used jointly with the patient health card.

Imaging and quantification in renal physiopathology
The use of imaging techniques such as CT, MR, and ultrasound, and the application of advanced image processing tools make it possible to perform non-invasive in-vivo quantitative analysis of biological phenomena. Within the Department of Biomedical Engineering, this approach is applied to the investigation of renal physiopathology.

Through CT and MR image-based quantification, new therapies for autosomal dominant polycystic kidney disease (ADPKD) are currently being evaluated. To this purpose, the Medical Imaging Unit has been involved in several clinical trials, some of which still ongoing, one funded by the Polycystic Kidney Foundation, aimed at reducing the overall kidney cyst volume with the use of novel therapies. Using specific automatic algorithms, it has been possible to
quantify on contrast-enhanced CT and MR images renal cyst volume, beyond total kidney volume only. Moreover, CT image quantification has recently led to the discovery of a fibrotic tissue component (named intermediate volume), highly correlated with both renal function and disease progression rate, showing for the first time a likely direct relationship between structure and function, thus opening the way to new therapeutic targets. Beyond ADPKD studies, new methodologies for non-invasive characterization of renal functionality and for the identification and quantification of fibrotic tissue from non-contrast enhanced MR images are currently under study.

Furthermore, the Medical Imaging Unit is involved in processing ultrasound images from an explorative pilot study, carried on in collaboration with Bracco, aimed at identifying by contrast-enhanced ultrasound (CE-US) perfusion patterns associated with key patterns of renal involvement characterizing acute rejection and other causes of acute renal dysfunction in patients receiving renal transplantation. Through a novel contrast agent, composed by microbubbles filled with gas spreading in the blood, it is possible to improve vessel representation and have access to structural and functional information on microcirculation, which could not be available by conventional clinical procedures.

Hemodynamics and vascular pathology
During the last twenty years, the existence of a tight relationship between hemodynamics and vascular pathology was confirmed in many investigations, either at basic or clinical research level. Thanks to innovative technologies developed in medical imaging and mathematical modelling, it is now possible to reproduce accurately patient-specific hemodynamic force distribution from computed tomography (CT), magnetic resonance (MR) and echo-color Doppler ultrasound investigations. Such typical applications in our Biomedical Engineering Department are the investigation of atherosclerotic lesions in the arterial circulation, of the intracranial aneurysm disease, and the effects of vascular access for hemodialysis creation. Analysing indicators of disturbed flow with numerical techniques, we found that disturbed flow develops in some sites in the radial-cephalic fistulae used as vascular access, with mechanisms similar to those observed in the carotid bifurcation. The localization of these zones depends from the geometry of the vascular access and distribution of blood volume flow, but predominant sites are on the inner wall of the juxta-anastomosis vein and on the anastomosis floor, where intimal hyperplasia and consequently the stenosis develop. Following these findings, we have recently shown that the angle of anastomosis influences the distribution of areas of disturbed flow, identifying in an acute angle (~30°) the geometry that better minimizes formation of neointima.

The Department has coordinated an international collaborative project funded by the European Commission within the Seventh Framework Programme (FP7-ICT-2007-2-224390, ARCH), aimed at ameliorating vascular access function for haemodialysis treatment. During ARCH project, computational tools for predicting patient-specific hemodynamic changes caused by vascular access creation based on pre-operative data have been developed. In particular, the Department actively contributed to the development of an open-source infrastructure (archTk, archtk.github.com), including a graphical interface (archNE), enabling to build vascular network models, and a solver (pyNS), enabling to simulate blood flow in the vascular network. After calibration and preliminary validation, these computational tools were embedded in a web-based clinical application (AVF.SIM) aimed at predicting vascular access function from pre-operative evaluations and thus helping surgeons to plan patient-specific best vascular access creation. A multicentric pilot clinical study aimed at proving AVF.SIM utility in support of vascular surgical planning in clinical practice is still ongoing.

Development of devices for the transplantation of immunoisolated islets
Transplantation of insulin-producing cells is a promising cellular-based therapy for type 1 diabetes and cell immunoisolation is one of the strategies used to prevent rejection of the
An immunoisolation system is able to separate the islets from the immune system of the host by a selectively permeable membrane without interfering with their physiological function. An immunoisolation device must have the most suitable geometry providing large surface area in a small volume. Moreover the semipermeable membranes chosen to protect the islets from the immune system must allow sufficient passage of nutrients, oxygen, and the therapeutic products, insulin. The project’s main objectives are to develop new immunoisolation devices for pancreatic islets that can be implanted in allo or xeno-transplantation models and to test the efficacy and in vivo resistance of the devices. We have developed a device made using parallel arrays of polysulfone hollow fibers that can be implanted with minimally invasive surgical procedures. The fibers have been chosen for their particular microstructure that allows the passage of glucose and insulin and prevents the passage of immunoglobulins and molecules involved in the rejection. We have also developed a method for subcutaneous implantation as alternative site for transplantation. In rats with diabetes induced by streptozotocin, implantation of islet-containing devices was able to reduce the severe hyperglycemia and to prevent the loss of weight of the animals without the use of immunosuppressors. The aims of our studies in the next months will be to improve functionality using nanotechnologies for material characterization.

Effect of pancreatic islet transplantation on diabetic complications

Type 1 diabetes is a form of diabetes mellitus that results from autoimmune destruction of insulin-producing beta cells of the pancreas. Diabetes is associated with increased risk of a number of microvascular, neurologic and macrovascular complications due to poor glycemic control. Diabetic complications contribute to morbidity and mortality rates and severely impair quality of life of patients. In recent years, to avoid diabetic complications, it has been developed a technique of beta-cell replacement thanks to the transplantation of pancreatic islets. The aim of this project is to evaluate whether transplantation of pancreatic islets can induce regression of diabetic complications in a model of allotransplantation in rats with chemically induced diabetes. For this study, we used four groups of rats: healthy controls, diabetics, diabetics with transplanted islet four months after induction of diabetes and diabetics treated with insulin to adjust glycemia under 200 mg/dl. Transplantation of islet induces a lowering of blood glucose within a few days after transplantation, accompanied by an increase in body weight. All the neurological parameters observed in diabetic rats significantly ameliorate in transplanted rats. We also observed a consistent reduction in proteinuria in islet-transplanted rats. When the blood glucose concentration was normalized by islet transplantation we observed an important regeneration of beta cells. Insulin treated rat group show a milder improvement of the above described parameters. Our results indicate that normalization of blood glucose by islet transplantation improved neural function, reduced proteinuria and induced partial regeneration of beta cells with islet-like organization in the pancreas. The next step of the project will be to investigate the transcription factors involved in the observed regeneration of beta cells. We will focus mainly on transcription factors such as PDX1, involved in the development of pancreatic beta cells, MafA, present in the adult beta cells and MafB that plays a critical role in beta cell differentiation. Preliminary experiments with these markers were performed to set up the immunohistochemical techniques.

Tendons and ligaments engineering

In the field of implantable biomedical devices, tissue engineering is probably still far from producing the ideal bioscaffold to replace, repair, or regenerate injured ligaments and tendons. Rupture of the anterior cruciate ligament (ACL) is a common injury, especially in Western countries. ACL injuries have been reported to occur in an estimated 1 in 3000 people each year. ACL surgery uses a graft to replace ligament. The most common grafts are autograft or allograft taken from a donor. However, this clinical approach shows some limits due to the onset of
surgical complications and the inability to fully restore knee joint function. In collaboration with “Stazione Sperimentale della Seta di Milano” and with “Politecnico di Milano” we used a silk fibroin textile structure as ACL substitute. We designed a bioreactor simulating the knee physiological environment with the goal to produce, in vitro, an engineered ligament. In particular, the aim of the project was to evaluate the effect of mechanical stimulation on a fibroin scaffold, previously seeded with rat mesenchymal stem cells. The morphological evaluation of the samples by scanning electron microscope showed that macroporosity of fibroin scaffold promotes adhesion of mesenchymal cells. Moreover, the cells were not damaged by the mechanical stress induced by the bioreactor. These results suggest that this procedure can be applied to long-term experiments to evaluate the proliferation, differentiation and the extracellular matrix production of rat mesenchymal stem cells seeded on a scaffold exposed to a mechanical stimulation.

Development of methodologies for kidney regeneration
Chronic kidney disease is a pathologic condition marked by deteriorating kidney function over time. When end stage renal failure ensues and renal replacement therapies with dialysis and/or kidney transplantation are required. However, alternative therapeutic solutions are needed to overwhelm the reduced access to dialysis, the scarce availability of donors and the side effects of immunosuppressive therapies for transplanted patients. The aim of this project is to recreate a new organ in the laboratory starting from a native kidney completely decellularized and subsequently recellularized with embryonic stem (ES) cells. To this purpose we have developed a perfusion system for decellularization and recellularization of intact rat kidneys. The experimental set-up allows to provide constant physiological flows and pressures to the organ in order to obtain optimal cellular removal and intact 3D architecture of renal extracellular matrix. Complete decellularization and preservation of matrix proteins were demonstrated by histological and histochemical analysis. The 3D ultrastructure and vasculature architecture preservation were assessed respectively by scanning electron microscopy (SEM) and microCT during infusion of a contrast agent in the renal artery. At the end of decellularization protocol, scaffolds were seeded with mES through renal artery and exposed to a physiological flow with culture medium to provide oxygen and nutrients to the cells and to promote cell proliferation and differentiation. The presence of seeded cells into the scaffold was confirmed by histological and histochemical analysis, demonstrating that rat renal ECM allows attachment and survival of ES cells. Cells infused through the renal artery were uniformly distributed in the vascular network and in glomeruli. Moreover, after 24 and 72 hours of perfusion with culture medium, cells progressively lost their stemness and started to differentiate in response to the signals contained in kidney ECM. Our findings indicate that kidney scaffolds can be suitable for adhesion, survival and differentiation of mouse ES cells. (In collaboration with the Department of Molecular Medicine).

Experimental evaluation of the shear stress on endothelial cells
We studied at a theoretical level the effects of endothelial cells that line the inner surface of blood vessels. The most recent studies allow the identification of the oscillating shear stresses acting on these cells as responsible for the formation of neointima, resulting in stenosis of blood vessels and occlusion. We developed experimental systems to submit endothelial cells to physiological or oscillating shear stress. The aim is to investigate whether these conditions activate gene expression of factors responsible for the vascular damage such as adhesive proteins, the oxidation state and vasoconstriction. To verify this, we investigated endothelial cells exposed to physical forces under controlled conditions. The system is based on a cone-plate geometry in which the cone is rotated at variable speed in time to obtain unidirectional or oscillating shear stress with the same dynamics of vascular access estimated by numerical simulations.
Aldo and Cele Daccò Center
Ranica (Bg)

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departments and laboratories
DEPARTMENT OF RENAL MEDICINE

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Paola BOCCARDO, Bio.Sci.D.
Piero Ruggenenti got his Medicine degree in 1983 at the University of Milan, Italy; he got his specialization in Cardiology in 1985 and in Clinical Nephrology in 1989 at the same University; he specialized in Pharmacological Research in 1988 at IRFMN.

**Educational training:** in 1980-1983 researcher at "Centro di Fisiologia Clinica e Ipertensione, Clinica Medica IV", Università degli Studi di Milano; in 1984 Researcher at IRFMN, Bergamo, Italy in 1987-1988 Honorary Registrar of the Unit for Metabolic Medicine, Division of Medicine (University of London) of Guy's and St. Thomas's Hospitals, London; in 1988-1989 Assistant Professor of the Division of Nephrology and Dialysis of the Ospedali Riuniti di Bergamo.

**Areas of interest:** mechanisms of chronic renal disease progression, diabetes and diabetic complications, clinical transplantation, thrombotic microangiopathies, cardiovascular complications of chronic renal disease, clinical trials, clinical pharmacology.

**Employment:** from 1990 Assistant Professor of the Division of Nephrology and Dialysis of the Ospedali Riuniti di Bergamo; in 1994-1999 Head, Unit of Advanced Development of Drugs, Daccò Center, Ranica, Bergamo, Italy; since 2000 Head, Department of Renal Medicine, Daccò Center, Bergamo, Italy.

**Selected publications:**
Paola Boccardo got her classic High School Diploma in 1979 and the Biol. Sci. Degree at the University of Pisa in 1985. In 1987 she passed the qualifying examination and got the license of Biologist. **Educational training:** she performed her training first at Mutagenesis and Differentiation Institute, CNR, of Pisa, and then at Mario Negri Institute for Pharmacological Research, where in 1990, got her diploma of “Specialist in Pharmacological Research”. Since 1987 has been working as full-time researcher at Mario Negri Institute, till 1995 at Molecular Medicine Department and then at Renal Medicine Department. **Area of interest:** since 1995 she is in charge of Regulatory Affairs and attends to the planning, organizing and conducting of clinical studies in accordance with the principles of Good Clinical Practice and with the laws in force. **Employment:** since June 2006 to October 2009 Responsible of Clinical Trials Office; since November 2009 Head, Laboratory of Regulatory Affairs for Clinical Studies. Member of Internal Staff for Security, since May 2008 she is Security Manager at Clinical Research Center for Rare Diseases Aldo e Cele Daccò. **Selected publications:**

Paolo Cravedi got his Medicine degree (cum laude) in 1999 at the University of Milan, Italy; he got his specialization in Nephrology (cum laude) in 2004 at the University of Parma. In 2009 got a Ph.D. degree from the Open University of London. **Educational training:** in 2005 Master on Organ Transplant at the University of Milano Bicocca; in 2006 researcher at the Mario Negri Institute, Bergamo; since 2007 to 2008 Research Fellow at the Transplant Branch of the National Institutes of Health (NIH) (Mentor Dr. Roslyn Mannon); since 2009 researcher at the Mario Negri Institute, Bergamo. **Areas of interest:** mechanisms of chronic renal disease progression, diabetes and diabetic complications, clinical transplantation, membranous nephropathy, clinical pharmacology. **Employment:** since 2010, Head Laboratory of Clinical Pathophysiology of Renal Disease and Transplantation. **Selected publications:**
Flavio Gaspari got his Chemistry degree in 1977 at the University of Milano, Italy, and the specialization in the same University in 1979. 

**Educational training:** in 1981-1985 Fellow and Researcher at IRFMN, Milan; in 1985-1991 at IRFMN, Bergamo, Italy.

**Areas of interest:** pharmacokinetics and the metabolism of xanthines in different animal species; drug pharmacokinetics in uremic patients and in subjects with different degrees of renal function; analytical methods to measure the most important immunosuppressive drugs to determine their pharmacokinetics in kidney, heart, and liver transplant recipients; evaluation of the renal function by using different approaches, in the study of renal disease progression, and in the comparison of different methods for albuminuria determination.

**Employment:** he is Head of Laboratory of Pharmacokinetics and Clinical Chemistry since January 2000 and he was Head of this Unit since 1991.

**Selected publications:**
Laboratory of Coordination and Conduction of Controlled Clinical Trials at IRFMN – Daccò Center. Since 2009 Head of the Laboratory of Coordination and Conduction of Controlled Clinical Trials at IRFMN – Daccò Center.

Selected publications:


Norberto Perico got his Medicine degree in 1983 at the University of Milano, Italy. He got his specialization in Pharmacological Research in 1986 at IRFMN, Bergamo and in Clinical Nephrology in 1989 at the University of Verona, Italy.

Educational training: in 1982 Fellow, Department of Pharmacology, New York Medical College, Valhalla, New York, USA; in 1984-1988 Post Doctoral Fellow, Laboratory of Kidney Diseases, IRFMN, Bergamo, Italy; in 1988-1989 Researcher in the same laboratory.

Areas of interest: pathophysiology and pharmacology of cyclosporine nephrotoxicity; new immunosuppressive strategies to prevent renal graft rejection; innovative approach to induce tolerance to organ transplantation; mechanism(s) and management of progression of chronic renal diseases, novel therapies for autosomal dominant polycystic kidney disease.

Employment: in 1990-1994 Head, Renal Physiology Unit, Laboratory of Kidney Diseases, IRFMN, Bergamo, Italy; in 1990-2000 Assistant Professor, Division of Nephrology and Dialysis, Ospedali Riuniti di Bergamo, Italy; in 1994 –1999 Head, Laboratory of Transplant Immunology, IRFMN, Bergamo, Italy; from January 2000 Head, Laboratory of Drug Development, Department of Renal Medicine, IRFMN, Bergamo, Italy; from September 2000 Health Director, Daccò Center, IRFMN, Bergamo, Italy. From October 2002 he’s Member, ISN-GO Research Committee of the International Society of Nephrology.

Selected publications:

Annalisa Perna obtained a degree in Statistical Sciences from the University of Bologna (Italy) and a Master of Science in Clinical Trials at the London School of Hygiene & Tropical Medicine - Faculty of Epidemiology and Population Health of the University of London (UK). Educational training: She completed her research training at IRFMN, Bergamo Labs. and at the Daccò Center. Areas of interest: Her research interests spread from statistical methodology of long-term randomised clinical trials, mainly in nephropathy and diabetes, to statistical methods for calculating sample size, to development of predictive models and to meta-analytic techniques. She is also involved in performing systematic reviews within the Cochrane Collaboration – Renal Review Group. Employment: she is Head of the Laboratory of Biostatistics - Department of Renal Medicine at Daccò Center, Ranica (Bergamo).

Selected publications:


Aneliya Parvanova Ilieva got her Medical Doctor degree at the Faculty of Medicine, Thracian University (former Higher Medical Institute), Stara Zagora, Bulgaria in 1988, and the specialization in Pharmacology in the Department of Pharmacology, University of Medicine, Sofia in 1992. Her medical degree is recognized in Italy in 2009. In 2013 she has been awarded the PhD degree by the Medical University of Sofia, Bulgaria. Educational training: in 1989-1998 teaching of 3rd, 4th and 5th-year medical students and 2nd and 3rd-year clinical nurses in a general pharmacology and clinical pharmacology, Thracian University, Stara Zagora, Bulgaria; examiner of these students in theoretical and practical, oral and written exams and tests and State examination. In 1993 Course on investigation of isolated organs – Bulgarian Academy of Sciences, Sofia. In 1998 visiting scientist, IRFMN, Ranica, Bergamo, Italy. In 1998 proficiency in the methods for insulin sensitivity evaluation (hyperinsulminemic euglycaemic clamp technique), in renal hemodynamic measurements - glomerular filtration rate (plasma clearance of iohexol and inulin), in renal plasma flow (plasma clearance of para-aminohippuric acid), glomerular size selectivity (plasma clearance of neutral dextrans) and in twenty four-hour blood pressure monitoring. Areas of interest: prevention and treatment of micro- and macrovascular diabetic complications; role of insulin resistance, arterial hypertension, dyslipidemia and hyperhomocysteinemia in micro- and macrovascular diabetic complications; clinical trials. Employment: Researcher at the Clinical Research Center for Rare Diseases Aldo e Cele Daccò, Ranica, Bergamo. She is Head of The Unit of Early Clinical Evaluation of Drugs at IRFMN since 2000. She is a member of the Union of Bulgarian Doctors (since 1989), of the Union of Pharmacologists in Bulgaria.

Selected publications:


INTRODUCTION TO THE DEPARTMENT’S ACTIVITIES

The Department of Renal Medicine was established on 1999 at the Clinical Research Center for Rare Diseases “Aldo e Cele Daccò” – Villa Camozzi, Ranica to coordinate the activities of 6 Laboratories and 2 Units.

The activities of the Department are mainly focused on the study of the mechanisms of progression of chronic nephropathies, of new prevention and intervention strategies for diabetic nephropathy, non diabetic chronic nephropathies, chronic allograft dysfunction, of cardiovascular complications of diabetes, chronic renal disease, dialysis and transplantation and of thrombotic microangiopathies.

The main aims of these activities are:
1. To identify screening and intervention strategies aimed to prevent the onset of nephropathy and of other chronic complications in subjects with diabetes and/or hypertension as well as in the general population.
2. To define intervention strategies to prevent or slow the progression of chronic nephropathies and eventually obtain remission/regression of renal dysfunction.
3. To optimize immunosuppressive protocols in kidney transplantation and to define new donor selection criteria in order to expand the pool of available organs.

These aims will be pursued through the following modalities:
1. Pilot pathophysiology and clinical pharmacology studies fully finalized at the Clinical Research Center to test new pathogenetic hypotheses and new treatment modalities.
2. National and international networks and multicenter trials aimed to verify the efficacy of treatments of potential interest identified as described at point 1.
3. Meta-analyses and probabilistic models to test new risk factors and treatments in large samples of patients and to transfer this information at individual level.
4. To identify novel treatments for primary glomerular diseases such as idiopathic membranous nephropathy, focal and segmental glomerulosclerosis and minimal change disease.
Many of these activities rest on the possibility of a tight cooperation with the Department of Molecular Medicine, the Department of Bioengineering and the Public-Private Department of Specialist and Transplant Medicine. This cooperation allows to plan the research activities of the Department on the basis of new information derived from basic research and of problems of major clinical relevance emerging from routine clinical activities.

**FINDINGS/MAIN RESULTS**

Definition and validation of specific treatments aimed to prevent the development and progression of nephropathy and related micro and macrovascular complications in subjects with type 2 diabetes.

Implementation of screening programs in the general population to early identify and treat subjects at risk of renal and cardiovascular events

Definition and validation of new integrated treatment protocols aimed to slow the progression and/or to achieve remission/regression of diabetic and non-diabetic chronic nephropathies.

Institution of a standardized protocol “on line” (The “Remission Clinics”) finalized to achieve regression/remission of chronic nephropathies and limit overall renal and radiovascular risk in hospital practice in the setting of a multicenter Network.

Characterization of the antiproteinuric, nephroprotective and cardioprotective effect of maximized and polypharmacologic renin-angiotensin system inhibition, intensified blood pressure and lipid control and identification of novel treatments to reduce the blood pressure and ameliorate insulin sensitivity in subjects at increased cardiovascular risk.

Identification of acquired or congenital risk factors for chronic complications of diabetes and cardiovascular morbidity and mortality

Identification and validation of early markers of acute kidney failure and of methods for direct and indirect measurements of kidney function and GFR decline.

Identification of safety and efficacy profile of new treatments for the Autosomal Polycistic Kidney Disease (APKD).

Definition and validation of new, specific treatments for idiopathic membranous nephropathy and for HUS forms associated with genetic defect of complement factors including the standardization of combined liver and kidney transplantation to prevent post transplant recurrence of genetic associated HUS.

Definition and validation of new laboratory procedures and predictive models to help monitoring and optimizing immunosuppressive therapy in clinical transplantation with particular focus on pharmacokynetic markers of drug exposure and genetic predictors of drug tolerability and efficacy.

Definition and validation of selection and allocation criteria of kidneys from marginal and old-very old donors to increase the donor pool and the transplant activity.

Finalization and activation of multicenter clinical trials aimed to prevent onset and progression of diabetic and non-diabetic chronic nephropathies, to achieve remission of the nephrotic...
syndrome in primary glomerular diseases, minimize maintenance immunosuppression in kidney transplantation and prevent cardiovascular morbidity and mortality in chronic hemodialysis.

Computerization of data acquisition and monitoring procedures for the conduction of controlled clinical trials.

**NATIONAL COLLABORATIONS**

- AO Bolognini Seriate, Ospedale Bolognini, Seriate (BG)
- AO Papa Giovanni XXIII, Bergamo
- AO Treviglio, Ospedale di Treviglio, Treviglio (BG)
- AO Treviglio, Ospedale SS. Trinità, Romani di Lombardia (BG)
- AO Treviglio, Poliambulatorio extra-ospedaliero, Brembate (BG)
- ASL Bergamo, Bergamo
- Istituto Humanitas Gavazzeni, Bergamo
- Policlinico San Pietro di Istituti Ospedalieri Bergamaschi, Gruppo Ospedaliero San Donato, Ponte San Pietro (BG)
- AO Spedali Civili di Brescia, Presidio Ospedaliero di Montichiari, Montichiari (BS)
- AO Spedali Civili, Spedali Civili, Brescia
- AO Ospedale Sant’Anna, Presidio Ospedaliero Sant’Anna, Como
- Azienda Ospedaliera Istituti Ospedalieri di Cremona, Cremona
- AO Provincia di Lodi, Presidio Ospedaliero di Lodi, Lodi
- AO di Desio e Vimercate, Vimercate (MB)
- AO Ospedale San Carlo Borromeo, Milano
- AO Polo Universitario, Ospedale Luigi Sacco, Milano
- AO San Gerardo, Ospedale Bassini, Cinisello Balsamo (MI)
- AO San Gerardo, Ospedale San Gerardo, Monza (MI)
- AO San Paolo – Polo Universitario, Milano
- ASL Provincia di Milano 2, Ospedale A. Uboldo, Cernusco sul Naviglio (MI)
- Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milano
- Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano
- IRCCS Fondazione Centro San Raffaele del Monte Tabor, Milano
- IRCCS Istituto Clinico Humanitas, Rozzano (MI)
- IRCCS Multimedica di Sesto San Giovanni, Sesto San Giovanni (MI)
- AO Guido Salvini, Ospedale Caduti Bollatesi, Bollate (MI)
- AO Ospedale Civile di Legnano, Ospedale Legnano, Legnano (MI)
- AO Ospedale Civile di Legnano, Ospedale G. Fornaroli di Magenta, Magenta (MI)
- AO Pavia, Ospedale Civile di Voghera, Voghera (PV)
- AO Valtellina e Valchiavenna, Ospedale di Sondrio, Sondrio
- AO Universitaria, Ospedale di Circolo e Fondazione Macchi, Varese
- ASO Santa Croce e Carle, Ospedale Santa Croce, Cuneo
- AO Ospedale Infantile Regina Margherita-Sant’Anna di Torino, Ospedale Infantile Regina Margherita, Torino
- AO Ordine Mauriziano, Ospedale Mauriziano Umberto I, Torino
- ASL TO2, Ospedale San Giovanni Bosco, Torino
- AULSS 17 Este, Ospedale di Monselice, Monselice (PD)
- AULSS 9 di Treviso, Ospedale Santa Maria di Cà Foncello, Treviso
- AO Universistaria degli Ospedali Riuniti di Trieste, Ospedale di Cattinara, Trieste
- IRCCS Materno-Infantile Burlo Garofalo, Trieste
- AO Universitaria di Bologna, Policlinico Sant’Orsola-Malpighi, Bologna
- AUSL Forlì, Ospedale G. B. Morgagni - L. Pierantoni, Forlì
- AO Universitaria di Parma, Ospedale di Parma, Parma
- AUSL Ravenna, Ospedale Santa Maria delle Croci, Ravenna
- AO Reggio Emilia, Arcispedale Santa Maria Nuova, Reggio Emilia
- AUSL Rimini, Ospedale Infermi, Rimini
- AO Universitaria Careggi, Firenze
- Università degli Studi, Firenze
- AUSL 2 Lucca, Ospedale Campo di Marte, Lucca
- AO Universitaria Pisana, Ospedale Santa Chiara, Pisa
- AUSL 3 di Pistoia, Ospedale SS. Cosma e Damiano di Pescia, Pescia (PT)
- ASUR Zona Territoriale 13, Ospedale Mazzoni, Ascoli Piceno
- IRCCS Pediatrico Bambino Gesù, Roma
- ASL Roma G, Ospedale San Giovanni Evangelista, Tivoli (RM)
- AUSL Rieti, Rieti
- AO Ospedale San Giuseppe Moscati, Avellino
- AO Antonio Cardarelli, Napoli
- AO Pediatrica Santobono-Pausilipon, Ospedale Santobono, Napoli
- Università Azienda Ospedaliera Universitaria Federico II, Napoli
- Università Azienda Ospedaliera Universitaria Federico II, Policlinico Nuovo, Napoli
- ASL Salerno, Ospedale Maria SS. Addolorata, Eboli (SA)
- ASL Teramo, Presidio Ospedaliero Giuseppe Mazzini, Teramo
- Centro Nazionale Ricerche, Reggio Calabria
- AS 3 Rossano, Ospedale Civile Nicola Giannettasio, Rossano (CS)
- ASP Agrigento, Ospedale San Giovanni di Dio, Agrigento
- AO Ospedale Cannizzaro, Catania
- AO Ospedale Garibaldi, Nesima (CT)
- AO Universitaria Policlinico-Vittorio Emanuele, Presidio Ospedaliero Vittorio Emanuele, Catania
- AUSL 3 Catania, Presidio Ospedaliero di Acireale, Acireale (CT)
- ASP 5 Messina, Ospedale di Milazzo, Milazzo (ME)
- AO Civico-Di Cristina-Benfratelli, Presidio Ospedaliero Civico e Benfratelli, Palermo
- AO Universitaria Policlinico Paolo Giaccone, Università degli Studi, Palermo
- Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione (IsMeTT), Palermo
- Fondazione Istituto San Raffaele – G. Giglio di Cefalù, Cefalù (PA)
- Azienda Ospedaliera, Ospedale Umberto I, Siracusa
- IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG)
- AUSL Le/1, Ospedale Vito Fazzi, Lecce
- ASL Taranto 1, Presidio Ospedaliero Valle D’Itria di Martina Franca, Martina Franca (TA)
- AO Brotzu, Ospedale San Michele, Cagliari
- ASL Sanluri, Presidio Ospedaliero Nostra Signora di Bonaria, San Gavino Monreale (VS)
- ASL 8, Cagliari
- ASL Olbia, Ospedale San Giovanni di Dio, Olbia (OT)
- ASL Sassari, Azienda Ospedaliero-Universitaria di Sassari, Sassari

INTERNATIONAL COLLABORATIONS

- University Medical Center, Ljubljana (Slovenia)
- Service de Pharmacologie Clinique, Faculté de Médecine, Lyon (France)
- Hospital Universitario de Canarias, La Laguna, Tenerife (Spain)
- Department of Primary Health Care, University of Oxford, Oxford (UK)
EDITORIAL BOARD MEMBERSHIP

Current Diabetes Reviews (Piero Ruggenenti)
Journal of Nephrology (Piero Ruggenenti)
Nephron (Norberto Perico)
The Open Hypertension Journal (Paolo Cravedi)
World Journal of Nephrology (Paolo Cravedi)

PEER REVIEW ACTIVITIES

Acta Diabetologica
Acta Pharmacologica Sinica
American Journal of Hypertension
American Journal of Kidney Diseases
American Journal of Pathology
American Journal of Transplantation
Archives of Medical Science
Bentham Science
Blood Purification
British Medical Journal (BMJ)
Circulation American Hearth Association (AHA)
Clinical Journal of the American Society of Nephrology (CJASN)
Clinical Nephrology
EMBO Molecular Medicine
Expert Opinion on Pharmacotherapy
Expert review of Clinical Immunology
Heart Failure Reviews
Indian Journal of Nephrology
Internal Urology and Nephrology
International Journal of Clinical Practice
Islets
Journal of the American Society of Nephrology (JASN)
Journal of Hypertension
Journal of Nephrology
Kidney International
Mediterranean Journal of Hematology And Infection Diseases
Nature Communications
Nature Reviews Nephrology
Nephrology Dialysis Transplantation
Nephron
New England Journal of Medicine
The International Journal of Artificial Organs
The Lancet
Translational research
Transplant International
Transplantation

PARTICIPATION IN EVENTS
IN WHICH THE DEPARTMENT WAS INVOLVED


Si può oggi rallentare o prevenire l’insorgenza del danno renale?. 45° Corso di aggiornamento in Nefrologia e Metodiche Dialitiche. Milano (Italy), December 6th-8th, 2013.


Si potrà un giorno fare un organo in laboratorio, e per il rene?. Advisory Board, Fresenius. Roma (Italy), October 25th, 2013.

RTX nella nefropatia membranosa dell’adulto. 24° Congresso Nazionale della Societa Italiana di Nefrologia (SIN). Firenze (Italy), September 26th, 2013.


What (not) to learn from meta-analyses. The Beijing Congress of Nephrology 2013 Beijing, Beijing Society of Nephrology. Pechino (Republic of China), September 20th-22nd, 2013.


Long-acting Somatostatin analogue in the treatment of ADPKD. ERA-EDTA CAM-ADPKD. Oxford (United Kingdom), September 12th-14th, 2013.

Fisiopatologia de la poliquistosis renal y nuevas alternativa terapéuticas. V Curso International. Sucre (Bolivia), August 16th, 2013.

Immunosupresion en la induccion y post trasplante renal. V Curso International. Sucre (Bolivia), August 16th, 2013.


Glomerulonefritis membranosa. V Curso International. Sucre (Bolivia), August 14th, 2013.

Heparin in pregnant women with adverse pregnancy outcome to improve the rate of successful pregnancy (HAPPY trial). AFFIRM Investigator and statistician meeting. Amsterdam, July 4th, 2013.

The role of remission clinics in the longitudinal treatment of CKD. Meeting Nephrologischer Sommer 2013. Salzburg (Austria), June 14th-15th, 2013.

Proteinuria as a surrogate marker of renal disease progression: is it a valid target for planning a trial?. Meeting 50° ERA-EDTA Congress. Istanbul (Turkey), May 18th-21st, 2013.

Novelties on therapy of ADPKD: recent CRTs. Meeting The Second International Renal Meeting and Mayo Clinic Day in Sardinia. Cagliari (Italy), May 2nd, 2013.

Le cellule mesenchimali stromali nel trapianto di rene. Rovereto, Trento (Italy), April 19th, 2013.

HUS/TTP: in the last 15 years all is changed. Meeting The Second International Renal Meeting and Mayo Clinic Day in Sardinia. Cagliari (Italy), April 30th May 3rd, 2013.


Approccio terapeutico dei pazienti con nefropatia diabetica. Meeting Spanish Society of Hypertension. Valencia (Spain), March 6th-8th, 2013.


GRANTS AND CONTRACTS

AIFA (Agenzia Italiana del Farmaco)
European Commission
Innovative Medicine Initiative Joint Undertaking (IMI JU)
International Society of Nephrology (ISN)
Regione Lombardia
AbbVie Srl
Alexion Pharmaceuticals
Baxter SpA
Boheringer Ingelheim Italia SpA
Bracco Imaging SpA
Genzyme Europe BV
Sanofi-Aventis SpA
Sigma-Tau Industrie Farmaceutiche Riunite SpA

SELECTION OF SCIENTIFIC PUBLICATIONS FROM 2013


**RESEARCH ACTIVITIES**

**Laboratory of Biostatistics**

Effect of long acting somatostatin analogue on kidney and cyst growth in autosomal dominant polycystic kidney disease (ALADIN): a randomised, placebo-controlled, multicentre trial

Autosomal dominant polycystic kidney disease slowly progresses to end-stage renal disease and has no effective therapy. A pilot study suggested that the somatostatin analogue octreotide long acting release (LAR) could be nephroprotective in this context. We aimed to assess the
effect of 3 years of octreotide-LAR treatment on kidney and cyst growth and renal function decline in participants with this disorder. We did an academic, multicentre, randomised, single-blind, placebo-controlled, parallel-group trial in five hospitals in Italy. Adult (>18 years) patients with estimated glomerular filtration rate (GFR) of 40 mL/min per 1·73 m² or higher were randomly assigned (central allocation by phone with a computerised list, 1:1 ratio, stratified by centre, block size four and eight) to 3 year treatment with two 20 mg intramuscular injections of octreotide-LAR (n=40) or 0·9% sodium chloride solution (n=39) every 28 days. Study physicians and nurses were aware of the allocated group; participants and outcome assessors were masked to allocation. The primary endpoint was change in total kidney volume (TKV), measured by MRI, at 1 year and 3 year follow-up. Analyses were by modified intention to treat. This study is registered with ClinicalTrials.gov, NCT00309283. Recruitment was between April 27, 2006, and May 12, 2008. In 2013 we performed final statistical analyses.

Effect on blood pressure of combined inhibition of endothelin-converting enzyme and neutral endopeptidase with daglutril in patients with type 2 diabetes who have albuminuria: a randomised, crossover, double-blind, placebo-controlled trial

Effective reduction of albuminuria and blood pressure in patients with type 2 diabetes who have nephropathy is seldom achieved with available treatments. We tested the effects of treatment of such patients with daglutril, a combined endothelin-converting enzyme and neutral endopeptidase inhibitor. We did this randomised, crossover trial in two hospitals in Italy. Eligibility criteria were: age 18 years or older, urinary albumin excretion 20–999 μg/min, systolic blood pressure (BP) less than 140 mm Hg, and diastolic BP less than 90 mm Hg. Patients were randomly assigned (1:1) with a computer-generated randomised sequence to receive either daglutril (300 mg/day) then placebo for 8 weeks each or vice versa, with a 4-week washout period. Patients also took losartan throughout. Participants and investigators were masked to treatment allocation. The primary endpoint was 24-h urinary albumin excretion in the intention-to-treat population. Secondary endpoints were median office and ambulatory (24 h, daytime, and night-time) BP, renal haemodynamics and sieving function, and metabolic and laboratory test results. This study is registered with ClinicalTrials.gov, number NCT00160225. We screened 58 patients, of whom 45 were enrolled (22 assigned to daglutril then placebo, 23 to placebo then daglutril; enrolment from May, 2005, to December, 2006) and 42 (20 vs 22) were included in the primary analysis. In 2013 we performed final statistical analyses.

ADAMTS13 predicts renal and cardiovascular events in type II diabetics and response to therapy: final analyses

In diabetics, impaired ADAMTS13 proteolysis of highly-thrombogenic VWF multimers may accelerate renal and cardiovascular complications. Restoring physiological VWF handling might contribute to ACE inhibitors (ACEi) renoprotective effects. To assess how Pro618Ala ADAMTS13 variants and related proteolytic activity interact with ACEi therapy in predicting renal and cardiovascular complications, 1163 normoalbuminuric type 2 diabetics from the BENEDICT trial were genotyped. In 2013 we performed final statistical analyses. Interaction between Pro618Ala and ACEi was significant in predicting both renal and combined renal and cardiovascular events. The risk [HR(95%CI)] for renal or combined events vs reference Ala carriers on ACEi progressively increased from Pro/Pro homozygotes on ACEi [2.80(0.849-9.216) and 1.58(0.737-3.379), respectively], to Pro/Pro homozygotes on non-ACEi [4.77(1.484-15.357) and 1.99(0.944-4.187)], to Ala carriers on non-ACEi [8.50(2.416-29.962) and 4.00(1.739-9.207)]. In a sub-study, serum ADAMTS13 activity was significantly lower in Ala carriers than in Pro/Pro homozygotes and in cases with renal, cardiovascular or combined events than in uneventful diabetic controls. ADAMTS13 activity significantly and negatively correlated with all outcomes. In diabetics, ADAMTS13 618Ala variant associated with less
proteolytic activity, higher risk of chronic complications and better response to ACEi therapy. Screening for Pro618Alapolymorphism may help identifying diabetics at highest risk who may benefit the most from early reno- and cardioprotective therapy.

A prospective, pilot, cross-over study to assess the efficacy of paricalcitol in reducing parathyroid hormone levels and ameliorating markers of bone remodelling in renal transplant recipients with secondary hyperparathyroidism (APPLE study): final analyses

Secondary hyperparathyroidism plays a major role in post-transplant mineral bone disease. Paricalcitol, a selective activator of vitamin D receptor, ameliorated mineral metabolism and even reduced proteinuria in renal patients with secondary hyperparathyroidism. This academic, internally-funded, single-center, prospective, randomized, cross-over study compared the effect of six-month treatment with paricalcitol (1 µg/day for 3 months then uptitrated to 2 µg/day if tolerated) or maintenance therapy only on serum parathyroid hormone levels (primary outcome), mineral metabolism and proteinuria in 43 consenting long-lasting renal transplant recipients with secondary hyperparathyroidism. Participants were randomized on a 1:1 basis according to a computer-generated sequence. This study is registered with ClinicalTrials.gov, NCT01220050. In 2013 we performed final statistical analyses.

A prospective, randomized, open label blinded end point (probe), Crossover study to compare the effects of Telmisartan and Losartan on metabolic profile of renal transplant patients (COSTANT study): final analyses

In renal transplant recipients, residual renal insufficiency combined to the effects of immunosuppressive therapy with steroids or calcineurin inhibitors may reduce insulin activity and may contribute to several of the abnormalities associated with the metabolic syndrome, such as hypertension, glucose intolerance and hyperlipidemia. In turn, insulin resistance, hypertension, hyperglycemia and dyslipidemia may importantly contribute to the excess cardiovascular risk of renal transplant patients (an excess comparable to that of diabetes subjects with over diabetic nephropathy) and may also accelerate progressive renal function deterioration and promote graft loss. Thus, amelioration of the insulin activity and of the related metabolic syndrome is a key component of treatments aimed to improve patient and graft survival in renal transplant recipients. Recently, drugs such as peroxisome proliferators-activated receptor-g (PPARg) activators, that ameliorate insulin sensitivity and metabolic syndrome, have become available. These agents, however, can provoke fluid retention, weight gain, edema and, in some cases, heart failure. Thus, the risk/benefit profile of PPARg activators is still uncertain, in particular in renal transplant patients where the risks of therapy may overwhelm the potential benefits. Recent studies showed that telmisartan, an angiotensin II (AII) type 1 receptor antagonist, in addition to block the AII receptor - a key surface receptor involved in the regulation of blood pressure - may also activate PPARg, thus improving some of the features of the metabolic syndrome, such as hyperglycemia and dyslipidemia in people with hypertension and/or diabetes. Thus, in addition to control high blood pressure and to limit some of the adverse effects of angiotensin II, including target organ damage, graft fibrosis and CsA nephrotoxicity, telmisartan may also substantially reduce the overall cardiovascular and renal risk of renal transplant recipients by ameliorating some of the modifiable components of the metabolic syndrome, such as hypertension, glucose intolerance and hyperlipidemia. On the other hand, telmisartan is devoided of the adverse effects of PPARg activators such as fluid retention, and has therefore a remarkably better risk/benefit profile. Thus, whether telmisartan in addition to the beneficial effects of a reference AII receptor antagonist (such as losartan) may offer adjunctive advantages related to improved insulin sensitivity in renal transplant patients on chronic therapy with steroids and/or calcineurin inhibitors, is worth investigating. This is an
academic, prospective, randomized, phase II, open label blinded end point (PROBE), cross-over was primarily aimed at comparing the short-term effects of Telmisartan and Losartan on insulin sensitivity in kidney transplant recipients with stable renal function and concomitant treatment with steroids and/or calcineurin inhibitors. Secondarily the trial compared changes in systemic (sitting systolic/diastolic blood pressure, 24-h blood pressure profile), metabolic (morning fasting blood glucose, glucose tolerance test, glicated hemoglobin, morning fasting insulin, HOMA index (HOMA IR), lipid profile and renal (AER, GFR/RPF, Albumin fractional clearance) variables. Participants were randomized on a 1:1 basis according to a computer-generated sequence. This study is registered with ClinicalTrials.gov, NCT01224860. In 2013 we performed final statistical analyses.

Long-term cardiovascular risk in proteinuric patients with non-diabetic chronic kidney disease from the Ramipril Efficacy in Nephropathy (REIN): data collection

We are collecting long-term data on major fatal or nonfatal cardiovascular events (i.e. myocardial infarction; stroke; coronary, carotid, or peripheral artery revascularization; or hospitalization for heart failure) are collected in order to conduct a post hoc analysis of the Ramipril Efficacy in Nephropathy (REIN) to evaluate the association of proteinuria and other baseline characteristics with proteinuria and cardiovascular risk among 352 CKD patients without diabetes who were treated with ramipril (5 mg/d). Multivariable survival analysis will be performed by modelling baseline proteinuria and other baseline characteristics with the fractional polynomial algorithm method (first and second degree), or a spline function, two flexible and informative approaches for the evaluation of possible nonlinear relationships between continuous variables and outcomes.

Laboratory of Coordination and Conduction of Controlled Clinical Trials

The main aim of the Laboratory is to implement and coordinate all the activities needed to fulfil the trials planned by the Renal Medicine Department, according to the study protocols and the Good Clinical Practice (GCP).

To Laboratory staff collaborates with all the Laboratories/Unit of the Mario Negri Institute involving in the clinical studies coordinated by the Renal Medicine Department taking care of: to guarantee the flow of the information between the Laboratories/Units of the Renal Medicine Department and to guarantee a continuous updating of the trial status; to develop the case report form of studies; to develop the database of studies; to implement and update a centralized system of data management easily enjoyable by researchers of the Renal Medicine Department; to promote training activities for young investigators; to implement and update the SOPs needed for the trial protocols.

Laboratory of Pharmacokinetics and Clinical Chemistry

Systemic and renal response of Gal-/- pigs to human angiotensin II

Transplantation is the best available treatment for many serious health problems including diabetes, kidney failure and heart disease. However, the shortage of donor organs severely limits the number of patients receiving transplants and the use of animals as a source of organs and tissues for xenotransplantation could overcome this growing problem.
Despite immunological aspects of xenotransplantation have been extensively studied in the last years, less emphasis has been given to investigate the physiology of engineered pig organs and their compatibility with human milieu.

In the perspective of clinical application of kidney xenotransplantation we generated 1,3-galactosyltransferase gene-knockout (GAL-KO) pigs and transgenic for human CD55 and CD59.

In our study, we aimed to establish whether engineered porcine kidneys properly respond to human vasoactive hormones that regulate renal function.

To this purpose we developed a model to measure in vivo the glomerular filtration rate (GFR) in anesthetized pigs and to characterize the systemic and renal function response of animals to increasing doses of human Angiotensin II (hAng II).

In 3 anesthetized Gal-/- pigs (weight range 60-165 kg), glomerular filtration rate was measured by renal clearance of unlabeled iohexol. Iohexol (1294 mg) was injected as an i.v. bolus into a peripheral vein followed by a continuous infusion by a volumetric pump at a rate of 9.61 mg/min. After 2 hour stabilization period, blood and urine samples were collected every 20 minutes for iohexol concentration assessment by HPLC and for the determination of creatinine, sodium, potassium, calcium, chloride, phosphorous, magnesium, by means of an automatic device. Urine protein to creatinine ratio (P/C) was calculated.

Baseline GFR, evaluated during three 20 min clearance periods averaged 151±19 mL/min/100kg. Thereafter, increasing doses of 5 μg/kg, 10 μg/kg and 35 μg/kg of hAng II, administered as i.v. bolus injection, each at 40 min intervals, resulted in a progressive GFR decline to 79 ± 19, 78 ± 21, and 69 ± 27 ml/min/100 kg, respectively (p<0.05 vs baseline), as a consequence of marked intrarenal vasoconstriction.

In parallel a significant decrease in urinary sodium excretion was found (baseline 1.2 ± 0.6, hAng II 5: 0.9 ± 0.6; hAng II 10: 0.9 ± 0.5; hAng II 35: 0.9 ± 0.5 mEq/min, p <0.05 vs baseline). Consistently with the known effect of Ang II on glomerular perm-selective properties to macromolecules, hAng II injection progressively increased urinary protein excretion (P/C baseline: 456 ± 49, hAng II 5: 742 ± 245; hAng II 10: 948 ± 307; hAng II 35: 969 ± 109 mg/g, p<0.05 vs baseline)

Together these findings indicated that, when given i.v to Gal-/- pigs, hAng II exerts acute physiologic renal responses as shown by reduced GFR and urinary Na excretion, as well as increased urinary protein excretion. This data suggested an acceptable compatibility between this human vasoactive hormone and engineered porcine kidney.

Glomerular hyperfiltration and renal disease progression in type 2 diabetes

Hyperfiltration (glomerular filtration rate, GFR ≥120 mL/min/1.73m²) plays a central role in the pathogenesis and progression of renal disease in experimental diabetes. Inconsistent data have been published up to now, probably due to the small number of patients participating in the clinical trials and to unreliable approaches to evaluate renal function, such as serum creatinine-based equations for GFR estimation. To address this issue, we longitudinally studied 600 hypertensive patients with type 2 diabetes and albuminuria <200µg/min enrolled in two randomized clinical trials (the BENEDICT Study and the DEMAND Study) testing the renal effect of trandolapril and delapril administration. The aim of our study was to describe the prevalence and determinants of hyperfiltration, GFR decline, and nephropathy onset or progression in type 2 diabetics with normo-and microalbuminuria. A total of 4593 GFRs were measured over a median (range) follow-up of 4 (1.75 -8.11) years by means of a gold standard procedure (plasma clearance of iohexol) centralized in our laboratory. GFR declined by 3.37 mL/min/1.73m² per year, a decrease 3-5 faster than in the general population. The GFR change was bimodal over time: a larger reduction at 6 months significantly predicted slower subsequent decline (coefficient: -0.0054, SE: 0.0009), particularly among hyperfiltering patients.
results indicated that 90 subjects (15%) were hyperfiltering at inclusion and 47 were persistently hyperfiltering despite adequate blood glucose and blood pressure control. In these patients, long-term GFR decline was faster and progression to micro- or macroalbuminuria was more than twice than in normofiltering subjects (23.4% vs. 10.6%, hazard ratio 2.16. CI 1.13 to 4.14). Amelioration of hyperfiltration was independent of baseline characteristics or ACE inhibition. It was significantly associated with improved blood pressure and metabolic control, amelioration of glucose disposal rate and slower long-term GFR decline on follow-up.

Our study demonstrated that despite intensified treatment, patients with type 2 diabetes have a fast GFR decline even before the onset of proteinuria. Results of our post hoc analyses suggest that in hypertensive type 2 diabetic subjects with normo- or microalbuminuria, persistent hyperfiltration is an independent risk factor for accelerated renal function loss and development or progression of nephropathy, whereas amelioration of hyperfiltration is renoprotective. In addition our data confirm that glomerular filtration rate should be directly measured by means of gold standard methods (i.e. plasma clearance of iohexol) rather than estimated by means of unreliable prediction equations.

A paper describing our study has been published in Diabetes Care.

Performance of GFR estimation equations in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

The evaluation of glomerular filtration rate (GFR) is of critical importance in the clinical management of ADPKD patients. This is the most common hereditary renal disease, responsible for the 8% to 10% of the cases of end-stage renal disease (ESRD) in Western Countries. The ADPKD progression is largely dependent on the development and growth of cysts and secondary disruption of normal tissue and, eventually, the renal function will decrease.

In clinical practice formula-derived estimates of GFR have been adopted that include serum creatinine and anthropometric indexes such as gender, age, and weight to account for between-individual differences in muscle mass and the consequent differences in creatinine generation. These formulas, however, has been repeatedly challenged and there is increasing evidence that their use might generate misleading information in particular in subjects with normal or near normal kidney function. Thus, direct measurements of the GFR by gold-standard techniques based on the use of exogenous markers of glomerular filtration such as inulin, iohexol or radio-labeled tracers would be needed to adequately assess a treatment effect on GFR decline in this population.

To test this hypothesis we compared GFR values centrally measured by iohexol plasma clearance with corresponding values estimated by Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) and abbreviated Modification of Diet in Renal Disease (aMDRD) formulas in a cohort of 111 ADPKD patients with a baseline GFR >30 mL/min/1.73m² (by aMDRD equation) prospectively monitored by serial GFR measurements and estimations in the setting of controlled clinical trials coordinated by the Mario Negri Institute for Pharmacological Research in Italy.

We evaluated the relationships between measured GFR values at inclusion and at one-year follow-up and the concomitant GFR estimates obtained by prediction formulas. Measured baseline GFRs averaged 78.6±26.7 mL/min/1.73m². CKD-Epi significantly overestimated and aMDRD underestimated renal function, (81.4±29.4 mL/min/1.73m² and 73.0±28.0 mL/min/1.73m² respectively, p<0.05). The accuracy of both formulas was poor: less than 50% the estimates were within ±10% actual values (i.e. estimates virtually identical to the measured values). Furthermore, a trend to greater errors was documented for higher levels of renal function.

Measured and estimated one-year GFR data were available in 71 of the 111 included patients. Consistently with data in the whole study group, baseline measured GFR values were significantly overestimated and underestimated by CKD-Epi and aMDRD formulas, respectively. Overall, at one-year, measured GFR decreased by 8.43 mL/min/1.73m² vs.
baseline, a reduction that CKD-Epi (4.99 mL/min/1.73m²) and aMDRD (4.53 mL/min/1.73m²)
significantly underestimated by 53% and 59%, respectively. Bias, mean percent errors and mean
absolute percent errors of estimated vs. measured one-year GFR changes were similar with the
two equations. However, only 8.57% and 5.71% of the CKD-Epi and aMDRD estimates
deviated by less than ±10% from actual values, respectively. The accuracy was poor for both
estimates, although the percentage of acceptable estimates was slightly higher with the CKD-
Epi than with the aMDRD formula.
These findings showed that in ADPKD patients, prediction formulas unreliable estimate actual
GFR values and fail to detect their changes over time. Our data indicated that the direct kidney
function measurements by appropriate techniques are needed to adequately evaluate treatment
effects in clinics and research.

**Laboratory of Advanced Development of Drug**

**Effects of long-acting somatostatin (octreotide LAR) on disease progression in patients with autosomal dominant polycystic kidney disease and normal renal function or moderate renal insufficiency (The ALADIN study)**

Autosomal dominant polycystic kidney disease (ADPKD), the most common hereditary renal
disease, accounts for 7-10% of patients on dialysis in developed countries. ADPKD shows
genetic heterogeneity, with two main genes implicated, the PKD gene 1 (85-90% of cases) and
the PKD gene 1 (10-15% of cases) which encode for proteins polycystin 1 and polycystin 2,
respectively. The disease is characterized by multiple cysts growth from distal and collecting
tubular epithelial cells producing progressive renal enlargement with relatively initial stable
renal function. Thereafter, both tubular and secondary interstitial damage lead to faster renal
loss and end-stage renal disease in approximately half of all patients affected in their sixth
decade of life. The precise molecular mechanisms of ADPKD are not completely understood,
but experimental models in renal tubule cells suggest that deficit in polycystin 1 or polycystin 2
carry out to decrease intracellular calcium and increased cAMP levels changing the cell toward
a proliferative and secretor phenotype. Cyst growth and enlargement are mainly consequence of
epithelial cell proliferation and fluid chloride secretion.

Somatostatin is an endogenous cyclic polypeptide present in two active forms (14 and 18
amino acids) with endocrine, paracrine and autocrine actions. The effects of somatostatin are
pleiotropic depending on the tissue and the types of receptors it binds to. Five subtypes of
somatostatin receptors have been described (sst1-sst5) but on the renal tubular epithelial cells
sst1 and sst2 receptors predominate. Of interest, it has been reported that somatostatin inhibits
the increase in intracellular cAMP and chloride secretion induced by arginine-vasopressin
hormone. Evidence that specific receptors for somatostatin are present in the kidney tissue,
arises the possibility that somatostatin treatment in patients with ADPKD might inhibit fluid
formation and eventually induce the shrinking of renal cysts. Octreotide-LAR, a synthetic
analogue of somatostatin with longer half-life and higher sst2 affinity than naïve polypeptides,
has been safely used for the treatment of acromegaly and some malignant tumours. To evaluate
the tolerability and the safety of octreotide in ADPKD patients, a prospective cross-over
controlled study has been recently performed. This pilot study demonstrated the safety of six
month treatment of octreotide-LAR in patients with ADPKD. Moreover, the percent increase of
total kidney volume was significantly lower in patients on octreotide than in placebo. These
findings provided the basis for designing a randomized, placebo-controlled trial to compare the
risk/benefit profile of 3 year treatment with octreotide-LAR or placebo in 79 ADPKD patients
with normal kidney function or mild renal insufficiency. We found that octreotide-LAR
significantly slowed yearly total kidney volume rate in 40 ADPKD patients as compared to 39
controls. Similar findings were observed for changes in total cyst volume. After a similar one-
year reduction in both groups, measured GFR stabilized in octreotide-LAR and continued to decline on placebo. On octreotide-LAR, one-year GFR reduction inversely correlated with subsequent chronic decline. Baseline total kidney volume and total cyst volume correlated with baseline GFR and absolute GFR changes at 3 years. Octreotide-LAR was safe and well-tolerated in all patients. The results of this study have been published in *The Lancet* 2013; 382: 1485-1495.

**Effects of long-acting somatostatin (octreotide LAR) on disease progression in patients with autosomal dominant polycystic kidney disease and severe renal insufficiency (The ALADIN II study)**

There is urgent need for renoprotection in ADPKD patients, particularly for those with more advanced renal dysfunction, for whom few clinical trials have been designed so far. In a pilot feasibility cross-over study we have previously demonstrated that in few patients with severe renal insufficiency 6 month octreotide-LAR treatment retarded the time-dependent increase in total kidney volume as compared to placebo. Moreover, the ALADIN study has recently demonstrated that three-year octreotide-LAR therapy slowed the increase in total kidney volume largely by blunting the growth of cyst volume in patients with ADPKD and relatively preserved renal function, with acceptable safety profile. Taken together, these observations made worth investigating the efficacy of octreotide LAR in slowing kidney enlargement and renal function decline even in ADPKD patients with moderate/severe renal failure. In particular, the aim of the trial is to assess the efficacy of one year treatment with Octreotide LAR compared with placebo in slowing kidney and liver growth in patients with estimated glomerular filtration rate 15-40 mL/min/1.73m² and to verify whether and to which extent this translates into slower renal function decline over 3-year follow-up. This trial (ALADIN II study) is currently ongoing.

**Ex-vivo expanded mesenchymal stem cells to repair the kidney and improve function in cisplatin-induced acute renal failure in patients with solid organ cancer**

Since its introduction into clinical trials, cisplatin has had a major impact in cancer medicine, changing the course of therapeutic management of several tumours, such as that of ovary. Unfortunately, this compound causes dose-dependent and cumulative nephrotoxicity sometimes requiring dose reduction or discontinuation of treatment. Present strategies for the treatment of acute renal failure induced by cisplatin have focused on targeting individual mechanisms thought to contribute to ischemic or toxic insults to the kidney. However, translational research efforts in patients have yielded disappointing results. An alternative possibility is to adopt a strategy aimed to regenerate the injured renal tissue. Attempts to accelerate recovery have focused on administration of growth factors. Although this strategy has been successful in experimental models, no beneficial effects have been observed in clinical trials. The ability of extrarenal cells to participate in the regenerative response following post-transplant acute renal failure may hold true for acute renal failure that develops in native kidneys after cisplatin therapy. The rationale for this approach rests on the recent demonstration in mice that MSCs infusion repairs acute tubular damage in animals given cisplatin. Similarly, consistent evidence of the beneficial effect of bone-marrow derived cell therapy has been recently reported in humans with ischemic heart disease. These observations raises the possibility that adult-derived bone marrow cells could be administered to enhance the recovery from renal injury. The aim of this study is to evaluate the efficacy of ex-vivo expanded donor mesenchymal stem cells infusion in the acceleration of tubular regeneration, and thus renal function recovery, in patients with cisplatin-induced acute renal failure, a disease that so far has not cure. Acute renal injury will be diagnosed by measuring urine concentrations of neutrophil gelatinase-associated lipocalin (NGAL).
Effect of induction immunosuppressive therapies on de novo anti-human leukocyte (HLA) antibodies development in kidney transplantation

Lymphocyte depleting agents are used to minimize chronic maintenance immunosuppression and to promote tolerance-permissive environment; however, high incidence of de novo donor-specific anti-HLA antibodies (de novo DSA) was observed. In this single-center matched-cohort study, we evaluated the phenotype of repopulating B cells and its correlation with donor-specific anti-HLA antibody (DSA) development and long-term graft function in 16 renal transplant recipients and 32 age- and gender-matched controls induced with alemtuzumab or basiliximab (Bas)/low-dose rabbit anti-thymocyte globulin (rATG), respectively. Alemtuzumab, but not Bas/rATG, profoundly depleted peripheral B cells in the first 2 months post-transplantation. In particular, early post-transplant naive B cells were significantly depleted, whereas Ag-experienced and memory B cells were partially spared. Transitional B cells transiently increased 2 months post-transplant. At month 6 pregerminal center B cells emerged, a process promoted by increased BAFF serum levels. Thereafter, B cell counts increased progressively, mainly due to expansion of naive B cells. Conversely, Bas/rATG did not modify the B cell phenotype throughout the follow-up period. Alemtuzumab was associated with a higher incidence of de novo DSA compared with Bas/rATG (50% vs 16%, respectively, p=0.011). DSA development was predicted by reduction in the count and percentages of B cells in the early post-transplant period, in particular the reduction of naïve B cells 15-30 days post-transplant correlated with DSA development 1 year post-transplant. Moreover, DSA production was associated with worse long-term graft function (4-year slope of GFR: DSA+: -3.57 ± 3.16 mL/min/1.73m²; DSA-: -1.07 ± 3.59 mL/min/1.73m²; p<0.05). Thus, alemtuzumab-induced B cell depletion/reconstitution may promote chronic humoral responses against the graft. The results of this study have been published in *J Immunol* 2013; 191: 2818-2828.

Efficacy of paricalcitol in reducing parathyroid hormone levels and ameliorating markers of bone marrow remodelling in renal transplant recipients with secondary hyperparathyroidism (APPLE study)

The risk of fracture for kidney transplant recipients is four times higher than that of the general population. The pathogenesis of post-transplant bone disease is multifactorial, but hyperparathyroidism plays a key role in the maintenance or development of bone remodelling. Vitamin D and its analogues are key components of treatment aimed to prevent or ameliorate secondary hyperparathyroidism in patients with chronic kidney disease (CKD). In hemodialysis patients, intravenous paricalcitol, a new vitamin D analogue, achieves a faster and more effective normalization of parathyroid hormone (PTH) levels than calcitriol, an effect that is associated with smaller changes in serum calcium and phosphorous levels. Preliminary evidence is also available that in pre-dialysis patients with CKD and secondary hyperparathyroidism, treatment with oral paricalcitol may also reduce urinary protein excretion, an effect that is independent of concomitant treatment with agents that block renin-angiotensin system and that in the long-term might translate into slower progression to end stage kidney disease and need for renal replacement therapy. Renal transplant patients are at high risk of hyperparathyroidism, largely because of long-lasting renal insufficiency before transplant, and of progressive deterioration of kidney function because of chronic allograft nephropathy (a disease of proteinuria and progressive decline of the glomerular filtration rate). The aims of this study are: 1) evaluate whether 6-month treatment with paricalcitol may achieve a prompt and effective reduction of PTH serum levels in stable renal transplant patients with secondary hyperparathyroidism; 2) verify whether amelioration of hyperparathyroidism (if any) may translate into an improvement of bone remodelling; 3) assess whether oral paricalcitol may also achieve reduction of urinary protein excretion. Compared to baseline, median serum PTH levels significantly declined after 6 months treatment with paricalcitol but not with maintenance therapy. Moreover, at 6 months, PTH concentrations significantly differed between treatments.
Serum bone-specific alkaline phosphatase and osteocalcin decreased on paricalcitol but not on maintenance therapy, and at 6 months significantly differed between treatments. At 6 months 24-hour protein excretion decreased on paricalcitol, but not on maintenance therapy. Paricalcitol was well tolerated. Thus, in renal transplant recipients with secondary hyperparathyroidism, 6-month paricalcitol supplementation, in addition to safely reduce PTH levels, attenuated bone remodeling and reduced proteinuria. These benefits might help improving long-term renal and cardiovascular outcomes in human kidney transplantation. These results were presented at the annual scientific meeting of the American Society of Nephrology, November 2013, Atlanta, USA.

Autologous Mesenchymal Stromal Cells to induce tolerance in Kidney Transplant Recipients

Mesenchymal stromal cells (MSC) abrogate alloimmune response in vitro, suggesting a novel cell-based approach in transplantation. Moving this concept toward clinical application in organ transplantation should be critically assessed. A safety and clinical feasibility study (ClinicalTrials.gov, NCT00752479) of autologous MSC infusion was conducted in two recipients of kidneys from living-related donors. Patients were given induction therapy with Basiliximab and low-dose Rabbit Anti-Thymocyte Globulin (RATG) and maintenance immunosuppression with cyclosporine and mycophenolate mofetil. On day 7 post-transplant, MSC were administered intravenously. A progressive increase of the percentage of CD4+CD25highFoxP3+CD127- Treg and a marked inhibition of memory CD45RO+RA-CD8+ T cell expansion were observed post-transplant. Patient T cells showed a profound reduction of CD8+ T cell activity. However, serum creatinine levels increased 7 to 14 days after cell infusion in both MSC-treated patients. A graft biopsy in patient 2 excluded acute graft rejection, but showed a focal inflammatory infiltrate, mostly granulocytes. It was hypothesized that subclinical inflammatory environment of the graft in the few days post-transplant surgery could have favoured the prevalent intragraft recruitment and activation of the infused MSC promoting a pro-inflammatory environment with eventual acute renal dysfunction (engraftment syndrome), as reported by others with combined kidney and bone marrow transplantation. This hypothesis has been confirmed back into a murine kidney transplant model showing that MSC administration before (day -1) but not few days after kidney transplantation avoided the acute deterioration of graft function, while maintaining the immunomodulatory effect of MSC (Casiraghi et al, American Journal of Transplantation 2012; 18:51-58). Based on the new data in the murine transplant models, in the second step of multi-step MSC-based clinical protocol in kidney transplantation, we examined in two living-related kidney transplant recipients whether pre-transplant (day -1) infusion of autologous MSC protected from the development of engraftment syndrome previously reported in patients given MSC at day 7 post-transplant. In this protocol we also assessed whether Basiliximab avoidance in the induction regimen might improve the MSC-induced Treg expansion previously reported with therapy including this anti-CD25 antibody. We found that pre-transplant infusion of MSC provided a safety advantage over post-transplant cell administration, in that the former protocol protected against acute graft dysfunction, and maintained MSC-immunomodulatory properties. Induction therapy without Basiliximab did not further expand the Treg pool as compared to induction regimen with this antibody, while exposing patients to the possibility of acute rejection early post-transplantation. The results of this study have been published in Transplant International 2013;26:867-879. As next step we plan a clinical protocol of pre-transplant infusion of autologous MSC with Basiliximab/low-RATG induction therapy.

A prospective, randomized, open label blinded end point (PROBE), crossover study to compare the effects of Telmisartan and Losartan on metabolic profile of renal transplant patients (COSTANT study)
In renal transplant recipients the effects of immunosuppressive therapy with steroids or calcineurin inhibitors may reduce insulin activity and contribute to several of the abnormalities associated with metabolic syndrome, such as hypertension, glucose intolerance and hyperlipidemia. In turn, insulin resistance, hypertension, hyperglycemia and dyslipidemia may importantly contribute to the excess cardiovascular risk of renal transplant patients (an excess comparable to that of diabetic subjects with over diabetic nephropathy) and may also accelerate progressive renal function deterioration and promote graft loss. Thus, amelioration of the insulin activity and of the related metabolic syndrome is a key component of treatments aimed to improve patient and graft survival in renal transplant recipients. Recently, drugs such as peroxisome proliferators-activated receptor-γ (PPARγ) activators, that ameliorate insulin sensitivity and metabolic syndrome, have become available. These agents, however, can provoke fluid retention, weight gain, edema and, in some cases, heart failure. Thus, the risk/benefit profile of PPARγ activators is still uncertain, in particular in renal transplant patients, where the risks of therapy may overwhelm the potential benefits. Recent studies showed that telmisartan, an angiotensin II (AII) type 1 receptor antagonist, in addition to block the AII receptor - a key surface receptor involved in the regulation of blood pressure - may also activate PPARγ, thus improving some of the features of the metabolic syndrome, such as hyperglycemia and dyslipidemia in patients with hypertension and/or diabetes. Thus, in addition to control high blood pressure and to limit some of the adverse effects of angiotensin II, including target organ damage, graft fibrosis and CsA nephrotoxicity, telmisartan may also substantially reduce the overall cardiovascular and renal risk of renal transplant recipients by ameliorating some of the modifiable components of the metabolic syndrome, such as hypertension, glucose intolerance and hyperlipidemia. On the other hand, telmisartan is devoid of the adverse effects of PPARγ activators such as fluid retention, and has therefore a remarkably better risk/benefit profile. Thus, whether telmisartan in addition to the beneficial effects of a reference AII receptor antagonist (such as losartan) may offer adjunctive advantages related to improved insulin sensitivity in renal transplant patients on chronic therapy with steroids and/or calcineurin inhibitors, is worth investigating. This study is currently ongoing.

Superpig project
Superpig is a Research & Development Program, co-financed by the Fund for the Promotion of Institutional Agreements of the Lombardy Region, Universities and Research. The Department of Renal Medicine of the Mario Negri Institute also takes part in this project. It aims to create a Technology Platform for the use of pig in biomedical field (organ and tissue transplants) and biotechnology (animal model). The project lasts 2 years and it includes 4 sub-projects; one of them is “Comparative physiology and Immunology”.

The generation of α-1,3-galactosyl transferase knock-out (Gal-KO) pigs transgenic for human CD55 and CD39 represents a remarkable step forward in xenotransplantation. Elimination of the sugar chain, that is the major xeno-antigen, and the transgenic expression of the regulatory complement protein CD55 and the inhibitor of platelet aggregation CD39 allowed to overcome the barriers of hyperacute humoral rejection and acute vascular rejection in the setting of xenotransplantation. However, the cell-mediated immune response to xenotransplantation remains ill defined. Thus, we characterized human T cell response to xenoantigens in mixed lymphocyte reactions (MLR). Human T cells showed a lower proliferative response (assessed by [3H]-thymidine uptake and by carboxyfluorescein diacetate succinimidyl ester dilution assay) to xenoantigens (peripheral blood mononuclear cells from wild-type pigs) compared to alloantigens (peripheral blood mononuclear cells from healthy individuals). Similarly, CD8+ T lymphocytes exhibited a lower activation, assessed by granzyme B ELISPOT assay, to xenoantigens compared to alloantigens. Both the proliferative response and the number of CD8+ T lymphocytes producing granzyme B were further reduced in response to cells isolated from GAL-KO pigs. Ex-vivo experiments were also performed. In particular, blood samples collected from healthy subjects were perfused for about 30 minutes in kidneys isolated from wild-type
pigs (n=3) or from GAL-KO pigs (n=3). After perfusion, blood mononuclear cells were isolated, and responses to xeno- and allo-antigens were assessed in MLR. Perfusion of human blood in kidneys of wild-type pigs was associated with an increase in the proliferative response and activation of CD8+ T cells assessed by spots for granzyme-B. CD8+ T cell activation was reduced following perfusion of human blood in kidneys of GAL-KO pigs. The lack of expression of galactose-α(1-3)-galactose on cell surface resulted in a further reduction of cell response. Taken together, these results suggest that cellular immune response may not represent a major barrier to xenotransplantation.

Global Burden of Disease Study 2010
The Global Burden of Disease Study 2010 (GBD 2010) is the largest ever systematic effort to describe the global distribution and causes of a wide array of major diseases, injuries, and health risk factors. In 2007, the Global Outreach Research & Prevention Committee of the International Society of Nephrology (ISN) was selected by the GBD consortium to lead the Genitourinary Disease Expert Group. This activity was coordinated by the Mario Negri Institute in Bergamo, Italy. Over 5 years, The GBD Core Team and the Genitourinary Disease Expert Group worked closely together to collect data and construct mathematical models to explore the epidemiology of renal and genitourinary disorders. The results of the GBD study 2010 show that infectious diseases, maternal and child illness, and malnutrition now cause fewer deaths and less illness than they did twenty years ago. As a result, fewer children are dying every year, but more young and middle-aged adults are dying and suffering from disease and injury, as non-communicable diseases, such as cancer and heart disease, become the dominant causes of death and disability worldwide. Since 1970, men and women worldwide have gained slightly more than ten years of life expectancy overall, but they spend more years living with injury and illness.

Noncommunicable diseases have replaced communicable diseases as the most common cause of premature mortality worldwide. Noncommunicable diseases also encompass chronic kidney disease, which is currently ranked 18th among the global causes of death, rising from 27 in 1990, so the number of deaths from chronic kidney disease has risen by 82.3% meanwhile. It’s the third largest increase among the top 25 causes of death, behind HIV/AIDS (396%) and diabetes (93%). It should be pointed out that these findings refer only to more advanced stages of chronic kidney disease. The proportion of patients with milder renal dysfunction is definitely larger, and it is known to contribute to overall mortality, especially cardiovascular mortality. Therefore, it could be anticipated even higher relevance of chronic kidney disease for healthy system in a given country. To date, there are no global estimates detailing the burden of primary chronic kidney disease mortality at country level. Thus, a study is underway with the aim to provide estimates for mortality of chronic kidney diseases as a primary cause of death for 187 countries by year, age and sex. These estimates are a crucial factor to help inform public health policy decisions, especially in countries previously lacking such estimates. The Department of Renal Medicine of the Mario Negri Institute takes part in this study.

Rapid rising rates of chronic kidney diseases globally portend a consequent rise in end-stage renal disease. As costs limits many nation’ abilities to develop maintenance dialysis programs, end-stage renal disease results in ultimately death and economic strain on health systems in many nations. In an effort to accurately report the trajectory and pattern of growth of availability of maintenance dialysis at the global, regional, and country levels, we are evaluating the changes in prevalence and incidence estimates between years 1990 and 2010.

Laboratory of Clinical Pathophysiology of Renal Disease and Transplantation
Main objective of the Laboratory of Clinical Pathophysiology of Renal Disease and
Transplantation is the study of pathophysiological mechanisms underlying the progression of chronic kidney disease and the identification of new therapeutic strategies for diabetic kidney disease and nondiabetic proteinuric nephropathies and chronic rejection. The multidisciplinary nature of these lines of research necessarily requires a close integration between various skills of clinical physiology and clinical pharmacology, and of molecular biology. For this reason, the laboratory includes not only researchers from the Department of Renal Medicine, which touch on the laboratory, but also the Department of Public-Private Medical Specialist and Transplants. From an operational standpoint, the laboratory interacts closely with the Laboratory for the coordination and conduct of controlled clinical trials which finalizes the protocols designed for clinical research within the Department of Renal Medicine.

A prospective, sequential study to assess the efficacy of rituximab therapy in maintaining remission of nephrotic syndrome after steroid and immunosuppressive therapy withdrawal in patients with steroid-dependant or multirelapsing minimal change disease or focal segmental glomerulosclerosis: the NEMO Study

Patients, especially children, with steroid-dependent or frequently relapsing nephrotic syndrome (NS) secondary to minimal change disease (MCD), even when associated with diffuse mesangial proliferation (MesGN), and focal and segmental glomerulosclerosis (FSGS), need continuous immunosuppression to limit or prevent recurrences, and are at increased risk of severe drug-related adverse events. Small uncontrolled studies suggested the B cell depleting monoclonal antibody Rituximab as safe and effective alternative to maintain remission and limit overall immunosuppression in this population.

This academic, prospective, multicenter, within-patient controlled trial was sponsored by the Italian Ministry of Health and was primarily aimed at evaluating whether Rituximab allows maintaining stable NS remission after tapering and withdrawal of steroid and other immunosuppressants in patients with steroid-dependent or frequently relapsing MCD, MesGN or FSGS. Secondarily, we evaluated whether ant to what extent Rituximab allows reducing overall immunosuppression and the burden of treatment-related side effects.

Overall, 30 patients (10 children) with MCD/MesGN or FSGS on steroid-induced remission were included. At one year all patients were on remission, and half of them had never relapsed. Compared to the year before, total number of relapses and per-patient relapse rate after Rituximab significantly decreased. Similar results were found after comparing the relapse rate during two years before and two years after Rituximab treatment. This finding suggests that Rituximab effect on relapse prevention is lasting over time.

In addition, both maintenance and induction steroids to treat each relapse after Rituximab significantly decreased, as well as the doses of other immunosuppressants. Growth, blood pressure and BMI significantly improved in children, an expression of reduced adverse effects of chronic steroid treatment. Renal function as revealed by the estimated GFR, ameliorated in all patients, especially in children and in those with FSGS. Furthermore, during three-year observation Rituximab was able to halt the progressive growth retardation observed in children over three years before Rituximab treatment. Treatment was generally well tolerated. These findings suggest that Rituximab therapy might help limiting the complications of the NS and of concomitant treatments, complications that are often devastating in this clinical context.

Efficacy and safety of rituximab second-line therapy for membranous nephropathy: a prospective, matched-cohort study

Idiopathic membranous nephropathy (IMN) is the most frequent glomerular disease in adult patients with nephrotic syndrome. About 20% of patients may achieve complete remission with non-specific interventions, while the remainder had some degree of proteinuria with stable or slowly declining renal function. These latter are at risk for progressive chronic kidney disease
up to ESRD in most cases. In addition, approximately 10% of those patients with persistent nephrotic syndrome die prematurely of cardiovascular events before progressing to ESRD. Non-specific immunosuppression with steroids and alkylating agents or calcineurin inhibitors in IMN patients at high risk of progression has led to a substantial reduction in ESRD incidence in the everyday setting. However, the burden of side effects of these medications is well documented and is a major clinical concern since patients eventually progressing to ESRD and receiving a kidney transplant are exposed to further immunosuppressive treatment to prevent allograft rejection.

Selective B lymphocyte depletion by the anti-CD20 monoclonal antibody Rituximab, may inhibit the production of autoantibodies involved in the pathogenesis of the IMN without the toxicity of non-specific immunosuppression.

Thus, we decided to offer this treatment option to patients referred to our nephrology unit who were expected to progress to ESRD or to die prematurely of cardiovascular events because of persistent nephrotic syndrome. Among 100 consecutive patients with IMN followed up for 29 months after Rituximab administration, 65 achieved complete or partial remission on average 7 months. Rates of remission were similar between patients with or without previous immunosuppressive treatment. Four patients died and 4 progressed to ESRD. Renal function improved in those who achieved complete remission. Serum albumin significantly increased and proteinuria decreased among those achieving complete or partial remission. Proteinuria at baseline and the follow-up duration each independently predicted the decline of proteinuria. No treatment-related serious adverse events occurred. In summary, Rituximab achieved disease remission and stabilized or improved renal function in a large cohort of high-risk patients with IMN.

Membranous nephropathy associated with IgG4-related disease

In collaboration with the Department of Molecular Medicine

IgG4-related systemic disease is a rare condition, characterized by high levels of circulating IgG4 and IgG4⁺ plasma cell infiltrates in various organs, including pancreas, salivary gland, biliary tract, liver, lung, and kidney. Herein, we describe the case of a 54-year-old man with IgG4-related systemic disease, presenting with autoimmune pancreatitis and Mikulicz’s disease. Steroid therapy reduced circulating IgG4 levels and promoted regression of clinical signs. Thereafter, an increase in serum IgG4 values was followed by occurrence of nephrotic-range proteinuria. A renal biopsy showed membranous nephropathy, with no IgG4⁺ cell infiltrates. Search for circulating immune complexes was negative, as well as that for anti M-type phospholipase A2 receptor antibodies. Western blot analyses identified circulating IgG reacting with some potential glomerular target antigens.

The present case suggests that membranous nephropathy represents an additional renal manifestation of IgG4-related systemic disease, whose pathogenesis is possibly associated with neoproduction of autoantibodies targeting podocyte antigen(s).

Study on genetics, autoantibodies against PLA2R (anti-PLA2R) and PLA2R staining on renal in biopsies in patients with membranous nephropathy on Rituximab.

In collaborazione con il Dipartimento di Medicina Molecolare

Recent evidences suggest that B cells dysfunction may have a role in the pathogenesis of idiopathic membranous nephropathy (IMN) through the secretion of autoantibodies directed against kidney constituents. Among them, autoantibodies against the phospholipase A2 receptor (PLA2R) seems to play an important role.

On the basis of this finding, 10 years ago we started to treat patients with IMN with Rituximab, a monoclonal antibody able to eliminate B cells from the circulation. Encouraging results were achieved in terms of both clinical efficacy and of treatment safety.
The observation of more than 100 IMN patients treated with Rituximab at our Center during the last 10 years, showed that more than 60% of patients achieved partial or complete remission of the nephrotic syndrome, while others did not respond or showed a limited response on proteinuria. In addition, some patients experience a relapse of disease after initial remission. Data on the pathogenic role of the anti-PLA2R antibodies are lacking, since IMN may present in patients who have no anti-PLA2R antibodies in the circulation. Further, recent studies on HLADQA1 and PLA2R1 gene polymorphisms suggest that genetic alterations may be responsible for the production of these antibodies.

Our aim is to evaluate: 1) the relationship between antibodies changes during follow-up and treatment response; 2) the predictive role of genetic alterations on relapse in patients with previous Rituximab treatment; 3) the enhanced renal expression of the PLA2R protein; 4) if HLADQA1 and PLA2R1 polymorphisms associate with clinical outcome. Serologic studies for anti-PLA2R antibody titers and expression of the PLA2R protein, is in collaboration with clinical researchers of the Tenon Hôpital, Paris.

Results from this study may suggest better understanding of disease insight and may suggest better treatment strategies.

A prospective, randomized, open, blinded endpoint (PROBE), clinical trial to assess the renal and humoral effects of sevelamer carbonate in patients with chronic kidney disease and residual proteinuria despite best available treatment (THE ANSWER STUDY)

In a post-hoc analysis on 331 patients with proteinuric chronic nephropathies included in the Ramipril Efficacy In Nephropathy (REIN) trial, increasing serum phosphate levels at inclusion, even within the normal reference range, were associated with an incremental risk of progression to End Stage Renal Disease (ESRD). Moreover, increasing levels of serum phosphate were associated with a progressively decreasing protective effect of ramipril therapy against progression to ESRD, to the point that the benefit of Angiotensin-Converting-Enzyme (ACE) inhibition was almost fully lost among patients with serum phosphate levels exceeding 4.5 mg/dL. Findings that the interactions observed between serum phosphate, disease progression and ACE inhibition did not change appreciably when the analyses were adjusted for all considered potential confounders - including proteinuria, GFR and other renal risk factors - provided convincing evidence that phosphate plays a direct pathogenic role in patients with progressive nephropathies. Moreover the excess phosphate exposure that limit or even blunt the renoprotective effect of ACE inhibitor therapy may have major clinical implications since rennin angiotensin-system (RAS) inhibition by ACE inhibitors and angiotensin receptor blockers (ARBs) is currently standard therapy for patients with proteinuric nephropathies with high-risk to ESRD.

Sevelamer carbonate is a newly approved phosphate binder for CKD patients not yet on maintenance dialysis. Treatment with Sevelamer, in addition to correct hyperphosphatemia, was also found to ameliorate abnormalities of the mineral metabolism associated with accelerated renal disease progression and increased cardiovascular risk. Furthermore, Sevelamer therapy reduces proteinuria in an animal model of uremia, an effect that in the long term might translate into significant renoprotection. These findings suggest that serum phosphate might be a specific target for renoprotective therapy in CKD patients and provide the background for randomized clinical trials to formally test whether reducing phosphate exposure by phosphate binding agents may serve to optimize the renoprotective effect of RAS inhibition in this population.

The ANSWER study was designed in order to assess whether the reduction of phosphate obtained by Sevelamer carbonate therapy may have an antiproteinuric effect in patients with CKD and residual proteinuria despite optimized RAS inhibitor therapy with Ramipril and Irbesartan.
The study will include 50 consenting subjects with residual proteinuria (>0.5 gr/24h) despite optimized RAS inhibitor therapy, GFR estimated with MDRD >15 ml/min/1.73m² and without concomitant treatment with phosphate binders.

The primary aim is to assess the effect of 3-month Sevelamer carbonate therapy on 24-h urinary protein excretion; secondary aims are to evaluate the effect of Sevelamer therapy on: office blood pressure; GFR as assessed by iohexol plasma clearance; 24-h urinary phosphate, calcium, magnesium, urea, sodium and albumin excretion; phosphate, calcium, magnesium, urea, sodium and albumin fractional clearance; venous pH and base excess; serum levels of FGF 23 (C terminal segment and the intact form) and other biomarkers of mineral metabolism (vitamin 25 OH D, 1-25 Vitamin D, calcium, phosphorous, PTH, ALP); serum levels of markers of inflammation such as hsCRP and IL-6; serum lipids (total, HDL and LDL cholesterol and triglycerides); parameters of arterial stiffness such as PWV and augmentation index as assessed non invasively via applanation tonometry.

Potentially eligible patients will enter two-month run-in treatment period with standardized and stable dose of renin angiotensin system (RAS) inhibitor therapy. Treatment will be titrated to blood pressure and proteinuria. Dose up-titration will be stopped when treatment targets are achieved or treatment-related side effects such as symptomatic hypotension, hyperkalemia or serum creatinine increase >30% vs baseline. Thus, at the end of the run-in period all patients will be on the maximum tolerated dose. At completion of the two month run-in period, subjects who fulfill the selection criteria will be stratified in two subgroups depending on serum phosphate (≤ 4mg/dl); each group will be randomized to receive three-month Sevelamer carbonate treatment period followed by four-month period without Sevelamer treatment or four-month period without Sevelamer treatment followed by three-month Sevelamer carbonate treatment period.

Throughout the study, patients will also be followed by a nutritionist who will verify adherence to a diet controlled for the amount of sodium and protein intake. Since study start date on 21/10/2013 at our Clinical Research Center till the end of 2013, the first five patients have been enrolled.

Community screening for early detection of kidney and cardiovascular disease in high-risk subjects in the Bergamo urban area

Chronic kidney disease (CKD) is a worldwide threat to public health, but the scale of the problem is probably not fully appreciated. Estimates of the global burden of diseases report that diseases of the kidney and urinary tract contribute to approximately 830,000 deaths annually. The aggregate cost for renal-replacement therapy (RRT), which consists primarily of kidney transplantation, hemodialysis and peritoneal dialysis during the coming decade will be more than US$1 trillion.

A survey of the local Health Authorities found that 2008 direct costs for chronic hemodialysis therapy approximated 25-thousand Euros for an average patient aged 50 to 69 years, and yearly treatment costs for 366 dialysis subjects in this age-range exceeded 9-million Euros in the Bergamo Province (see Attachment). This economic burden is even larger if indirect costs are considered. Thus, early intervention programs aimed to prevent or limit the onset of CKD and eventual progression to terminal kidney failure and need for renal replacement therapy would have major implications for health care providers.

Patients on RRT can be regarded as the tip of an iceberg, as the number of those with CKD not yet in need of RRT is much greater. However, only rough estimates exist for the prevalence of pre-dialysis CKD. Importantly, patients with reduced kidney function represent a population not only at risk for progression of kidney disease and for ESRD, but also at even greater risk for cardiovascular diseases and that this frequently leads to death before ESRD is reached. Early detection of CKD and its risk factors may help to establish timely renoprotective intervention to slow or prevent progression to ESRD and reduce cardiovascular (CV) risk.
CKD is defined as the presence of either micro-macroalbuminuria, hematuria or abnormalities on renal ultrasound; or an impaired estimated glomerular filtration rate (GFR). Microalbuminuria is defined as the urinary albumin excretion of 30–300 mg/24 h or 20–200 μg/min or 20–200 mg/ml or 30–300 mg/g creatinine, in the spot morning urine sample. Microalbuminuria is not just a biomarker of kidney injury but also an important predictor of both cardiovascular damage and renal disease.

There are only few studies of screening normal population for early signs of CKD such as microalbuminuria in Europe. The Prevention of End Stage Renal and Vascular End-points (PREVEND) study (Groningen - The Netherlands) evaluated almost 40,000 individuals of which the 7.2% had microalbuminuria. In these subjects a positive dose-response relationship between increasing urinary albumin concentration and mortality was found (22.5% related with CV deaths and 16.6% with non-CV deaths). Subjects from the general population that were found to have increased urinary protein levels were shown to represent more than half of the patients who started dialysis or had a kidney transplant during nine years follow-up. Restricting screening to those individuals with hypertension, diabetes, cardiovascular disease history, or age >55 years having increased urinary protein levels identified nearly all cases needing kidney disease treatment during follow-up.

In Italy, the prevalence estimates for stage 3 to 5 CKD are around 4 million, yet less than 30% of these subjects are believed to be followed at nephrology clinics. This means that in Italy for every dialyzed patient there are about 85 individuals with possibly progressive kidney disease, while fewer than five (mainly stage 4 and 5 patients) are actually followed by a nephrologist. Therefore, implementation of new diagnostics and therapeutics strategies to reduce risk factors for development of CKD and their associated complications are important. Several interventional studies are targeted to reduce microalbuminuria with the ultimate objective to lower CKD progression. Experimental evidence in animal models and humans demonstrates that angiotensin-converting-enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) reduce microalbuminuria and slow the progressive loss of renal function. The multicenter double-blind, randomized Bergamo Nephrologic Diabetes Complications Trial (BENEDICT) found that in patients with type 2 diabetes, arterial hypertension, and normoalbuminuria, angiotensin-converting-enzyme inhibitor (ACEi) therapy with trandolapril also prevented the onset of microalbuminuria in 50% of the study population. With this background, in 2008 local Health Authorities started a screening program for microalbuminuria in the Province of Bergamo (see Attachment). To enhance the cost-effectiveness of the program, the screening was restricted to subjects aged 50 to 69 years, that is the age-group expected to have the highest incidence of considered outcome and at the same time to benefit the most of reno- and cardio-protective intervention.

Beyond microalbuminuria, other urinary markers may help identifying subjects at enhanced renal and CV risk, including glycosuria that might unmask subjects with unknown diabetes (who have blood glucose levels exceeding renal reabsorption threshold), hematuria, a marker of glomerular disease, urological abnormalities, lithiasis, or urinary tract infection, and increased sodium excretion that may identify subjects at risk because of excess salt intake. Indeed, high sodium intake is associated with hypertension, hyperfiltration and activation of tissue ACE inhibitor activity. Moreover, high sodium intake may sustain chronic renal injury not only through increased systemic BP and glomerular pressure, but also by directly inducing tissue fibrosis. Thus, the present Community Screening Program aims at early detecting renal and cardiovascular disease in high-risk subjects in the Bergamo Urban Area with the final goal of preventing/limiting renal and cardiovascular events in this population and reducing direct and indirect costs of these complications.

In January 2012 the Local Health Authority of the Province of Bergamo, in collaboration with the Mario Negri Institute for Pharmacological Research in Bergamo, the primary care physicians from Bergamo district and the Ospedali Riuniti di Bergamo, formally launched the screening project. Phase 1 of the project which involved the operational phase was
completed in June 2012. Since September 2012 the screening program has been formally
launched. Up to December 2012 more than 1200 at risk patients have been identified which
represents about 25% of the 6000 subjects included in the project. The patient’s recruitment
phase foresees to complete the enrolment of all estimated subjects in December 2013.

**Laboratory of Pharmacovigilance and Monitoring for Clinical Investigations**

The target of the Laboratory of Pharmacovigilance and Monitoring for Clinical Investigations is
to ensure that the clinical trials are performed according to the experimental protocol, the Good
Clinical Practice (GCP), and the rules required.
The task of Laboratory researchers is to arrange and planning the clinical trials following a
correct methodology in order to ensure studies with a high scientific value, which is guaranteed
through the monitoring of all the stages of research activities.
The trial design phase provides documentations, informations and scientific support to the
researchers in order to allow them to take a part in projects, programs and financial
opportunities for the research sponsored by regional, national and international authorities.
Researchers are informed about suitable announcements and they are supported during the
preparation and submission of the “applications”; and the relationship with suppliers, too.
Laboratory collaborates with the trial promoters for: identifying project candidate Centres and to
arrange national and international team work networks for each research area with meetings
which are useful for sharing and divulging scientific information.
It’s very important the commitment taken for the arrangement of singular meetings or seminars
for subjects who participate on the clinical studies, through them each participant is informed
about the results of the studies where he took a part and also about the new therapeutic outlooks
on his state of health.
The constant coaching and updating activity of the investigators involved in the clinical studies
is one of all the activities of the monitor researchers, who provide to them the basic
methodologies for a clinical research, focusing in particular on the scientific foundation which
govern it, and the information needed for the development of the project where they are
involved in. Moreover, they follow the progress of the studying phase, discussing any possible
problem about the enrollment with the promoter and the investigator in order to find an
agreement for the corrective actions focusing on the target, in terms of procedures and times,
provided at the beginning of the study.
About the data management phase, the Laboratory, in collaboration with the Clinical Research
Informatics Unit, has developed a specific interest for the implementation of telematic tools for
the management and the data collection with a remote device related to collaborative clinical
studies. The remote data collection device is an important and innovative tool for the
improvement of the clinical research which could be useful in order to help the collaboration of
more national and international team works. Using a “web based” electronic database, the data
monitoring activities are made in a centralized way and the activities on the Centers are limited
to source data verification, drug accountability and Investigator’s File control.
The essential task of the Laboratory is also to manage the Pharmacovigilance of clinical studies
that consisting in different activities which have to guarantee a safe and appropriate utilization
of the study drug during the test phase, for example:
- protocols auditing for the valuation of the product security during the testing phase;
- auditing and processing of the SAEs and follow up related;
- communication of the SUSARs to the EMA, Authorities and Ethics Committees;
- security recurring reports;
- consistence between the clinical and the pharmacovigilance database.
Coaching and updating the new monitor researcher is a part of the job of the senior personnel through the arrangement of individual lessons, group lessons and monitoring activities supported by senior monitors.

The relationship among different people (with different skills as doctors, nurses, biologists, statisticians and engineers) involved in a clinical project give to the monitor the capability to have a global vision of the different phases of a clinical research guaranteeing the right management of the resources, rules and scientific standards as provided by the current regulations.

During 2013 were coordinated and monitored the follow studies:

ALADIN 2 Study - A prospective, randomized, double-blind, placebo controlled clinical trial to assess the effects of long-acting somatostatin (octreotide Lar) therapy on disease progression in patients with autosomal dominant polycystic kidney disease and moderate to severe renal insufficiency

APPLE Study - “Paricalcitol in reducing parathyroid hormone levels and ameliorating markers of bone modelling in renal transplant recipients with secondary hyperparathyroidism”

ANSWER Study – “A prospective, randomized, open, blinded endpoint (PROBE), clinical trial to assess the renal and humoral effects of sevelamer carbonate in patients with chronic kidney disease and residual proteinuria despite best available treatment.”

ARCADIA Study - "A prospective, randomized, open label, blinded end-point (PROBE) trial to evaluate whether, at comparable blood pressure control, ACE inhibitor therapy more effectively than non RAS inhibitor therapy reduces cardiovascular morbidity and mortality in chronic dialysis patients with left ventricular hypertrophy”

ATHENA Study - "A randomized, prospective, multicenter trial to compare the effect on chronic allograft nephropathy prevention of mycophenolate Mofetil versus Azathioprine as the sole immunosuppressive therapy for kidney transplant recipients”

CE-US Study – “A pilot, explorative study to identify contrast-enhanced ultrasound (CE-US) patterns that characterize acute allograft rejection and other causes of acute allograft dysfunction in renal transplant recipients”

COSTANT Study – “A prospective, randomized, open label blinded end point (PROBE), crossover study to compare the effects of telmisartan and losartan on metabolic profile of renal transplant patients”

CRESO2 Study – Long-term effects of caloric restriction on metabolic, renal and retinal health in subjects affected by obesity and type 2 diabetes.

DIABASI Study - "A prospective, randomized, double-blind, placebo-controlled trial to evaluate the effect of 6-month acetyl-L-carnitine therapy on arterial blood pressure, lipid and metabolic profile, and kidney function in hypertensive patients with type 2 diabetes on background simvastatin therapy”

EAGLE Study – Evaluating the morphofunctional effects of Eculizumab therapy in primary membranoproliferative glomerulonephritis: a pilot, single arm study in ten patients with persistent heavy proteinuria”

LIMONE Study - "A prospective, randomized, open blind endpoint (PROBE) trial to assess the possibility to prevent stone recurrence by lemon juice supplementation in patients with recurrent calcium oxalate nephrolithiasis”

MSC-CIS Study - “Ex-Vivo Expanded Mesenchymal Stem Cells To Repair The Kidney And Improve Function In Cisplatin-Induced Acute Renal Failure In Patients With Solid Organ Cancers”

MSC-KTX Study - “Mesenchymal stem cells under basiliximab/low dose RATG to induce renal transplant tolerance”

PREDICTION Study – “A prospective study to compare early graft function or recipients of single or dual kidney organs stored in ice cold solution or pulsed perfusion.

PROCEED Study - "A prospective, randomized, cross-over, double-blind, placebo controlled study to assess the antiproteinuric effect of selective vitamin D receptor activation by Paricalcitol"
in type 2 diabetes hypertensive patients on low or high sodium diet and stable RAS inhibitor therapy”

REMISSION CLINIC Study: “Proposta di un registro multicentrico, prospettico, informatizzato “ON-LINE” per il monitoraggio di pazienti con nefropatie croniche progressive afferenti a diversi ambulatori di nefrologia trattati sulla base di un protocollo di intervento multimodale standardizzato finalizzato alla normalizzazione della proteinuria ed alla stabilizzazione della funzione renale”

SIRENA II Study - “Effects of Sirolimus on disease progression in patients with Autosomal Dominant Polycystic Kidney Disease and severe renal insufficiency”

VALID Study – “A prospective, randomized, open label blinded end point (PROBE) trial to evaluate whether, at comparable blood pressure control, combined therapy with the ACE inhibitor Benazepril and the angiotensin II receptor blocker (ARB) Valsartan reduces progression to ESRD more effectively than Benazepril or Valsartan alone in high risk patients with type 2 diabetes and overt nephropathy”

VARIETY Study – “A prospective, randomized, open label blinded end point (probe) trial to evaluate whether, at comparable blood pressure control, combined therapy with the ACE inhibitor Benazepril and the angiotensin II receptor blocker (ARB) Valsartan reduces the incidence of microalbuminuria more effectively than Benazepril or Valsartan alone in hypertensive patients with type 2 diabetes and high-normal albuminuria”.

**Laboratory of Regulatory Affairs for Clinical Studies**

Since 1999, the Department of Renal Medicine, in collaboration with other Departments of the Institute and with the Hospital of Bergamo, within the framework of activities of the Public-Private Department of Specialist and Transplant Medicine, designs, promotes and coordinates projects of clinical research. These are mainly independent research projects; some of them are carried out exclusively in the Daccò Centre, but most of them are multi-centre trials, in collaboration with Hospitals, Research Centres and Universities, both Italian and foreign. The Laboratory of Regulatory Affairs for Clinical Studies has been created in relation to this activity, to verify that the studies are conducted in compliance with patient’s safety and rights and according to the legislation in force.

In the execution of its activities, the Laboratory, interacts with several interlocutors, among them the Ministry of Health, the Italian Agency for Drugs (AIFA), the Local Ethic Committees, the ASL and the Pharmaceutical companies.

The Laboratory, supported by a secretariat, participates in all the phases of planning, organizing, conducting and managing the clinical studies.

During the preparatory phase, preceding the onset of the study, the Laboratory collaborates with the researchers in order to establish the protocol and the study’s documents in accordance with the principles of Good Clinical Practice. In addition it verifies the adherence of these documents to regulatory and ethical aspects. Once the protocol has been established, the Laboratory proceeds to the input of the trial in the National Monitoring Centre on Clinical Research with Medicines (OsSC) and subsequently submits all the documents to the competent authorities, in order to obtain the ethical and administrative approvals and manages all the bureaucratic procedures.

During the course of the study, the Laboratory is responsible for complying with the legal obligations to keep AIFA and the Ethic committees constantly updated on the progress of the experimentation. It takes care of sending communications relating to the beginning and conclusion of the experimentations, and periodic reports (usually annual) on the progress of the studies and adverse events that have occurred. Is also responsible for the collection and maintenance of all essential documents for the conduction of clinical studies (preparation and
updating of the Trial Master File for each study and an electronic protocol archive, accessible to all users of the Centre Daccò).

Since 2005, according to the rules set by the International Committee of Medical Journal Editors, the Laboratory ensures that all the clinical studies promoted by the Centre Daccò are registered to the NIH international registry, “clinicaltrials.gov”. At the same time it deals with updating the Register of Clinical Trials on the website of the Institute.

In accordance with the Ministerial Decree of 17 December 2004, credits for Continuing Medical Education (CME) provided by the National Commission for continuing education could be allocated to medical doctors and nurses involved in no profit trials. Concerning this issue, as the Institute is a regional ECM Provider, the staff of the Laboratory is responsible for all activities related to the allocation of ECM to doctors, nurses and technicians who participate in no profit trials coordinated by the site of Ranica.

In respect to the recent sanitary accreditation of Daccò Centre, the Laboratory has maintained relations with ASL and other competent authorities, for everything concerning the maintenance of requirements and other legal purposes.

During 2012, the Laboratory has carried out the activities described above for all the ongoing studies at the Departments of Bergamo and Ranica and in collaboration with the Hospital of Bergamo and other centers, both Italian and foreign, participating in our research projects. The studies performed in 2012 are about 30 and involve more than 80 centers. In the year 2012 have been performed several new ethical submissions and clinical studies closure procedures.

In particular, during 2012 have been prepared the final reports of these studies supported by AIFA:

- A randomized, prospective, multicenter trial to compare the effect on chronic allograft nephropathy prevention of mycophenolate mofetil versus azathioprine as the sole immunosuppressive therapy for kidney transplant recipients (ATHENA Study);
- A prospective, randomized, open label blinded end point (probe) trial to evaluate whether, at comparable blood pressure control, combined therapy with the ACE inhibitor Benazepril and the Angiotensin II receptor blocker (ARB) Valsartan reduces Progression to ESRD more effectively than Benazepril or Valsartan alone in high risk patients with type 2 diabetes and Overt nephropathy (VALID Study);
- A prospective, randomized, open label blinded end point (probe) trial to evaluate whether, at comparable blood pressure control, combined therapy with the ACE inhibitor Benazepril and the Angiotensin II receptor blocker (ARB) Valsartan, reduces the Incidence of microalbuminuria more effectively than Benazepril or Valsartan alone in hypertensive patients with type 2 diabetes and high-normal albuminuria (VARIETY Study);
- A prospective, sequential study to assess the efficacy of rituximab therapy in maintaining remission of nephrotic syndrome after steroid and immunosuppressive therapy withdrawal In patients with steroid-dependant or multirelapsing Minimal Change Disease or Focal Segmental GlomeruloSclerosis (NEMO Study).
RARE DISEASES DOCUMENTATION
AND RESEARCH

STAFF

Head Erica DAINA, M.D.

Genetics for Clinical Research

Head Elena BRESIN, M.D.

Network Development for Rare Diseases

Head Sara GAMBA, Research Nurse
CURRICULA VITAE

Erica Daina got her degree in Medicine at the University of Milan in 1987 and the specialisation in Medical Nephrology in 1990 at the same University.
She performed her training at the II° Medical Division - San Raffaele Hospital - Milan, and at the Division of Nephrology and Dialysis - Riuniti Hospital - Bergamo.
In March 1988 she started her collaboration with the Mario Negri Institute and since June 1993 she works as full-time clinical researcher at the Clinical Research Center for Rare Diseases Aldo e Cele Daccò.
1996 – 2009: Chief, Information Center for Rare Diseases
June 2009: Chief, Laboratory of Rare Diseases Documentation and Research
Areas of interest: Rare diseases, Takayasu’s Arteritis, Hemolytic Uremic Syndrome/Thrombotic Thrombocytopenic Purpura, genetic kidney diseases.

Selected publications


Elena Bresin got her degree in Medicine at the University of Padua in 1994 and the specialisation in Medical Genetics at the University of Verona in 2000.
She performed her training at the Department of Pediatrics of the University Policlinic of Padua, then at the Department of Biology and Genetics of the University Policlinic of Verona and since 2000 at the Clinical Research Center for Rare Diseases Aldo e Cele Daccò.
Since January 2001 she works as full-time clinical researcher at the Clinical Research Center for Rare Diseases Aldo e Cele Daccò and since June 2009 she is Unit Head of Genetics for Clinical Research.
Areas of interest: thrombotic microangiopathies, membranoproliferative glomerulonephritis, familial focal segmental glomerulosclerosis.

Selected publications


Sara Gamba got her Nurse Diploma on 1994 at Bolognini Hospital Nurses School, Seriate (Bergamo).

Areas of interest: rare diseases studied by the Laboratory staff. She is involved with the Italian rare diseases patients’ Associations and she coordinates the documentation service addressed to the patient with rare conditions, their relatives and the health care professionals.

Employment: In 1997-2003 she was involved as co-organizing, speaker, co-speaker and tutor for the Clinical Research Course for Nurses at IRFMN – Daccò Center.

Since 2009 she is Chief of the Unit Network Development for Rare Diseases.

From 2005 she collaborates for training of students that partecipate at the First Level Master in Clinical Research - Milan University.

Since 2007, she collaborates with Turin University for lessons to the students of the Second Level Master in Rare Diseases.

Selected publications


ACTIVITIES

Rare Diseases (RD) represent about ten percent of all human medical illnesses and infirmities. It is difficult to define what exactly is intended as a RD. The US Congress in the Orphan Drug Act has given the first definition in 1983. Under this law it is considered rare a disease that affects less than 200 000 Americans (prevalence 0.75 per 1 000).

The European Parliament adopted a more strict definition; they consider rare a condition that affects not more than five individuals per 10 000 in the European Community (prevalence 0.5 per 1 000).

According to the WHO, there are 5-7 000 rare diseases and most of them (about 4 000) are of genetic origin.

Rarity often brings a difficult and/or late diagnosis, and represents a difficulty in implementing experimental and clinical research studies.

RD comprehend heterogeneous groups of diseases and often require a multidisciplinary approach.

The greatest barrier to prevention, diagnosis and treatment of RD is inadequate knowledge. Once a diagnosis of RD is made, a major complaint of patients and of those involved in their care is the difficulty to obtain pertinent information about causes, symptoms and either established or experimental treatments. Often, patients with RD are willing to participate in clinical studies, but they do not know where and how, and physicians or health authorities are seldom able to help them.

RD is not a very attracting field for basic and clinical investigators for several reasons: it is difficult to find adequate animal models for many rare disorders; clinical trials may require more patients than available; financial support is insufficient.
Few countries have a central body or system to disseminate information on RD. Accurate information on the incidence and prevalence of RD is extremely important for both basic and clinical investigators. Invaluable help to research advances in RD would come from the availability of registries and databases containing diagnostic, clinical and biological data of patients with rare disorders.

The Laboratory has been established with the aim to collect different skills to support patients with rare diseases.

Since the beginning of the activities, a Database for rare diseases was created with updated information for patients, their families and professionals. Specific research projects has been developed for some rare conditions such as familial and recurrent forms of Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP), Fabry disease, Alport Syndrome, Takayasu arteritis, hereditary nephrotic syndromes.

During the years the Laboratory role has changed, because of the major attention on rare diseases developed at the Institutional level.

In 2001 it has been nominated Coordinating Centre of the Regional Network for Rare Diseases in the Lombardy Region, an area of 9 million people in Northern Italy. As Coordinating Centre, it is also working with the National Centre of Rare Diseases at Istituto Superiore di Sanità in Rome. All the information regarding the activities of the Coordinating Centre are available at the web site: [http://malattierare.marionegri.it](http://malattierare.marionegri.it)

In 2009 the Genetics for Clinical Research Unit was established with the aim to support research projects on hereditary rare diseases ongoing at the Daccò Centre, through a close collaboration with the Laboratory of Immunology and Genetic of Rare Diseases and Organ Transplantation and the Laboratory of Cell Biology and Xenotransplantation.

The Network Development for Rare Diseases Unit (also established in 2009) represents a suitable tool for the enhancement of collaborations already activated in the past years and for the development of new network on rare diseases. In addition, very relevant are the collaborations with Italian patients’ organisations and with national (UNIAMO-FIMR) and international (NORD, EURORDIS) Federations for rare diseases. Moreover, particular attention is dedicated to keep up-to-date documentation and scientific bibliography on rare diseases. An help-line service to the public it is also maintained.

### MAIN FINDINGS

The database of the Information Centre for Rare Diseases contains data about 12783 patients affected by 935 different rare disorders. This database represents an important tool to implement clinical research projects for some rare diseases.

In the Bank of biological materials, samples from 2612 patients with rare conditions and their families have been collected. Disease registries include 1330 cases.

The Centre has established contacts with 373 Italian Associations for rare diseases. It was even possible that patients with 91 different rare diseases - for which no Associations have been established in Italy yet - to meet among themselves.

In December 2001 (Delibera della Giunta Lombarda n. 7328), the Centre was identified as "Coordinating Centre of the Regional Network for Rare Diseases".

The Laboratory coordinates the International Registry of Recurrent and Familial Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP), since 1996. The
research projects developed in collaboration with the Laboratory of Immunology and Genetic of Rare Diseases and Organ Transplantation and with the Laboratory of Cell Biology and Xenotransplantation, have allowed to better comprehend the pathogenesis of these diseases.

The Laboratory coordinates the Italian Registry of Membranoproliferative Glomerulonephritis and the Registry of Steroid-Resistant Nephrotic Syndrome.

**NATIONAL COLLABORATIONS**

Italian National Institute of Health  
Centro di Ricerche di Immunopatologia e Documentazione su Malattie Rare - Struttura Complessa a Direzione Universitaria di Immunologia Clinica, Torino  
Coordinamento Interregionale delle Malattie Rare del Piemonte e della Valle d’Aosta  
Papa Giovanni XXIII Hospital (Riuniti Hospital), Bergamo  
Assessorato alla Sanità, Lombardia Region  
University of Turin, School of Clinical Pathology, Faculty of Medicine and Surgery  
Italian Network for Promotion of Folic Acid to Prevent Birth Defects  
University of Turin, Department of Experimental Medicine and Oncology, 2nd Level Master in Rare Diseases  
Italian Society of Neonatology (Lombardy section), Rare Congenital Respiratory Diseases Study Group  
“BergamoScienza” Association  
AO Niguarda Cà Granda, Milan, Nursing course degree

**INTERNATIONAL COLLABORATIONS**

EURenOmics: European Consortium for High-Throughput Research in Rare Kidney Diseases – Coordinator: Heidelberg University Hospital, Germany  
Podonet: Consortium for Clinical, Genetic and Experimental Research into Hereditary Diseases of the Podocyte – Coordinator: Heidelberg University Hospital, Germany  
Information Centre for Rare Diseases and Orphan Drugs – ICRDOD, Bulgaria

**EDITORIAL COMMITTEE MEMBERSHIP**

Quaderni di Farmacoeconomia

**NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP**

Network for Rare Diseases – Lombardy Region (Delibera Regione Lombardia N°7328, 11/12/2001)  
Working group “Classification and coding of rare diseases” coordinated by Italian National Institute of Health  
Working group “National Registry of Rare Diseases” coordinated by Italian National Institute of Health  
Working group “Indipendent Research” (DG Sanità – Lombardy Region)  
Working group “Biobanks for diagnosis and research” (DG Sanità – Lombardy Region)  
Working group “Rare diseases and orphan drugs” (DG Sanità – Lombardy Region)
EVENT ORGANIZATION

Open Day: information, research and treatments
A journey dedicated to rare diseases
Ranica, (Bergamo) February 26, 2013

Meeting of Italian Society of Nephrology (SIN)
Ranica (Bergamo), June 25, 2013

CONFERENCE AND WORKSHOP CONTRIBUTIONS

16 ° Convegno Patologia Immune e Malattie Orfane 2013
Torino, January 17-19, 2013

Il Registro nazionale e i registri regionale ed interregionali delle malattie rare
Roma, February 25, 2013

Giornata Internazionale delle Malattie Rare
Cremona, February 28, 2013

Novità clinica e sperimentazione nella nefrologia moderna
Cipro, February 28 – march 04, 2013

Meeting Annuale GIPF/AIFA
Firenze, March 09, 2013

17th Convention Scientifica Telethon
Riva del Garda, March 11-13, 2013

Le malattie renali rare: dalla genetica ai percorsi digestivo-assistenaizli multidisciplinari
Taormina, April 05-06, 2013

Immunoglobuline endovenosa e sottocute nelle neuropatie immunomediante: quando e come usarle
Vimercate, April 12-13, 2013

Congresso Nazionale Associazione Italiana Osteogenesi Imperfetta (As.It.O.I): Osteogenesi
Imperfetta, il percorso diagnostico, terapeutico e assistenziale dal bambino all’adulto
Milano, May 02-03, 2013

Cusano Milanino città giardino e dei servizi: qualità della vita e disabilità:
Cusano Milanino, May 18, 2013

Corso avanzato sulla malattia di Fabry
Monza, May 27, 2013

Le malattie metaboliche rare dal bambino all’adulto
Pavia, June 01, 2013

ION Torrent User Meeting Italia
Bologna, June 05-06, 2013
4th International Conference HUS-MPGN-TTP & related diseases
Innsbruck, June 09-11, 2013

Convengo Malati rari in tempo di crisi
Pisa, June 15, 2013

La Rete delle malattie Rare
Milano, June 26, 2013

14th European Meeting on Complement in Human Diseases
Jena, August 17-21, 2013

Il complemento nella malattie Glomerulari: passato presente e futuro
Torino, September 05-07, 2013

1st Meeting on OMICS towards the systems biology approach in renal diseases and transplantation
Bari, September 12, 2013

47° Convegno di cardiologia 2013
Milano, September 24, 2013

54° congresso nazionale SIN
Firenze, September 25-29, 2013

Congresso di Nefrologia: Genetica e rene
Grado (GO), October 04/05, 2013

2nd International workshop: Rare disease and orphan drug registries
Roma, October 20/22, 2013

Festival della scienza: 20 anni di ricerca sulle malattie rare
Genova, October 29, 2013

Neurofibromatosi tipo I: il rischio oncologico
Milano, November 08-09, 2013

Corso Teorico pratico sulla biopsia renale
Viterbo, November 21-23, 2013

Ambasciata italiana a Washington: Incontro sulle malattie rare
Washington, December 14-16, 2013

GRANTS AND CONTRACTS

Fondazione Aiuti per la Ricerca sulle Malattie Rare - ARMR, Bergamo
Lombardy Region
Telethon Foundation
Health Local Unit (ASL), Bergamo

**SCIENTIFIC PUBLICATIONS (2013)**

Combined complement gene mutations in atypical hemolytic uremic syndrome influence clinical phenotype  
*J Am Soc Nephrol* 2013; 24: 475-486

A case of familial glomerulopathy with fibronectin deposits caused by the Y973C mutation in fibronectin  
Ertoy Baydar D, Kutlugun A A, Bresin E, Piras R  

Two patients with history of STEC-HUS, posttransplant recurrence and complement gene mutations  
*Am J Transplant* 2013; 13: 2201-2206

Post-transplant recurrence of atypical hemolytic uremic syndrome in a patient with thrombomodulin mutation  
Sinibaldi S, Guzzo I, Piras R, Bresin E, Emma F, Dello Strologo L  
*Pediatr Transplant* 2013; 17: E177-E181

Unbiased next generation sequencing analysis confirms the existence of autosomal dominant Alport syndrome in a relevant fraction of cases  
*Clin Genet* 2013; E-pub:

Frontiers in Liver Transplantation  
Monogenic diseases that can be cured by liver transplantation  
Fagiuoli S, Daina E, D'Antiga L, Colledan M, Remuzzi G  

Atypical haemolytic uraemic syndrome with underlying glomerulopathies. A case series and a review of the literature  
Vanenti L, Gnappi E, Vaglio A, Allegri L, Noris M, Bresin E, Pilato FP, Valoti E, Pasquali S and Buzio C  

Successful long-term outcome after renal transplantation in a patient with atypical haemolytic uremic syndrome with combined membrane cofactor protein CD46 and complement factor 1 mutations  
Pabst WL, Neuhaus TJ, Nef S, Bresin E, Zingg-Schenk A, Sparta G  

**OTHER PUBLICATIONS (2013)**

Farmaci orfani: cosa sono?  
Cardiologia 2013: Atti del 47° convegno Dipartimento cardiotoracovascolare “A. De Gasperis”, Milano  
Daina E

**RESEARCH ACTIVITIES**

Bank of biological samples and description of hereditary nephropathies
The aim of this project is to collect clinical data and biological samples from patients and their families with rare genetic conditions. A database with clinical data and a Bank for biological samples collection and preservation has been created.

The availability of clinical data and biological samples is useful to perform new biochemical and genetic tests within specific research projects aimed to better reveal the mechanisms of the diseases, their manifestation and therapeutic opportunities.

In particular, the attention is focused on rare genetic disorders of the kidney. A thorough clinical evaluation, including clinical data collection, medical physical examination, renal ultrasonography, laboratory tests of blood and urine is offered to patients, affected by hereditary nephropathies (Alport syndrome, Fabry disease, Familial Focal Glomerulosclerosis, Glomerulopathy with Fibronectin deposits, Membranoproliferative glomerulonephritis, Medullary Cystic Kidney disease, Cystinuria), who are addressing our Centre. After obtaining a written informed consent, biological samples from patients and their relatives are collected, labelled with specific codes to assure the anonymity and conserved in the Bank for biological samples. In case the responsible gene for a hereditary nephropathy is known (Alport syndrome, Fabry disease, Medullary Cystic Kidney disease, Cystinuria), the blood samples are redirected to the relevant Laboratory of reference. For other nephropathies, where the identification of the gene mutation is unknown or still in course, the blood samples are conserved with the aim to be used in specific future research projects.

International Registry of Recurrent and Familial Hemolytic Uremic Syndrome and Thrombotic Thrombocytopenic Purpura

Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP) are two closely related rare diseases characterized by microangiopathic hemolytic anemia and thrombocytopenia with signs of renal (most prevalent in HUS) and cerebral (most prevalent in TTP) damage. An even more rare subset of these diseases (called atypical forms, approximately 10% of all forms) are familial and are characterised by frequent relapses, leading to permanent renal and neurological sequelae. In these familial and recurrent forms of HUS and TTP, the attention is concentrated mainly on the genetic predisposition to the disease.

Since 1996 the Laboratory coordinates the ‘International Registry of Recurrent and Familial HUS/TTP’, with the following aims: to collect clinical data of patients and their relatives; to study genetic and biochemical abnormalities of HUS/TTP; to provide up-to-date information to physicians and families; to explore new therapeutic approaches.

Clinical and laboratory data of all patients referred to the Registry are collected by a dedicated Case Report Form. The family history and also the personal data of the unaffected relatives are also recorded, when possible. Biological samples are collected from all patients and available relatives, for the biochemical and genetic analyses. All participants receive detailed information on the purpose and design of the study and give their informed consent. Genetic counseling is also provided to patients and relatives, when appropriate.

Through this Registry, data of about 1042 patients referred from around 100 Italian and 80 European and extra-European Centres have been collected.

Many research projects are in collaboration with the Laboratory of Immunology and Genetic of Rare Diseases and Organ Transplantation and the Laboratory of Cellular Biology and Xenotransplantation – Kilometro Rosso (Mario Negri Institute, Bergamo). Molecular analyses have demonstrated that a genetic predisposition in complement regulatory factors (Factor H, Factor I, Membrane Cofactor Protein, Complement factor C3, Factor B, Thrombomodulin) accounts for the majority of atypical HUS and provided a detailed description of both known and new mutations and polymorphisms involved in sporadic and familial forms. These studies provided data showing that clinical phenotype, response to treatment, and long-term outcomes are predicted by individual gene abnormalities. They also supported the rationale for new therapies such as preemptive plasma exchange, combined liver–kidney transplantation and eculizumab treatment to efficiently prevent or treat disease recurrences of atypical HUS. The
maintenance of a centralised bank of biological samples ensures the availability of clinical material for new investigative approaches as they will be developed. Another particularly significant result is represented by the possibility to identify TTP patients at risk for recurrence and effectively provide pre-emptive rituximab treatment. Since 2012, the Registry participates also to EUReOmics, the European Consortium for High-Throughput Research in Rare Kidney Diseases.

Identification of new genes associated to Steroid-Resistant Nephrotic Syndrome
Steroid-Resistant Nephrotic Syndrome (SRNS) is an uncommon cause of chronic renal disease (consisting of massive proteinuria, hypoalbuminemia, edema, and hyperlipidemia) that affects 3–4 of every 100,000 children under the age of 16 and accounts for about 30% of primary forms of glomerulonephritis in adults. SRNS cases are thought to be caused by a primary defect in the glomerular filtration barrier, since do not respond to immunosuppressive therapy (the first-line treatment in NS). Patients with SRNS have an unfavourable prognosis and usually develop end stage renal disease (ESRD) within 10 years from the onset. Minimal Change Disease (MCD) accounts for around 90% of cases; the remaining ones are mostly diagnosed as Focal Segmental Glomerulosclerosis (FSGS), while rarely as Diffuse Mesangial Sclerosis (DMS). In some cases of SRNS, a familial form of IgA Nephropathy can also be recognized. In a subgroup of cases of SRNS, a rare form of glomerulopathy with fibronectin deposits (GFND) can also be diagnosed. Besides isolated SRNS, several syndromes characterized by SRNS with associated extra-renal manifestations have been described, as Denys-Drash syndrome, MYH9-related disorder, and Renal-Coloboma syndrome.

In literature, SRNS is defined as familial when at least two members of the same family are affected by the disease. Among familial forms of SRNS, both autosomal recessive and dominant inheritance patterns have been reported. The autosomal dominant form of SRNS is generally less severe and patients present at a later age (usually in adulthood) than with the autosomal recessive form. Genetic studies have shown that mutations in genes encoding proteins important for the podocyte homeostasis and function can cause SRNS (NPHS1, NPHS2, PLCE1, MYO1E, PTPRO, LAMB2, CD2AP, INF2, ACTN4, TRPC6, WT1, and ARHGAP24). Mutations in these genes are found in about 60% of childhood-onset patients and in about 20% of adolescent- or adult-onset patients. Since 2007 the Laboratory coordinates an International Registry dedicated to SRNS, with the following aims: to collect clinical data of patients and their relatives; to study genetic abnormalities of SRNS; to provide the best therapeutic approach for each patient. Clinical and laboratory data from patients with SRNS and available unaffected relatives are collected by a dedicated Case Report Form and re-evaluated by a multidisciplinary team with expertise in rare diseases. Biological samples are stored from all participants. All participants receive detailed information on the purpose and design of the study and give their informed consent. Genetic counselling is also provided to patients and relatives, when appropriate. Data and biological samples of 315 patients, referred from 15 Italian and 4 international Nephrology Units, have been collected. So far, the genetic cause of the disease has been identified in about 17% of the overall patients. The establishment of the SRNS Registry allows a better characterization of the patients. A great number of SRNS patients has been recruited increasing the potential to perform epidemiologic, genetic and clinical studies. The Registry participates also in PodoNet, a European research consortium funded under E-Rare first joint call for podocyte affecting diseases, that follows 1469 patients from 34 different countries.
Coordinating Center for rare Diseases - Lombardy Region
The Regional Network for Rare Diseases of Lombardy is currently made up of 35 Centres of reference, a Coordinating Centre and the 15 Local Health Units (called ASL) in the area. The Centres of reference are among those identified with documented experience in diagnostic or therapeutic activities for specific diseases or groups of rare diseases, as well as proper allocation of support structures and services (eg: genetic diagnosis process). The role of Coordination Centre was assigned to the Clinical Research Centre for Rare Diseases Aldo e Cele Daccò, Mario Negri Institute for Pharmacological Research (Ranica, BG). The regional network is also implemented by a “Working Group with functions of operational coordination, discussion and sharing of common strategies for rare diseases”. This Working Group is composed by representatives of the Lombardy Region, Coordinating Centre, Centres of reference, ASL, and Associations of patients.

Roles of the Coordinating Centre are: (1) Management of the Regional Register of rare diseases (italian acronym: ReLMar), in collaboration with the National Institute of Health register; (2) Coordination of the Centres of reference, in order to ensure early diagnosis and appropriate treatment, if any, through the adoption of specific agreed therapeutic protocols; (3) Advice and support to physicians of the National Health Service in order to rare diseases and the availability of appropriate treatment; (4) Collaboration and implementation of educational programmes for health care professionals and Associations; (5) Information to Associations of patients and the general public in order to rare diseases and the availability of drugs and treatments. The Coordinating Centre also provides for the exchange of information and documentation on rare diseases with the relevant national and international institutions, through collaboration with other centres with regional or inter-regional coordination function, relating to the National Network for Rare Diseases (National Institutes of Health, Rome); participation in the Working Group "Classification and codification of rare diseases" coordinated by the National Institute of Health (Rome); participation in the “Technical Working Group for the scientific analysis and processing of data from the National Register of Rare Diseases”.

Operators of the Regionale Coordinating Centre for Rare Diseases are part of the following technical study groups (called GAT) set up by the Lombardy Region: Independent Research GAT; Biobanks GAT for diagnosis and research.

The Register of Rare Diseases of Lombardy
The Register of Rare Diseases of Lombardy (italian acronym: ReLMar) has the objectives of providing information for health planning, monitoring and studying the epidemiology of Rare Diseases. The Register collect demographic, public assistance and clinical information. Data are collected by specialists of the Reference Centres of the Rare Diseases Network; at present the implementation of the Register is web-based using a specific software called “Sistema Malattie Rare”. The Coordinating Center is in charge of Register management which consists in analyzing data and sending “minimum dataset” (data required by the National Register of Rare Diseases) to Istituto Superiore di Sanità (Rome). Periodic Review Reports of ReLMaR has been implemented and are available at the Coordinating Centre web site (http://malattierare.marionegri.it).

These reports describe registered data by medical specialists of Regional Centres of Reference, which are validated by the operators of the Coordinating Center for Rare Diseases. On the bases of periodic report updated at 30 June 2013, it has been possible to define the prevalence of rare diseases monitored in Lombardy region as 489/100.000 inhabitants. The Register of Rare Diseases of Lombardy, represent a remarkable resource for the study of epidemiology and health planning for rare diseases. To note that in these evaluations it has not been considered the celiac diseases, because its prevalence is more than 5/10.000 inhabitants.
Complement abnormalities in primary Membranoproliferative glomerulonephritis

Primary membranoproliferative glomerulonephritis (MPGN) is a rare kidney disease characterized by nephrotic syndrome, primarily of children and young adults. The term MPGN refers to a pattern of glomerular injury with characteristic histopathologic findings. No specific cure exists and usually the outcome at long-term is poor. To date, there are insufficient epidemiological data on MPGN, then a primary aim of this project is to collect clinical information and biological samples from well-characterized patients to improve diagnostic process, treatment options and clinical research.

Causes of MPGN are unknown; prominent feature of MPGN is hyperactivation of the complement alternative pathway, associated with the presence of complement regulatory gene mutations and/or Nephritic Factor (C3NeF), leading to the formation of the membrane attack complex (MAC, C5b-9) on cell surfaces.

Identification of genetic or acquired defects that cause hyperactivation of complement alternative pathway in MPGN patients and to correlate the link between clinical spectrum, is another aim of this project.

High levels of the complement complex (sC5b-9) in plasma indicate massive formation of the terminal C5b-9 lytic complex. This marker of complement activation could be potentially used to identify patients who could benefit from treatment with Eculizumab, an anti-C5 humanized monoclonal antibody that prevents C5 cleavage and formation of the terminal C5b-9.

Since 2006, the Laboratory coordinates the 'Italian Registry of Primary Membranoproliferative Glomerulonephritis', with the following aims: to collect clinical data of patients with MPGN; to study the genetic and biochemical abnormalities of MPGN; to provide the best therapeutic approach for each patient.

Clinical and laboratory data from patients with MPGN are collected by a dedicated Case Report Form. The family history is also recorded and biological samples are stored from all patients to perform biochemical and genetic analyses. All participants receive detailed information on the purpose and design of the study and give their informed consent. Genetic counselling is also provided to patients and relatives, when appropriate.

Ongoing studies, in collaboration with the Laboratory of Immunology and Genetic of Rare Diseases and Organ Transplantation, are focused on functional consequences of complement genetic abnormalities and in searching for new gene mutations/variants that may be involved in the predisposition to MPGN. Candidate genes are those encoding proteins of the complement system, due to the evidence that MPGN is a disease of complement hyperactivation.

Increased levels of the terminal complement complex sC5b-9 have been also found in around 40% of the evaluated patients. This marker of complement activation should be potentially used to identify patients who could benefit from treatment with Eculizumab, a humanized monoclonal antibody that binds to complement factor C5 and inhibits the generation of the terminal lytic complement complex. In this regard, among patients with MPGN who have been recently treated with Eculizumab, a good clinical response was observed in two patients from the Registry who showed high levels of sC5b-9 before treatment and normalization thereafter.

Thus, preliminary findings support a pathogenic role for complement dysregulation in the pathogenesis of MPGN and suggest that Eculizumab could be an effective treatment in the subgroup of MPGN patients with hyperactivation of the terminal pathway of complement.

Since 2012, the Registry participates also to EURenOmics, the European Consortium for High-Throughput Research in Rare Kidney Diseases.

**EURenOmics - European Consortium for High-Throughput Research in Rare Kidney Diseases**

The Laboratory take part of EURenOmics, a Consortium will integrate several established consortia devoted to rare kidney diseases with eminent need and potential for diagnostic and
therapeutic progress (i.e. steroid resistant nephrotic syndrome, membranous nephropathy, tubulopathies, complement disorders such a haemolytic uraemic syndrome, and congenital kidney malformations). The Consortium has access to the largest clinical cohorts assembled to date (collectively >10,000 patients) with detailed phenotypic information and comprehensive biorepositories containing DNA, blood, urine, amniotic fluid and kidney tissue. The project aims to (1) identify the genetic and epigenetic causes and modifiers of disease and their molecular pathways; (2) define a novel mechanistic disease ontology beyond phenotypical or morphological description; (3) develop innovative technologies allowing rapid diagnostic testing; (4) discover and validate biomarkers of disease activity, prognosis and treatment responses; and (5) develop in vitro and in vivo disease models and apply high-throughput compound library screening. For these purposes the Consortium will integrate comprehensive data sets from next generation exome and whole-genome sequencing, ChiP-sequencing, tissue transcriptome and antigen/epitope profiling, and miRNome, proteome/peptidome, and metabolome screening in different body fluids within and across conventional diagnostic categories. These data will be combined in a systems biology approach with high-resolution clinical phenotyping and findings obtained with a large array of established and novel in vitro, ex vivo and in vivo disease models (‘functiomics’) to identify disease-associated genetic variants involved in monogenic or complex genetic transmission, disease-defining molecular signatures, and potential targets for therapeutic intervention. These efforts will converge in the development of innovative diagnostic tools and biomarkers and efficient screening strategies for novel therapeutic agents.
INTERNATIONAL RELATIONS OFFICE
OF RARE DISEASES

STAFF

Head

Arrigo SCHIEPPATI, M.D.
Arrigo Schieppati

Arrigo Schieppati got his degree in Medicine at the University of Milan in 1978 and the specialisation in Medical Nephrology in 1984 at the same University. He performed his training at the Mario Negri Bergamo Laboratories with Dr. Remuzzi, and completed it with stages at the laboratories of prof. Patrono (Catholic University in Rome), prof. John Gordon (Cambridge, GB), and at the Division of Renal Diseases - University of Colorado Medical School, directed by Dr. Schrier (Denver, USA). Since 1982 he works at the Division of Nephrology and Dialysis – Papa Giovanni XXIII Hospital (previous denomination: Riuniti Hospital) – Bergamo, where he is in charge of Outpatients Clinic and Day Hospital.

1991-1995: Chief, Information Center for Rare Diseases
1996-2008: Chief, Laboratory for Coordination of Information and Diagnosis of Rare Diseases
2009 to date: Chief, International Relations Office of Rare Diseases.

Areas of interest: diagnosis and therapy of chronic renal diseases, hypertension and rare kidney diseases.

Affiliations: ethical committee Papa Giovanni XXIII Hospital (previous denomination: Riuniti Hospital) - Bergamo; member of the working group of the regional network for rare diseases in Lombardy; scientific committee Bolognini Hospital – Seriate (BG); member of the Task Force on Rare Diseases (DG Health and Consumer Protection); International Society of Nephrology; American Society of Nephrology; Editorial Board Journal of Nephrology.

Principali pubblicazioni


INTRODUCTION TO THE LABORATORY’S ACTIVITIES

The Mario Negri Institute has focused its attention on rare diseases since 1992, with a dedicated site at Ranica (Bergamo), and increasing research projects for a specific condition or groups of rare diseases within different departments inside the Institute. Rare diseases include illnesses very heterogeneous, involving virtually all areas of medical interest; the peculiarities of the activities implemented is right in bringing together different skills, with the aim to help patients and families, to promote the availability of information and the knowledge advancement in this field. Every research activity takes place through multidisciplinary projects ranging from basic research to the epidemiological, clinical research, with a strong commitment in the dissemination of scientific information, training and educational programmes for professionals and citizens.

At the international level, the commitment of the Institute in the field of rare diseases and orphan drugs has remained constant over the years, as evidenced by the organization of high level meetings, starting with the first "International Symposium on rare diseases and orphan..."
drugs" (Lancet 1994 343:8912 , 1560-1561), from participation in numerous projects on rare
diseases, the recognition in 2000 as the "Postgraduate training on rare diseases" by the European
Commission (Contract No. QLK4 - 1999-50547), the participation in first Rare Disease Task
Force working group.
The International Relations Office of Rare Diseases was created as an evolution of the previous
laboratory dedicated to information on rare diseases and represents the needs to the international
dimension, especially in Europe, of our initiatives on rare diseases.

**NATIONAL COLLABORATIONS**

Italian National Institute of Health
Assessorato alla Sanità, Lombardy Region
UNIAMO - Rare Diseases Italian Federation
Papa Giovanni XXIII Hospital (previous denomination: Riuniti Hospital), Bergamo
“BergamoScienza” Association

**INTERNATIONAL COLLABORATIONS**

ICORD Society - International Conference on Rare Diseases and Orphan Drugs
EURORDIS Rare Diseases Europe, non-governmental patient-driven alliance
ECRIN - European Clinical Research Infrastructures Network
ICRDOD - Information Centre for Rare Diseases and Orphan Drugs, Bulgaria

**EDITORIAL COMMITTEE MEMBERSHIP**

Journal of Nephrology

**NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP**

External Advisory Board E-Rare (ERA-Net for research programs on rare diseases).
Network for Rare Diseases – Lombardy Region (Delibera Regione Lombardia N°7328,
11/12/2001)
Scientific Committee A.O. Bolognini di Seriate
Ethical Committee, A.O. Ospedali Riuniti di Bergamo

**EVENT ORGANIZATION**

Open Day: information, research and treatments
A journey dedicated to rare diseases
Ranica, (Bergamo) February 26, 2013

Meeting of Italian Society of Nephrology
Ranica (Bergamo), Jun 25, 2013
PARTICIPATION IN EVENTS IN WHICH THE LABORATORY WAS INVOLVED

Workshop ECRIN
Milano, February 06-07, 2013

Final Meeting of the BURQOL_RD
Santa Cruz, Tenerife, September 26-27, 2013

GRANTS AND CONTRACTS

European Commission (DG SANCO)

SELECTION OF SCIENTIFIC PUBLICATIONS FROM 2013

Immunosuppression for membranous nephropathy: A systematic review and meta-analysis of 36 clinical trials

Kidney failure: aims for the next 10 years and barriers to success
Lancet 2013; 382: 353-362

RESEARCH ACTIVITIES

European Clinical Research Infrastructures Network – ECRIN
The European Clinical Research Infrastructures Network (ECRIN) is a sustainable, not-for-profit infrastructure supporting multinational clinical research projects in Europe. ECRIN provides information, consulting and services to investigators and sponsors in the preparation and in the conduct of multinational clinical studies, for any category of clinical research and in any disease area. The activity is particularly significant in supporting academic initiatives or small and medium enterprise-sponsored clinical trials, and for clinical research on rare diseases where international cooperation is a key success factor.
ECRIN is based on the connection of coordinating centres for national networks of clinical research centres and clinical trials units, able to provide support and services to multinational clinical research.
Among the areas in which ECRIN is involving at European level we can mention: research and innovation, with particular attention to the biotechnologies and pharmaceuticals; development of new models of health care; rare diseases; translational research; appropriateness of treatment; patient safety; health care costs; promotion of evidence-based medicine and prevention.
The project aims to reduce health inequalities in Europe and to ensure the sustainability of health systems, taking into account national contexts and population characteristics.
More information are available at http://www.ecrin.org/

Social economic burden and health-related quality of life in patients with rare diseases in Europe - BURQOL-RD
BURQOL-RD is a 3 year project under the 2nd Programme of Community Action in the Field of Public Health, that commenced in April 2010 and is promoted by the DG Sanco. The main aim of BURQOL-RD is to generate a model to quantify the socio-economic costs and Health Related Quality of Life (HRQOL), of both patients and caregivers, for up to 10 rare diseases in different European countries. This model will be adaptable and sufficiently sensitive to capture the differences in the distinct Health and Social Care Systems in the EU Member States. The information generated by the BURQOL-RD consortium will help to: design future policies in the area of rare diseases, which will ultimately have positive benefits for EU citizens health, both that of patients and of their caregivers; readily transfer the protocols established to other RD and to other countries; compare the availability and access to specific health resources for specific RD in each country; explore the potential relationships between HRQOL and access to healthcare resources.

After a thorough selection process, a set of 10 rare diseases to be targeted was decided: cystic fibrosis, Prader-Willi syndrome, haemophilia, Duchenne muscular dystrophy, epidermolysis bullosa, fragile X syndrome, scleroderma, mucopolysaccharidosis, juvenile idiopathic arthritis, histiocytosis.

The questionnaires have been implemented by FUNCIS and translated for the Italian reality by the Italian partners of the BURQOL project (Mario Negri Institute, Italian National Institute of Health, Bocconi University).

For each of these diseases has been developed a questionnaire designed to be administered to the patients and/or their caregivers to assess the economic and social burden on patients suffering from a rare disease and their families. The questionnaires were developed by FUNCIS, and translated and adapted to the Italian situation by Italian partners of the project (Mario Negri Institute, National Institute of Health and Bocconi University). The survey was promoted in collaboration with associations of patients and was completed in the current year. Data processing is ongoing.

The Transplant Research Center

Chiara Cucchi De Alessandri e
Gilberto Crespi

The Transplant Research Center (CRT) was set up in 2002 to support and promote the work of outstanding research scientists throughout the world and to carry out major organ transplant research programs.

The Center is housed in the Villa Camozzi, at Ranica, under the same roof as the Mario Negri Institute in Bergamo and is managed in collaboration with the Institute.

The Center’s staff is mainly made up of senior and junior researchers that were trained in the laboratories of the Mario Negri Institute in Bergamo, focusing on transplant immunology, research for less toxic immunosuppressant drugs, and new gene therapy techniques to prevent acute rejection of transplanted organs.

Information on the Center’s activities can be found in the sections addressed to the Department of Molecular Medicine (Laboratory of Immunology and Genetics of Organ Transplantation and Rare Diseases) and the Department of Renal Medicine (Laboratory of Pharmacokinetics and Clinical Chemistry).
EDUCATIONAL ACTIVITIES

Dean, Educational Activities – Dr. Enrico Garattini

The Mario Negri Institute holds a well established expertise in educational training of young post-degree students in biomedicine, that, since 1963, when the Institute started its activities, amount to more than 6000. Excellence of the educational courses is confirmed by the fact that Mario Negri Institute diplomas are widely considered a guarantee of an excellent theoretical and practical training, and students who earn their specialization title at the Mario Negri can easily find positions in academic and industrial research laboratories both in Italy and abroad.

The Pharmacological Research Specialists, Recognized by Lombardy Region, was the first educational program of the Institute.

In 2009 the Lombardy Region started reviewing its occupational training courses and established an ad hoc register for “Regional Occupational Standards” (Quadro Regionale degli Standard Professionali - QRSP), This lists all the training courses and the standards reached by pupils. The Lombardy Region Decree No. 14355 dated 22/12/2009 approved the new occupational profile known as Biomedical Research Specialists (formerly Pharmacological Research Specialists) presented by the Mario Negri Institute. The Biomedical Research Specialists will receive diplomas issued officially by the Lombardy Region and the Mario Negri Institute for Pharmacological Research. These have legal value throughout Italy, and are recognised in competitions for public posts, where they are worth a certain number of points.

In the last fifteen years new post-degree courses have been introduced. In particular, in 1996 the International Graduate Program has been introduced in the three Institute locations (Milano, Bergamo, Ranica (BG)), organized in collaboration with the Open University, UK (Milton Keynes, UK). More recently, in the Bergamo campuses, a collaboration with the university of Groningen and Maastricht (The Netherlands) have been started. These courses confer a PhD title recognized worldwide. The Research Degree Coordinator acts as the local contact person and is responsible for all communications with the foreign universities, coordinates and supervises the teaching, training, financial and administrative activities of the School. According consolidated European procedures, the viva defense of the thesis is done in English language, with external examiners non involved in the student projects. These procedures further confer excellence to the Institute educational activities. The Open University PhD degree earned at the Institute has legal value throughout Europe and in the USA.

Since January 2009 the Institute started a two-year Advanced School in Applied Pharmacology (SAFA). The course provides advanced teaching and practical experience with experimental work in the laboratories. The SAFA course is aimed at preparing young researchers and enabling them to specialize and work for the pharmaceutical industry, for other research institutes and for public institutions.

In January 2009, the Institute started running courses for Pharmacological Research Doctorates,
recognised by the Ministry for the University and Research with Ministerial Decree dated 11 November 2008.

The main feature of these courses is that students receive their training "on site". They work full-time in research programs of a high scientific standard, using advanced equipment and learning the latest methods, in regular contact with colleagues in different countries. Besides its scientific value, this approach provides an excellent preparation on the human and personal scale.

Students are usually assigned to one of the Institute’s laboratories, where they gradually gain specialized skills by working on specific research projects. They are expected to attend lessons, seminars, courses and congresses and learn to make full use of the Institute’s well-stocked library. Students all have access to the internet and to biomedical databases and can print out articles they need to consult, from major international journals. Should the opportunity arise, students are expected to be available for trips abroad, to participate in conferences or courses.

Students enrolled in formal courses are assigned study grants. **Between 1963 and 2011 the Mario Negri Institute awarded 7,653 grants,** 793 of them to foreign researchers who came to the Institute for special training. Everything possible is done to help students find work once they finish the course.

At the moment the following courses are available:

- Three-year course for graduates, in Milan or Bergamo, leading to a diploma as **Biomedical Research Specialist.**
- Three-year course for diploma-holders, in Milan or Bergamo, leading to a diploma as **Biochemical Research Technician.**
- Research doctorates (PhD), run under an agreement with the Open University (UK) and the Universities of Maastricht and Groningen (NL).
- Two-year **Advanced School in Applied Pharmacology (SAFA)** for graduate students, in Milan and Bergamo.
- Three-year course for **Pharmacological Research Doctorates**, in Milan or Bergamo, recognised by the Ministry for the University and Research.
- First Level Master in Clinical Research, in collaboration with the University of Milan.
- Second Level Master Course in Rare Diseases, in collaboration with the University of Turin.

Other training opportunities

**PREPARING A DEGREE THESIS**

Students can prepare their thesis in scientific subjects at the Institute, with the approval of their university faculty. These students must work at the Institute for at least two years.

**SUMMER STUDENTS**
In June and July each year the Institute accepts a certain number of students in their last two years at high school, to give them experience as part of school/work programs.

Since 2003 the Institute’s training schemes have been certified according to UNI EN ISO 9001:2008 requirements for the “Design and provision of specialized training courses in biological and medical fields”
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Prof. Silvio Garattini

Silvio Garattini was born in Bergamo (Italy) on 12 November 1928. He earned a diploma in chemistry, then a degree in medicine, and was appointed lecturer in chemotherapy and pharmacology. He held the post of Assistant then Deputy Professor at the Milan University Institute of Pharmacology until 1962. In 1961 he founded the Mario Negri Institute for Pharmacological Research, and was nominated by Mario Negri as its director, the post he has held since it opened in 1963. The Institute now has four locations - Milan, Bergamo, Ranica (Bg), St. Maria Imbaro (Ch) - and more than 950 people work there. Professor Garattini is a member of the Gruppo 2003 (a group of the most cited Italian scientists in international scientific literature) and has published hundreds of articles in Italian and English in international scientific journals, and texts on pharmacology. He was a founder of the European Organisation for Research and Treatment of Cancer (EORTC).

Over the last decades Professor Garattini has acted in various organizations, including the Italian National Research Council (CNR) - Committee on Biology and Medicine; the National Health Council, the Committee for Italian Research Policy, set up by the Presidency of the Council of Ministers; the Ministry of Health CommissioneUnica del Farmaco (CUF). He has held the following posts: President of the UICC Committee on Antitumoral Chemotherapy, President of the European Organisation for Research and Treatment of Cancer (EORTC), consultant to the World Health Organisation (WHO), member of the Board of the Italian IstitutoSuperiore di Sanità (ISS), President of the European Society of Biochemical Pharmacology, member of the Committee for Proprietary Medicinal Products (CPMP) of the European Agency for the Evaluation of Medicinal Products (EMA), the Committee of Experts for Research Policy (CEPR) at the Italian Ministry for University and Scientific and Technological Research, and the Scientific Committee of the LegaItaliana per la LottaContro i Tumori (LILT); Vice-president of the ConsiglioSuperiore di Sanità (CSS), Chairman of the Commission for Research and Development of the AgenziaItaliana del Farmaco (AIFA), President of the Technical Commission for Pharmaceutical Assistance of the Sardinian RegioneAutonoma; member of the Lombardy Region Strategic Committee for Welfare, of the AISLA Scientific Committee, the International Scientific Committee of the Centro di RiferimentoOncologicoAviano, the Scientific Committee of the Council of Ministers Presidency Antidrug Policy Department, the Advisory Board, ADAMO Onlus, Milan, and the Committee of the “Recommendation for the Call”, Wemos Foundation, Amsterdam.

He currently holds other important posts, such as President of the Angelo and Angela Valenti Foundation, of the "Via di Natale" Association, Honorary President of the AuxiliaOnlus, permanent member of the CSS, member of the National Bioethics Committee, and of the Sicilian Regional Bioethics Committee, President of the Scientific Committee of the Italian National Center for Disease Prevention and Control (CCM).

Professor Garattini has received numerous awards for his work, including the French Legion d'Honneur for scientific merit, the Medal of the SocietàItaliana di Chimica “Giulio Natta”, the Grand Ufficiale della Repubblica Italiana, honorary degrees from the Universities of Białystok in Poland and Barcelona in Spain and in Milan, and the Medaglia d’Oro al MeritodellaSanitàPubblica of the Italian Ministry of Health.

Other awards include the HippocratesPrize, Mens Sana in Corpore Sano, Nuova Spoleto, Angelo dell’Anno, Alkmene International Prize, Sant’Agostino Città di Bergamo International Prize, Il Campione della Scienza, Coppola Prize, Scienza e Società in the framework of the Premio Città di Firenze, Premio Rana d’Oro, Premio Barocco Città di Lecce, Premio Nazionale TV L’Altra Italia, Premio Chirone, Premio “Testimonial” LILT.

Silvio Garattini is a Fellow of the New York Academy of Sciences, the American Association for the Advancement of Science, Honorary Fellow of the Royal College of Physicians (Pharmaceutical Medicine), London, Honorary Fellow of the Italian Society of Pharmacology and a member of many other Italian and international scientific societies.
In over 50 years, the Mario Negri Institute for Pharmacological Research, under Professor Garattini’s leadership, has published more than 12,000 scientific papers and more than 250 books, on topics ranging from cancer and its treatment to tumor immunology, neuro-psycho-pharmacology, cardiovascular, renal pharmacology and rare diseases. More than 7000 young researchers and technicians have qualified as specialists at the Institute.
Prof. Giuseppe Remuzzi

Prof. Giuseppe Remuzzi was born in Bergamo, Italy in 1949. Upon completion of his medical training at the University of Pavia in 1974, he received specialty training in Hematology and Nephrology at the University of Milan. Since 1975, he has pursued his academic career at the Papa Giovanni XXIII hospital in Bergamo (previous Ospedali Riuniti di Bergamo), where he was appointed Professor of Nephrology and Director of both the Department of Immunology and Clinical Transplantation (1996) and the Department of Medicine in 2011. Since 1999, he is Director of the Division of Nephrology and Dialysis of the same hospital. Since 1984 he also coordinates the Negri Bergamo Laboratories of the “Mario Negri” Institute for Pharmacological Research and the affiliated Clinical Research Center for Rare Diseases “Aldo e Cele Daccò” in Ranica (BG), a group of basic scientists, physiologists, pharmacologists, molecular and cellular biologists, pathologists and clinicians devoted to the study of human renal diseases and their corresponding animal models from the perspective of pathophysiology and therapeutic intervention. He touched major advances in many areas of nephrology. For example, his studies have led to new insights into many disorders, including the interactions between platelets and endothelium, pathophysiology of glomerular diseases and the factors that influence the progressive loss of kidney function. Work focused on improving the outlook for patients with end stage renal disease. Giuseppe Remuzzi pays tribute to the work of pioneers such as Barry Brenner, who delved deep into the processes behind glomerular function and their possible reversibility. Early work on the use of angiotensin-converting enzyme inhibitors to slow the decline of glomerular filtration rates proved dialysis was avoidable, not inevitable. Studies on immunologic mechanisms that influence the survival of transplanted organs, understanding of immunologic tolerance in the disorders that are linked to autoimmunity and finally, genetic diseases of the kidney have also been areas of investigation. Concerned by kidney donation shortages and deploring the current practice of discarding suboptimal donor kidneys, his team has shown that transplanting such kidneys in pairs is feasible and have set up an international effort to validate this approach. Giuseppe Remuzzi is investigating the kidney's ability to regenerate itself.

Prof. Remuzzi serves on editorial boards of numerous journals and is member of the International Advisory Board of The Lancet. He served as Editorial Board member of the New England Journal of Medicine from 1998-2013. In recognition of his achievements, he has been awarded in 1998 honorary memberships of the Association of American Physicians and the British Royal College of Physicians. In 2001 he was nominated Chairman of the Research and Prevention Committee of COMGAN (Commission on Global Advancement of Nephrology) of the International Society of Nephrology (ISN). In 2005 during the World Congress of Nephrology in Singapore he received the ISN Jean Hamburger Award. In 2006 he was invited by the Italian Health Minister to become member of the Commission: “Consiglio Superiore di Sanità”. In 2007 he received during the annual American Society of Nephrology Congress in San Francisco the prestigious ASN John P. Peters Award and in 2011 he was awarded with the ISN AMGEN Award (WCN 2011, Vancouver). In November 2011, he received the Third Edition of the International Award "Luis Hernandez" assigned by the Iñigo Alvarez de Toledo Renal Foundation (FRIAT) in Madrid, Spain. He is, since June 2013, President of the International Society of Nephrology (ISN).

Prof. Remuzzi has authored and co-authored more than 1220 scientific articles, reviews and monographs and regularly collaborate through the preparation of scientific articles with the Italian national newspaper “Corriere della Sera”.

Prof. Giuseppe Remuzzi
Mario Negri Institute Milan

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