PREFACE

ANNUAL REPORT

MARIO NEGRI INSTITUTE, MILAN

www.marionegri.it

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PREFACE

In 2014 the Mario Negri Institute for Pharmacological Research celebrated 53 years since its foundation. The date marked the first year of the Institute as IRCCS. In fact 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 420 articles were accepted in international scientific journals in 2014.

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 2014 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to watch the Italian Parliament display its ‘disdain’ for science with laws in which that researchers’ opinions had been replaced by pseudo-arguments put forward by charlatans.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. We had many delays in the authorization for the use of animals in experimentation.

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 the Health care Commission did some serious self-criticism as the 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, using quantitative morphology techniques, together with genomics, proteomics and metabolomics, cell biology, molecular biochemistry. 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 500 people aged over 80, investigated in the Monzino 80+ 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. Particularly, we obtained interesting results about the use of new drugs, among which Ketamine.

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 used for studying cerebral and peripheral amyloidosis, using the worm C. elegans. We found interesting analogies with the behaviour of mammals. Promising results have already been achieved with the use of nanoparticles to improve drug entry into tumors, and to pass the blood/brain barrier. Particularly interesting are some nanoparticles that convey paclitaxel towards the cancer cells.

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. The results are particularly interesting and encouraging, because of the possibility to have in the future a kidney that will not be rejected. 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 of the controlled clinical trials conducted at the Mario Negri Institute is available, 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).

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 (964 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 90 students have passed); there is also a research doctorate recognized by the Ministry of University and Research in Italy (15 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 2014, a total of 1611 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
Director
Mario Negri Institute for Pharmacological Research Milan

Annual Report 2014
Departments and Laboratories
DEPARTMENT OF ONCOLOGY

STAFF

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Maurizio D’INCALCI, M.D.

Oncological Studies Office and Documentation
Scientific Documentalist
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Laboratory of Cancer Pharmacology
Head
Maurizio D’INCALCI, M.D.

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Head
Paolo UBEZIO, Phys.D.

Flow Citometry Unit
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Eugenio ERBA, Biochem.D

Translational Genomic Unit
Head
Sergio MARCHINI, Biol.Sci.D., Ph.D

Cancer Clinical Pharmacology Unit
Head
Massimo ZUCCHETTI, Chem.Pharm.D.

Preclinical Experimental Therapeutics Unit
Head
Roberta FRAPOLLI, Chem.Pharm.D.

Laboratory of Molecular Pharmacology
Head
Massimo BROGGINI, Ph.D.

Molecular Genetics Unit
Head
Mirko MARABESE, Biol.Sci.D., Ph.D.

DNA Repair Unit
Head
Giovanna DAMIA, M.D.

Laboratory of Biology and Treatment of Metastases
Head
Raffaella GIAVAZZI, Biol.Sci.D., Ph.D.

Tumor Angiogenesis Unit
Head
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Head Maria Rosa BANI, Biol.Sci.D., Ph.D.

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Laboratory of Cancer Cachexia AIRC Start-Up
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Head Roldano FOSSATI, M.D.

Pain and Palliative care Research Unit
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 Candilo. From 2014 he is member of the Board of Directors of the Controlled Release Society (CRS) Italy Chapter, as representant of the medical-biology area. From September 2014 he is member of the IFOM Ethics Committee for Biomedical Research. 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 490 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.
Since 2014 she is Associate Editor of Endocrine. Since 2013 she is chairman of Ethics Committee of Lecco Como e Sondrio. Since 2011 he is on the editorial board of World Journal of Methodology. From 2010 to 2013 she was member of Ethics Committee of Azienda ospedaliera della Valtellina e Valchiavenna. From 2004 to 2013 she was member of Ethics Committee of Azienda Ospedaliera Sant’Anna of Como (from 2010 to 2013 as Chairman). From 2002 to 2013 she was member of Ethics Committee of Azienda Ospedaliera San Paolo of Milan. From 1999 to 2006 she was member of Ethics Committee of Istituto Scientifico Eugenio Meda. Since 1998 she is member of Ethics Committee of Fondazione IRCCS Carlo Besta Neurological Institute (from 2002 to 2013 as vice-president).

Luca Clivio has a Master degree in Informatics Engineering and a Post degree Master in Evidence Based Medicine and Methodology of Sanitary Research. Senior developer in the fields of Clinical Research distributed databases and Bioinformatics. he is the developer of an open source software for handling eCRF for Clinical Trials and Biobanks and has realized with that about 150 eCRF in various fields. Currently all of the Clinical Trials and Biobanks in the Department of Oncology at Mario Negri Institute make use of his software covering the data collection and validation. He has been working on the implementation of High Performance Computer centres in collaboration with the University of Aberdeen, Maastricht and the INRIA in Rennes. He is currently responsible for the Cluster Room at the Mario Negri Institute in Milan.

His collaborations range between the Department of Oncology, Cardiovascular, Neurosciences for managing Clinical Trials and Biobanks.

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).

Since 1998 she is member of Ethics Committee of Fondazione IRCCS Carlo Besta Neurological Institute (from 2002 to 2013 as vice-president). From 1999 to 2006 she was member of Ethics Committee of Istituto Scientifcico Eugenio Meda. From 2002 to 2013 she was member of Ethics Committee of Azienda Ospedaliera San Paolo of Milan. From 2004 to 2013 she was member of Ethics Committee of Azienda Ospedaliera Sant’Anna of Como (from 2010 to 2013 as Chairman). From 2010 to 2013 she was member of Ethics Committee of Azienda ospedaliera della Valtellina e Valchiavenna. Since 2011 he is on the editorial board of World Journal of Methodology. Since 2013 she is chairwoman of Ethics Committee of Lecco Como e Sondrio. Since 2014 she is Associate Editor of Endocrine.
Since 2011 he is on the editorial board of World Journal of Methodology and since 2014 of Endocrine. The main areas of interest include statistical and methodological aspects of clinical research, with focus on randomized clinical trials in oncology; systematic reviews of medical literature and methodological aspects of diagnostic test evaluation. She is author of approximately 100 papers published in peer reviewed international journals.

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 member of the Board of Directors (CdA) at the University of Trento.


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 Junico 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. 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**


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 summa laude, University of Milano; 1988 Post-Doctoral Degree in Pharmacological Research, Mario Negri Institute, Milano; 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 Milano; 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 the 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 co-author of 38 peer reviewed publications, 2 book chapters and 72 abstracts of which 17 selected for oral presentations at international meetings.

Selected publications


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 Cochran 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 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 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 Lombarďa) 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 AIFF 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


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 postdoctoral 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 to1995 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 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 Prassi, Institute for Research on teta, 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 of 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

- Pérez B, Pérez A, Llerena M, Rodríguez OM, Álvarez S. Aseguramiento de la calidad en el sitio de ejecución de los ensayos clínicos coordinados por el CENCEC. Revista Cubana de Farmacia 2003;37(Suplemento Especial).

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 strog collaboration with clinician in orpsitals, 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 "MIGLIORE 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 and 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
- Wiring miRNAs to pathways: a topological approach to integrate miRNA and mRNA expression profiles. Calura, Enrica; Martini, Paolo; Sales, Gabriele; Beltrame, Luca; Chiorino, Giovanna; D’Incalci, Maurizio; Marchini, Sergio; Romualdi, Chiara. NAR doi: 10.1038/nar.2014.354 First published online: May 6, 2014

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, data management and local monitoring 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

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, development of predictive models in Oncology, 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, for preclinical and translational research

Selected Publications
- Califano R, Karamouzis MV, Banerjee S, de Azambuja E, Guarnieri V, Hutka M, Jordan K, Kompisioras K, Martinelli E, Corral J, Postel-Vinay S, Preusser M, Porcu L, Torri V Use of adjuvant chemotherapy (CT) and adjuvant therapy (RT) in incompletely resected (R1) early stage Non-Small Cell Lung Cancer (NSCLC): a European survey conducted by the European Society for Medical Oncology (ESMO) young oncologists committee. Lung Cancer. 2014 Jul;85(1):74-80
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 preclinal studies of antiangiogenic and vascular disrupting compounds, including tubulin-targeting agents. She is author of more than 80 articles published in peer reviewed international journals (h index 43). 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

Eliana Ruli 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.

Selected publications

Dr. Zucchetti is the author of more than 110 papers on pre-clinical and clinical cancer pharmacology published in peer-reviewed international journals.

Pharmacological Research Specialist at the Mario Negri Institute for Pharmacological Research in 1986.

Selected publications

- Since 1991, he is Head of the Unit of Biophysics at the Mario Negri Institute.

Dr. Zucchetti is the author of more than 110 papers on pre-clinical and clinical cancer pharmacology published in peer-reviewed international journals.

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 the author of more than 110 papers on pre-clinical and clinical cancer pharmacology published in peer-reviewed international journals.

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) Mathematical modelling to support interpretation of data at different experimental levels of the pharmacological research: receptor binding, cellular drug uptake, drug resistance, dose-response in vitro, drug resistance, pharmacokinetics, biodistribution, tumor expansion in vivo. ii) Studies of tumor proliferation during/after treatments using in silico models based on the cell cycle; iii) Development of new methods and data analysis tools in flow cytometry and in time-lapse imaging of living cells; iv) Integration of experimental and computational methods to analyse separately cytostatic and cytotoxic effects of anticancer drug and ionizing radiations, towards optimization of the schemes of single and combined anticancer treatment; v) Cellular uptake of nanoparticles loaded with anticancer drugs.

Since 1991 he 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
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- Pharmacokinetic and pharmacodynamic studies in humans in GCP and GLP conditions
- Pharmacokinetic, toxicokinetic and metabolic studies in animals
- Pharmacokinetic drug interaction

Dr. Zucchetti is the author of more than 110 papers on pre-clinical and clinical cancer pharmacology published in peer-reviewed international journals.

Selected publications

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.

In frame with the development of new technologies for generating and integrating genomic data, in February 2014 an omic core facility was established within the activity of the Traslational genomic Unit and with informatic support of the Life Science Informatic.

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.

miR-181a, acts on the TGFB pathway by increasing the activity of the TGBreceptor and drive tumor cell transformation from an epithelial to amesenchymal phenotype. At primary surgery, differences in the expression levels of miR-181a have been associated in multivariate analysis to overall and Porgresison free survival.

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 patient swith 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 toEGFR 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.

In myxoid liposarcoma, trabectedin inhibits angiogenesis by stimulating the production of endogenous inhibitors by endothelial and tumor cells, in association with the adipocytic differentiation of tumors.

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 pivotal role in orchestrating tumor-endothelial cell motility.
A new antiangiogenic domain of thrombospondin-1 (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.

Thrombospondin-1 has pleiotropic activities in tumor progression, depending on the tumor type. In cutaneous melanoma, thrombospondin-1 cooperates with other pro-invasive genes in promoting tumor cell motility and metastasis.

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

The response of EOC-Xenografts to bevacizumab (an antibody anti-VEGF used for the treatment of ovarian cancer) is heterogeneous: the final outcome depends upon the treatment schedule and the combination with the standard-of-care chemotherapy.

The “normalization” of tumor tissue architecture after the angiogenesis inhibitor bevacizumab improves the delivery and distribution of paclitaxel in the tumor, resulting in greater antitumor activity of the combination.

Cediranib, a new tyrosine kinase inhibitor with antiangiogenic proprieties, affects tumor progression and metastases and increases survival of mice bearing ovarian cancer patient derived xenografts (EOC-PDX) with different response to cisplatin.

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

Tyrosine kinase angiogenesis inhibitors, such as sunitinib and sorafenib, prevent tumor cachexia and prolong survival of mice bearing renal carcinoma, by interfering with the activation of STAT-3 and MuRF-1 in the muscle.

Five stroma/extracellular matrix related molecules were identified as potential early biomarkers in plasma of two independent cohorts of pancreatic ductal adenocarcinoma (PDAC) patients. These biomarkers were further validated using genetically modified mouse models carrying the oncogenic hits for human PDAC and patient derived PDAC xenografts.

Preclinical studies have shown that bevacizumab combined with chemotherapy not only affects ovarian carcinoma progression, but when administrated as maintenance regimen significantly prolonged mouse survival, reducing ascites and tumor dissemination.

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 vargatef 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 or 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).

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. We have recently found that anti-angiogenic drugs, such as Sorafenib and Sunitinib, may prevent and even revert cancer cachexia in renal tumor-bearing mice, greatly extending their life-span. This therapeutic effect was independent of anti-tumoral activity and implied attenuation of catabolic pathways in muscle involving STAT3 an MuRF-1.

**NATIONAL COLLABORATIONS**

Agenzia Italiana del Farmaco (AIFA), Roma  
Alleanza Contro il Tumore Ovarico (ACTO), Milano  
Associazione Italiana di Ematologia Pediatrica (AIEOP)  
Associazione Italiana per lo Studio del Glaucoma (AISG), Torino  
Associazione Italiana di Oncologia Medica (AIOM)  
Associazione Volontari Assistenza Pazienti Oncologici (AVAPO)  
Azienda Ospedaliera “Guido Salvini”, Ospedale “di circolo” Rho, (MI)  
Azienda Ospedaliera di Reggio Emilia Arcispedale S. Maria Nuova  
Azienda Ospedaliera San Gerardo, Università Milano-Bicocca, Monza  
Azienda Ospedaliera Naz. SS. Antonio e Biagio, Alessandria  
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 Humanitas per la Ricerca - ROZZANO (MI)
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
Fondo Edo Tempia, Laboratorio di Bioinformatica e Farmacogenomica, Biella
Gruppo Italiano Sarcomi
I.R.C.C.S. Istituto Ortopedico Galeazzi (Cell and Tissue Engineering Laboratory), Milano
Istituti Ospitalieri di Cremona
Istituto Clinico Humanitas, Rozzano MI
Istituto Europeo di Oncologia (IEO), Milano
Istituto di Chimica del Riconoscimento Molecolare, CNR, Milano
Istituto di Genetica Molecolare CNR, Pavia
Istituti Ortopedici Rizzoli, Bologna
Istituti Ospitalieri di Cremona
Istituto Nazionale Tumori Fondazione G. Pascale, Napoli
Istituto Oncologico Veneto - IRCCS
Istituto Regina Elena, Roma
Istituto per lo Studio delle Macromolecole, CNR, Milano
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 S. Maria, Terni
Ospedale Papa Giovanni XXIII, Bergamo
Politecnico di Milano
Rete Oncologica Lombarda (ROL), Milano
Rete Tumori Rari
Spedali Civili di Brescia
Università Cattolica del Sacro Cuore, Roma
Università degli Studi di Brescia
Università degli Studi di Catania
Università degli Studi di Milano
Università degli Studi di Modena e Reggio Emilia
Università degli Studi di Monza
Università degli Studi di Napoli
Università degli Studi di Pavia
Università degli Studi di Padova
Università degli Studi di Pisa
Università degli Studi di Torino
Università degli Studi di Verona
Università degli Studi “La Sapienza” di Roma

INTERNATIONAL COLLABORATIONS

AstraZeneca Ltd, UK
Barts and The London School of Medicine & Dentistry, Londra, Gran Bretagna
Breakthrough Breast Cancer Center, Instutute of Cancer Reasearch, Londra, Gran Bretagna
Cancer Biomarkers and Prevention Group, University of Leicester, Gran Bretagna
Cancer Research UK, Londra, Gran Bretagna
Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland
ECRIN (European Clinical Research Infrastructure Network)
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, Norvegia
European Society of Medical Oncology (ESMO), Svizzera
Executive Board of GCIG (Gynecologic Cancer Intergroup)
Fundació Institut de Recerca del Hospital de la Santa Creu y Sant Pau (IR-HSCSP)- Institute of Biomedical
Research (IIB Sant Pau)
Goteborg University, Lundberg Laboratory for Cancer Research, Goteborg, Svezia
Gynecologic Cancer Intergroup (GCIG)
INSERM (Institut national de la santé et de la recherche médicale), Francia
Institute of Pathology, Friedrich Schiller University, Jena, Germania
Institut Villejuif, Parigi, Francia
Istituto Oncologico della Svizzera Italiana
Johns Hopkins University, USA
Klinik und Poliklinik für Kinder- und Jugendmedizin, Muenster, Germania
Ludwig Institute for Cancer Research, Londra, Gran Bretagna
National Cancer Center, Singapore
Swiss Federal Institute of Technology, Zurigo, Svizzera
National Cancer Institute (NCI), Bethesda and Frederick, MD, USA
Ospedale San Giovanni, Bellinzona, Svizzera
Rosalind Franklin University, Chicago, IL
SAKK (Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung)
University of Athens (UAE), Department of Mathematics, Atene, Grecia
University College, London Medical School, Londra, Gran Bretagna
University of Newcastle, Gran Bretagna
University of Ulm, Germania

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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)
PEER REVIEW ACTIVITIES


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Comitato Scientifico, Fondazione Pezcoller, Trento
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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 Tecnico-Scientifico, Mario Negri Gynecologic Oncology Group (MaNGO)
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, Trento

EVENT ORGANIZATION

Meeting with scientists “Studio clinico multicentrico, osservazionale sulla qualita’di vita del paziente glaucomatoso in Italia”. XXIX Riunione AISG, Naples (Italy), March 29, 2014

Convention “Terapia personalizzata nei pazienti con tumore avanzato del polmone non a piccole cellule premesse biologiche, contesto internazionale e contributo degli studi italiani”. IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Milan (Italy), May 19, 2014

Meeting “Implicazioni cliniche delle novità nel carcinoma ovarico” e 11° MaNGO Group Meeting. Sestri Levante (Italy), May, 23-24 2014
CONFERENCE AND WORKSHOP CONTRIBUTIONS

Conference: VI International Conference on Angiogenesis in Memory of Judah Folkman, Rome (Italy), January 24-25, 2014
“Translational Research -Round Table: Predictive factors”

Congress: La nutrizione artificiale nelle cure palliative, Selinute (Italy), February 8, 2014
“La terapia del dolore in corso di NPT”

Conference: Quale progetto per la ricerca e la cura dei Tumori Rari in Puglia. Bari (Italy), March 20, 2014
“La ricerca scientifica: le motivazioni partono dal cuore”

Conference: Studiare per contrastare il cancro - Colloqui per la Scienza, Milano. Milan (Italy), March 27, 2014
“Possibili strategie per migliorare le terapie antitumorali”

Meeting: AACR Annual Meeting, San Diego (USA), April 05-09, 2014
"Cediranib affects tumors progression and survival of mice bearing patient derived ovarian carcinoma xenografts (EOC-PDX)"
“OTX015, a novel pan BET-BRD inhibitor is active in non-small-cell-lung cancer cell (NSCLC) lines bearing the fusion protein EML4-ALK”

Seminar ISAL: Il dolore in oncologia, Rimini (Italy), April 11, 2014
“La realtà italiana nella cura del dolore da cancro”

“Caratterizzazione molecolare dei tumori ovarici”

Course: Rete tumori rari in Sicilia: 1° Corso educazionale regionale sui sarcomi dei tessuti molli. Ragusa Ibla (Italy), May 9-10, 2014.
“Letta Magistrale: Aspetti critici della terapia diretta a target molecolari specifici”

Conference: XI Convegno Regionale SICP Lombardia, Milan (Italy), May 16, 2014
“Focus sugli oppioidi”

Congress: Carcinoma ovarico, approccio multidisciplinare, Rho MI (Italy), May 16, 2014
"Angiogenesis Inhibitors in Ovarian Cancer: Preclinical Investigation"

"Circulating stroma-related molecules as potential biomarkers for pancreatic ductal adenocarcinoma".

Meeting: “Implicazioni cliniche delle novità nel carcinoma ovarico”. Sestri Levante (Italy), May 23, 2014
“Implicazioni cliniche delle firme molecolari”

Meeting: ASCO Annual Meeting, Chicago (USA), May 30 –June 3, 2014
“Trabectedin and indole-3-carbinol combination in heavily pre-treated metastatic breast cancer. results of a pilot clinical study”

Course: VII Corso di aggiornamento Oncologia Toracica, Criticità ed Evidenze Scientifiche nelle neoplasie pleuro-polmonari, Naples (Italy), June 5, 2014
“La gestione del dolore”

Congress: 23rd Biennial Congress of the European Association for Cancer Research EACR-23: From Basic Research to Personalised Cancer Treatment, Monaco (Germany), July 5-8, 2014
"Heterogeneous response to bevacizumab Combined with chemotherapy in PATIENT-DERIVED ovarian cancer xenografts"
"Identification of potential biomarkers in pancreatic ductal adenocarcinoma associated to tumor-stroma interaction"
"Inhibition of angiogenesis promotes a homogeneous intra-tumor distribution of chemotherapy associated with better antitumor response"

Conference: ESGO State of the Art Conference 2014. Follow-up in gynaecological malignancies, Turin (Italy), September 11-13, 2014
“Mutation profiling of longitudinal epithelial ovarian cancer biopsies using next-generation sequencing”

Meeting: 56° Meeting della Società Italiana di Cancerologìa, Ferrara (Italy), September 11-13, 2014
"Thrombospondin-1 TS3R domain potentiates response to chemotherapy of human ovarian carcinoma by increasing drug delivery into tumors"
"Establishment of a platform of patient-derived tumor xenografts (EOC-PDX) to study the biology and therapy of epithelial ovarian cancer"

Congress: La spiritualità dinanzi al morire: dal corpo malato alla salvezza, Padua (Italy), September 26, 2014
“Il dolore in oncologia: indirizzi terapeutici e variabilità delle risposte”

Congress: ESMO Madrid (Spain), September 26-30, 2014
“Trabectedin and pegylated liposomal doxorubicin (PLD) versus carboplatin and PLD in partially platinum-sensitive ovarian cancer patients: INOVATYON study”

Congress: 2nd Cancer Cachexia Conference, Montreal (Canada), September 26-28, 2014
“The p97/VCP ATPase is critical in muscle atrophy and for the accelerated degradation of most muscle proteins”

Congress: XI Interuniversity Institute of Myology (IIM) Meeting, Siena (Italy), October 2-5, 2014
“Does CXCR4 pathway play any role in muscle loss induced by cancer?”

Symposium: 26th EORTC-NCI-ACR symposium on "Molecular targets and Cancer therapeutics", Barcelona (Spain), November 18-21, 2014
“A dose dense schedule improves antitumor activity of trabectedin in myxoid liposarcoma with type III FUS-CHOP chimera”
“Trabectedin and lurbinectedin are effective against leukemic cells derived from patients affected by chronic and juvenile myelomonocytic leukemia”

Conference: Settimana di (in)formazione, a cura dei ricercatori dell’Istituto di Ricerche Farmacologiche Mario Negri. Noto, CT (Italy), October 6-10, 2014
“Perché è difficile curare i tumori?”

Congress: Dolore e sofferenza: tra medicina, cultura ed etica delle cure, Florence (Italy), October 17, 2014
“Gli analgesici oppioidi e le loro interazioni con altri farmaci”

Course: Analisi della sopravvivenza ed applicazioni in oncologia: un percorso dalle basi agli ultimi aggiornamenti, BIAS – Biometristi dell’Industria Associati, Genua (Italy), October 30-31, 2014
“The Cox regression model for survival data”

Course: Highlights in Ginecologia Oncologica. Standard, innovazione e ricerca nei tumori maligni dell’ovaio. Camogli, GE (Italy), November 27-29, 2014.
“La genomica dei tumori epiteliali dell’ovaio”
“Firma Molecolare della Resistenza”
GRANTS AND CONTRACTS

Actavis Italy SpA
Agenzia Italiana del Farmaco
AIRC Associazione Italiana per la Ricerca sul Cancro
ArQule USA
Astra Zeneca Ltd
AVAPO (Associazione Volontari Assistenza Pazienti Oncologici)
Azienda Ospedaliera Fatebenefratelli e Oftalmico- Milano
Celgene Italy, SrL
CLOVIS Oncology
Comitato Emme Rouge in ricordo di Mara Nahum Onlus
FIRC Fondazione Italiana per la Ricerca sul Cancro
Fondazione Buzzi Unicem
Fondazione Cassa di Risparmio delle Province Lombardie (Carpil)
Fondazione "Eugenio Morandi" Onlus per lo studio e la cura dei tumori del pancreas
Fondazione Monza e Brianza per il Bambino e la sua Mamma
Fondazione Nerina e Mario Mattioli Onlus
Fundació Institut de Recerca del Hospital de la Santa Creu y Sant Pau (IR-HSCSP)- Institute of Biomedical Research (IIB Sant Pau)
GISCAD (Gruppo Italiano Studi di Carcinomi Apparato Digerente)
Grunenthal Italia, Milano
Gruppo Italiano Sarcomi
Indena SpA
Innmedica, Berna
Istituto Nazionale dei Tumori, Milano
Istituto Regina Elena
Italian Sarcoma Group
Marie Curie International Reintegration Grant
Medac
Ministero della Salute
Mundipharma EDO GmbH Novartis
Novartis Farma SpA
Oncoethix
O.T.D. – Oncology Therapeutic Development s.a.r.l.
Pfizer Global Research and Development
Pharm Mar, SA
Roche SpA
SAKK (Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung)
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)

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Thrombospondin-1 is part of a slug-independent motility and metastatic program in cutaneous melanoma, in association with VEGFR-1 and FGF-2

Drago (KIAA0247), a new DNA damage-responsive, p53-inducible gene that cooperates with p53 as oncosuppressor
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Mode of action of trabectedin in myxoid liposarcomas
Oncogene 2014 33 : 5201-5210

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Marchini S
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Edimes, Pavia, 2014; 26-29

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-Jo ining (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 derives 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.

Combination studies with trabectedin and agonists of PPARγ are ongoing in order to promote differentiation even in models of myxoid liposarcoma less sensitive to trabectedin.

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.

The absence of the protein Merlin, frequently in mesotheliomas, seems to predict sensitivity to inhibitors focal adhesion kinase. The expression of this protein in our models is being evaluated. In order to study the role of inflammation in mesothelioma resistance to chemotherapy we are characterizing mouse models of this disease in immunocompetent mice.

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 high levels are characterized by poor prognosis and reduced overall survival. When we integrated miRNA and gene expression, we idendtifyed 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. In matched tumor biopsies taken before and after different lines of chemotherapy, we observed differences in gene expression that resemble the epithelial to mesenchymal transition. One of driving force we have identified so far is miR-181a, that affects directly the TGFβreceptor firing. We noted that at onset,
differences in miR-181a expression correlate with clinical parameters like overall and progression free survival. 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 tumors) 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 context, 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 performs analytical 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 tumor 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 clinical phase I / IIa study with this drug in patients with solid tumor and solid tumor, bearing FGFR 1, 2 amplification, started in late 2011 and ended in 2014. After the development of the LC-MS/MS method for measuring the drug in plasma as well as in plasma ultrafiltrate, we also developed the methods for measuring Lucitanib in blood, urine and tumor tissue always using HPLC-MS/MS technique.

This year we have completed the assessment of the pharmacokinetic profile in the patients enrolled the Phase I/II study (dose escalation phase -DE- and expansion phase –E-) and also in an additional expansion phase with intermittent administration schedule. The study that included a total of 112 patients (76 in DE and E, plus 36 in the intermittent phase) has documented sixteen partial responses and has shown that Lucitanib, administered orally for 21 or 28 consecutive days was well tolerated also ensuring a high plasma exposure. The drug reaches concentrations at steady state pharmacologically active already after one week of therapy.

In a tumor biopsy obtained on day twenty one of therapy we measured 4.9 μg/g (11μM) of Lucitanib.

Always in clinical setting, we continued in 2014 the therapeutic drug monitoring of Oncaspar (a new formulation of pegylated Asparaginase from E. Coli) in children with Acute Lymphoblastic Leukemia. This is a large multicenter study in collaboration with AIEOP (Associazione Italiana Emato Oncologia Pediastrica) and the corresponding German Association (BFM). The study included children treated in the protocol of poly-chemotherapy AIEOP-BFM-LLA 2009. The study shows that this new formulation is better tolerated in children and allows durations of asparagine depletion during therapy greater than those of produced by native Asparaginase.

Quality assurance program

During the year 2014 the quality system of the Laboratory of Cancer Pharmacology became in compliance with the Good Laboratory Practice (GLP). To date we completed the first revision of the procedures and merged together the activities of two Units of the Department of Oncology, the Clinical Pharmacology Unit and the Experimental Preclinical Unit, in a single test facility which now is named "Centre of Bioanalysis and Pharmacokinetic".

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Antitumoral activity and pharmacokinetic properties of new drugs and combinations

It is a study project that focuses on the study of the pharmacokinetics of new oriented anticancer drugs (eg. different kinase inhibitors and derived marine compounds) and conventional drugs (taxanes and camptothecin derivatives) linked to the evaluation of the antitumor activity. The studies were conducted in tumors of mice and rats and in human tumors transplanted into immune-deficient mice. One of the aims was to study the distribution of known drugs (for example paclitaxel and doxorubicin) and/or new analogues in tumors, particularly on tumors refractory to chemotherapy for their particular composition of the extracellular matrix and aberrant angiogenesis. To better study the distribution of the drugs we have developed a method based on Imaging Mass Spectrometry that allows the visualization of drugs distribution in a two-dimensional space. The method was able to visualize paclitaxel in tumor tissues.

**Laboratory of Molecular Pharmacology**

**Checkpoints proteins and cell cycle regulation**

Chk1 and the synthetic lethality with Wee1 in lymphomas non Hodgkin.

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 we identified and characterized the synthetic lethality of Chk1 and Wee1. The two protein kinases are involved in the control of cell cycle transitions and in ensuring a faithful initiation and progression of DNA replication. The effects of combined Chk1 and Wee1 inhibitor treatment was tested at first in human cancer cell lines from solid tumors and more recently in aggressive non-Hodgkin lymphomas. Specifically the effects of a Chk1 inhibitor (PF-00477736) and a Wee1 inhibitor (MK-1775) have been investigated in a large panel (about 40) of mature B cell lymphoma cell lines comprising the mantle cell lymphoma (MCL) and diffuse large B cell lymphomas (DLBCL). To seed and perform treatments we used an automated handle system. We found that MCL cells lines are considerably more sensitive to Chk1 and Wee1 inhibitors as single agents than other lymphoma cell lines and epithelial tumor cell lines.

**Mantle Cell Lymphoma.** The Chk1 and Wee1 inhibitors combination presented a strong synergism at very low concentrations both in *in vitro* and in an *in vivo* setting in MCL providing the rationale for a new therapeutic approach to treat MCL patients. In the last year to better investigate the molecular mechanisms at the basis of the Chk1 inhibitor activity in MCL, a MCL cell line, JEKO-1, resistant to a Chk1 inhibitor was isolated and characterized. The JEKO-1-R cell line is more resistant to PF-00477736, to another Chk1 inhibitor (AZD-7762) and to the Wee1 inhibitor MK-1775. It has a shorter doubling time than JEKO-1 likely due to a faster S phase. Interestingly cyclin D1 expression levels, which in MCL is constitutively expressed due to the presence of the t(11;14), were decreased in the resistant cell line. CyclinD1 over-expression by lentiviral infection, partly restored cyclin D1 protein level and partially re-established PF-00477736 sensitivity. These data suggest that the cyclin D1 expression level is inversely correlated to PF-00477736 resistance. A gene expression profile analysis is undergoing and will provide additional details on the mechanisms of sensitive/resistance of MCL to a Chk1 inhibitor.

**Diffuse Large B Cell Lymphomas.** Among the DLBCL cell lines, the germinal-centre B-cell like (GCB) subtype resulted significantly more sensitive to Chk1 inhibitor than the the activated B-cell like (ABC) subtype. We aim to investigate which molecular feature in GCB subtype may justify the higher sensitivity, focusing on the somatic hyper mutation event which occurs in GCB and not in ABC subtype. Moreover it is known that about 20% of DLBCL cases present recurrent gains affecting chromosome 11, very close to where Chk1 is mapped. We recently found that Chk1 gene is indeed amplified and its expression increased in these DLBCL cases. We aim to investigate the role played by Chk1 in this subgroup, taking advantage of cell lines available in the lab and displaying Chk1 gene amplification and over-expression.

**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.

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.

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 c-Met 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 evidenced 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 tivantib 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 metastasis 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.

Characterization of patients derived human ovarian xenografts

In collaboration with the lab of Biology and Therapy of Metastasis, more than 40 new patients derived human ovarian carcinomas (ovarian PDXs) have been characterized from a histological, molecular point of view. We have demonstrated that these PDXs photocopy the human tumor from which they were derived.
The pharmacological characterization allowed us to define the spectrum of antitumor activity of the drugs commonly used to treat human ovarian cancer. Specifically the antitumor activity of cisplatin, taxol and trabectedin has been studied and some tumors have been found to be resistant, to be sensitive and very sensitive. All these data reinforce the idea that ovarian PDX available in our laboratory retain the phenotypic and genomic characteristics of their original tumor. This PDX platform offers an instructive framework for molecular target discovery/validation studies, for the identification of biomarker of platinum resistance and for testing new investigational therapeutic agents.

**Ovarian cancer stem cell**

In the last year we have isolated and characterized the ovarian cancer stem cell from ovarian fresh tissue obtained from San Gerardo Hospital, Monza, with whom we have a long standing collaboration. We obtained two ovarian enriched cancer stem cells that have been used to screen, through a high-throughput methodology, a chemical library of more than 500 compounds: this chemical library has also been tested in the differentiated cell lines derived from the cancer stem cell lines and we found some compounds that were specifically active on stem cells, some specifically active on the differentiated cells and some active against both cell population. The most interesting compounds are now under study.

**Studies on the mechanism at the basis of resistance to cisplatinum: role of epithelial mesenchymal transition and staminality**

Using our PAdx platform, we investigated their sensitivity to a platinum based therapy, mimicking the clinical setting. Specifically, tumor bearing mice were treated with two cycles of cisplatinum (DDP) each cycle consisting of the drug given once a week for three weeks; the second cycle of DDP was given at tumor re-growth. These experiments clearly demonstrated how DDP had a wide range of efficacy and that the tumors were less sensitive to as second DDP cycle. These models represent a unique tool to study not only the mechanisms at the basis of the intrinsic and acquired resistance to DDP in ovarian cancer, but ways to therapeutically overcome it. We are analyzing xenograft tumor tissues from mice not treated or treated with one or two DDP cycles for the differential expression of genes involved in epidermal mesenchymal transition (EMT) and staminality with the final goal to identify genes potentially predictive of response and genes potentially associated with lack of response that could be pharmacologically targetable.

**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, which are the mainstay of NSCLC treatment. 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 need to be generated. The model will be generated through the use of a new technology, (CRISPR) 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. Once obtained, these models will be investigated with the aim of better understand and bypass the resistance to chemotherapy sustained by KRAS.

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 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 vascular endothelial growth factors (VEGF) released in the tumor microenvironment is accompanied by an impaired response to some chemotherapeutics. It is well known that angiogenesis inhibitors, such as bevaczumab, but also many others, affect the morphology and functions of the tumor microenvironment, thus affecting the response to chemotherapy. On one side we are studying molecular modifications of the tumor stroma caused by an angiogenic microenvironment, on the other side, we are studying how the treatment with these drugs that “normalize” the tumor microenvironment, alters drug distribution and ultimately the outcome with the combined chemotherapy.

**Tumor associated vasculature**

Blood vessels play a key role in the development of cancer, understanding qualitative and functional differences between endothelial cells (EC) lining the vasculature of tumors (tum-EC) with respect to the EC of normal blood vessels is important in order to develop new pharmacological interventions. Among the molecules identified in this laboratory as preferentially expressed by the tum-EC and/or vascular endothelial growth factor (VEGF) rich tumor stroma, we have recently demonstrated i) that the protein RGS5 (regulator of G-protein signalling 5) co-localises with the vasculature of ovarian carcinoma specimens, but is not expressed in human healthy ovaries and its expression by tum-EC is sustained by a milieu of pro-angiogenic factors (including VEGF); ii) the pivotal role of PRSS3/TrypsinogenIV in regulating the migration capability of tum-EC, promoted by the angiogenic microenvironment.

**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 with distinctive morphologic and molecular genetic features. The laboratory has been involved since the '90s in the establishment and continuous updated of preclinical models derived from EOC patients and transplanted in immudeficient mice (EOC-Xenografts). The EOC-xenograft molecularly, biologically and pharmacologically characterized,
which resemble the original patient tumor, together with a large bio-bank of EOC-Xenografts derived biological materials, provide basis for the study of novel selective pharmacological interventions. As an example, inhibitors of angiogenesis are being investigated on EOC-Xenografts. We have shown that bevacizumab, the antibody anti-VEGF, is active on EOC-Xenografts, but the response is heterogeneous between the different EOC-xenografts and it depends from their chemo-sensitivity and the schedule of treatment. The research is in progress to understand mechanisms of resistance and to identify biomarkers of response. The study of these classes of molecules in combination with chemotherapy is one of the main interests of the laboratory. Studies have been conducted and more are in progress to optimize the best combinations with angiogenesis inhibitors, accordingly to the mechanism of action of the drugs and their pharmacokinetics and pharmacodynamics.

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), 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.

Biomarkers for early diagnosis and risk assessment of pancreatic cancer
Pancreatic ductal adenocarcinoma (PDAC) is characterized by intense fibrotic reaction called dysplasia in which extracellular matrix reorganization occurs in term of composition and structural organization. Studies are in progress to i) study extracellular matrix remodeling (synthesis, organization, composition) during PDAC progression; ii) identify extracellular matrix related molecules in plasma of PDAC patients and, in vivo, in genetically modified mouse model of PDAC, biomarker of tumor diagnosis and progression; iii) develop patient derived PDAC xenograft preclinical models to understand the relative tumor and host contribution to the production of selected biomarkers; iv) validate selected molecules as biomarkers predicting the risk of PDAC development and progression. Parallel studies will engage the PDAC xenografts to study novel pharmacological interventions.

Life Science Informatics Laboratory
Projects
- software development and IT management of Clinical Trials, Registries and Biobanks.
- development of software technologies (cluster computing, pipeline for data analysis) for bioinformatics.

A new approach
Several diseases such as cancer or neurodegenerative diseases do not stop to reveal their complexity. To understand the mechanisms that govern such diseases, researchers are increasingly using quantitative approaches that involve advanced knowledge of physics and mathematics. Furthermore, the technical progress - particularly in the field of miniaturization, computers computing power or automation - open new perspectives concerning both the search tools and the therapeutic possibilities.

The growing importance of technology
Understanding the communication between the billions of neurons in the nervous system or the mechanical forces involved in the migration of cancer cells through a tissue requires both an understanding of biological phenomena and a thorough understanding of physics and mathematics to analyze and modelize these processes.

Information Technology plays a fundamental role not only to manage the thousands of data collected
through an experiment deducing significant results from them, but also to modify the complex algorithms that allow to reproduce an image from a microscope data or to interpret an image of a microarray or a next generation sequencing.

Being able to modelize phenomena is essential to make predictions and therefore to plan the experiments to be performed.

A modern education at the intersection of biology, engineering and medicine.

Modern Biology has changed. The advancement of knowledge depends now on mastery of technical tools and, vice versa, research requirements encourage the development of new technologies.

Research in both Functional Genomics and Informatics Life Sciences fields is part of this evolution. The skills to be developed in this area are twofold and inseparable: a thorough training in biology associated with solid knowledge of engineering, not forgetting the critical sense and the ethical dimension.

Technologies

The “-Omics” research relies increasingly on that part of the Informatics Science called Knowledge Engineering, and the integration of genomic, clinical and biological data is the cornerstone of evolution of new knowledge in this area.

The objective of the LSI lab is the development of software and technologies for the integration of data coming from different areas of research.

HeavyBase

In the field of the data sharing infrastructures, in the last 10 years most of the clinical trials have been handled by home-made web based remote data entry engines or even commercial ones but with a common web based centralized view.

Luca Clivio himself has achieved in 2000 GCPBase, based on this kind of model, to manage all clinical trials sponsored by the departments of Oncology and Neurosciences between 2000 and 2008.

However this kind of technology has evidenced some limitations such as the need for a permanent "fast enough" network connection (impossibility of working offline), the need for a "always well configured" web browser (Internet Explorer, Mozilla Firefox, etc. Usually some pop-up blocker as well as others virus protection systems become a problem when using electronic case report forms, forcing investigators to contact their IT Department for a technical intervention to configure the browser).

Furthermore, web-based applications are hardly compatible with the guidelines of GCP (Good Clinical Practices) which establish that the original copy of the data should be maintained at the investigator's site. In fact, web-based applications can't offer to the investigators the possibility to analyze their data without recording information simultaneously in their own local database, causing practical problems regarding computer security issues (accidental damage protection, protection against data theft). Finally, it's not so easy for an application that works as a web site to offer extensive data processing services because of the exclusively interactive nature of this kind of technology.

HeavyBase is an integrated database for clinical data management validated against FDA 21 cfr part 11. This system is able to manage simultaneously multiple clinical trials tracking all the changes that are made on data, and allowing users to work both on-line, same as a web-based system, and off-line (without internet connection) thanks to the capability to store in a local database the original copy of all the data, which can then be transmitted to the Study Coordinating Center when the computer on which data have been saved is connected to the network.

The system does not need any former installation. No administrator rights are requested, the system is provided for download in a dedicated web page (it's a single executable file) and it could be saved on the computer's desktop and used directly.
HeavyBase functions with all versions of Windows from Windows XP to Windows 8.1, as well as on Mac OS-X (versions of reference: from Leopard to Mountain Lion), the system functions also on Linux (reference platform: Ubuntu). Due to its portability can be copied for example on a pendrive (generally it does not exceed 0.2G of space) and used directly from there. Therefore it could be hypothesized for example to use the system for data-entry for some time in the hospital on the institution's computers and for some time at home or in another doctor's office, simply carrying the pendrive.

The security of data is ensured by the redundancy of the network, because once the database is activated on a computer connected to a network, HeavyBase automatically replicates a copy of the information (in protected form) on all the others active nodes. Thanks to a strong encryption data are unreadable for an unauthorized user.

Database achieved and managed with HeavyBase

- Oncology Department – Clinical Research Laboratory
  - ALC - Randomized, 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
  - ATREUS - Phase II study on the activity of trabectedin in pretreated epithelialoid or biphasic/sarcomatoid malignant pleural mesothelioma (MPM)
  - B490 - Cetuximab and Cisplatin with or without Paclitaxel in recurrent/metastatic head and neck cancer
  - ECTvsICT - Extra-capsular tonsillectomy (ECT) vs intra-capsular tonsillectomy (ICT) in children with symptomatic tonsillar hypertropy. An italian, randomized, comparative, multicenter clinical study
  - GLAUCOMA - Studio clinico multicentrico, osservazionale sulla qualità di vita del paziente glaucomatoso in Italia
  - GREAT (Good REsponse with Appropriate Treatment) - Fattori associati alla risposta analgesica nel tempo della terapia combinata ossicidone-naloxone nel trattamento del dolore in pazienti oncologici
  - INOVATYON - Phase III international, randomized study of trabectedin plus pegylated liposomal doxorubicin (pld) versus carboplatin plus pld in patients with ovarian cancer progressing within 6-12 months of last platinum
  - ORCHIDEE - Multicenter, randomized, non-comparative, open-label phase II trial on the efficacy and safety of the combination of bevacizumab and trabectedin with or without carboplatin in adult women with platinum partially sensitive recurring ovarian cancer
  - ORTATAVELX - Multicenter, randomized, non-comparative, open-label phase II trial on the efficacy of Ortataxel and Fotemustine in recurrent glioblastoma
  - PACT-18 (PAncreatic Cancer Trials) - Salvage therapy with trabectedin in metastatic pancreatic adenocarcinoma: A single-arm phase II trial
  - 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
  - RER - Studio osservazionale, prospettico, longitudinale per valutare le caratteristiche cliniche ed i trattamenti a base di oppioidi in pazienti affetti da breakthrough cancer pain (btcp)
  - TERAPIE ORALI - Farmaci antitumorali orali: interventi infermieristici per migliorare la gestione delle terapie e la sicurezza del paziente
  - BEVATRABE - Multicenter, randomized, non-comparative, open-label phase II trial on the efficacy and safety of the combination of bevacizumab and trabectedin with or without carboplatin in adult women with platinum partially sensitive recurring ovarian cancer
  - TRAVELL - A Phase II study on trabectedin in advanced retroperitoneal leiomyosarcoma and
well differentiated/dedifferentiated liposarcoma

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

- Oncology Department – Antitumoral Pharmacology Laboratory
  - PANDORA - Biobanca di Tumori Ovarici in collaborazione con l'Ospedale San Gerardo di Monza
  - PANDORA_LECCO - Biobanca di Tumori Ovarici in collaborazione con l'Ospedale di Lecco
  - PANDORA_BRESCEA - Biobanca di Tumori Ovarici in collaborazione con l'Ospedale di Brescia
  - C3 – Biobanca di Linee Cellulari
  - MC3 – Biobanca di Linee Cellulari
  - MITO-MANGO – Biobanca di Tumori Ovarici
  - XENO-HOC – Biobanca di Linee Cellulari

- Neuroscience Department
  - ANACONDA - Neurodegeneration and Dementia – Network multicentrico dedicato alla registrazione di pazienti con Alzhaimer
  - El-Escorial - Validation Study
  - MBL-Stroke - Mannose binding lectin, un nuovo target per la cura dell'ictus
  - FFI - Fatal Familial Insomnia - Preventive treatment with doxycycline of at risk individuals
  - LONG-TERM PROGNOSIS OF EPILEPSY - A multi-center retrospective survey of prognostic patterns in newly diagnosed patients
  - RF2009_1502045 - Valutazione dei bisogni assistenziali geriatrici e dei percorsi clinici nei pazienti anziani con neoplasia urogenitale (prostata, vescica, rene, pene) dopo la terapia iniziale

**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 and 2014, 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.

We are also characterizing such models at the levels of multiple organs through sophisticated technology involving in vivo bioluminescence-based imaging, micro-Computerized Tomography and ultrasounds-based imaging. Additionally, various targeted drugs are the matter of our research for their possible ability of blocking cachexia in vivo or in vitro. To this regard in 2014, in collaboration with Dr Giavazzi's group, we have found that anti-angiogenic drugs, such as Sorafenib and Sunitinib, may prevent and even revert cancer cachexia in renal tumor-bearing mice, greatly extending their life-span. This therapeutic effect was independent of anti-tumoral activity and implied attenuation of catabolic pathways in muscle involving STAT3 an MuRF-1.

Finally, we are actively involved to understand at the molecular level how physical exercise protects from tumors.
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. Moreover it is dedicated to identify more efficient designs and type of analysis for preclinical studies, in order to improve the predictive value of preclinical results on clinical effects.
Laboratory of Clinical Research

The Laboratory of Clinical Research sponsors and coordinates clinical trials, especially in oncology, in collaboration with national and international research groups, joining the mission and identity of a non-profit research organization to high quality standards. Its staff includes medical doctors, computer scientists, study coordinators, statisticians, responsible of pharmacovigilance and quality assurance, certificated clinical monitors.

In the last years we dealt with clinical trials in the most frequent cancer sites such as breast, colorectal and lung, head and neck, ovary, endometrium, kidney but also in sarcoma, mesothelioma and glioblastoma. Besides oncology area, we have developed a large experience in the field of ophthalmology, focusing on the treatment of glaucoma.

The Laboratory houses two groups, MaNGO (Mario Negri Gynecologic Oncology Group) involved in the research on gynecological cancers, and CERP (Center for the Evaluation and Research on Pain) involved in the evaluation of cancer pain management.

In collaboration with several Italian and International research groups, and with the investigators of Mango and CERP, the laboratory is actively engaged in support of non-profit clinical research. Our goal is also the training of young researchers, training them on planning, conduct and analysis of clinical trials. We also conduct systematic reviews and meta-analyses in different medical areas with physicians and researchers.

The Laboratory gives also statistical and methodological advice.

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 2014 two studies conducted in patients with breast cancer, the TRIPLE NEGATIVE and PAINTER studies have been activated.

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

It is a multicenter, Italian, single-arm, phase II study, which plans to recruit 40 patients.

The aim of this study is to investigate the antitumor activity of zoledronate 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 zoledronate 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 zoledronate 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 of the research project is the analysis of expression of critical genes/proteins related to the mevalonate pathways (p53/PIN1 and YAP/TAZ) in tumor samples collected at diagnosis and at the time of definitive surgery in order to investigate the modulation of gene expression according to the zoledronate activity. The study will also address the safety and tolerability of the drug. The project is funded by AIRC 5 per mille and it is sponsored by the IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milano. The experimental sites are the coordinating center at AO Giovanni XXIII, Bergamo (Principal Investigator Alberto Zambelli) and the Fondazione S. Maugeri, Pavia (Principal Investigator Gian Antonio Da Prada).

**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 Halicondrina 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, sponsored by Fatebenefratelli Hospital in Milan, foresees 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 study will enroll approximately 200 patients with metastatic breast cancer treated with Eribulin accordance with official recommendations, involving 20 Italian centers.

**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 38,000 new cases in people aged up to 84 years, of which 27,000 men and 11,000 women. The annual mortality from lung cancer amounts to approximately 34,000 persons, representing the leading cause of cancer death in men and the third in women, after colorectal carcinoma and breast cancer (the leading cancer death in women).

In fact, lung 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 three histological sub-classes with different prognoses as follows:
  1. squamous cell carcinoma, arises from the cells that line the airways and is often caused by smoking
  2. adenocarcinoma: is currently the most common type. Develops from cells that secrete mucus in the lining of the airways
3. large cell carcinoma, also called large cell undifferentiated carcinoma.

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. Despite the development of targeted anticancer agents and the possibility of replacing the traditional chemotherapy approach which provides the same treatment for all patients with a strategy which takes into account the molecular characterization of the tumor in order to choose the most appropriate therapy for each patient, the available treatments for lung cancer are not very effective.

In 2014 the Acetylcarnitine study in patients with non-small lung cancer cells in advanced stage was closed.

**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

This is 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 was closed.

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 was 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 2014, at the end of the follow-up period, the study was closed and after data validation statistical analysis was done. The report of the study results is expected by January 2015.

**Malignant pleural mesothelioma**

This tumor is a relatively rare and very aggressive form of cancer originating from the mesothelium. Among all forms of malignant mesothelioma, malignant pleural mesothelioma 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, malignant pleural mesotheliomas 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**

There is no active second-line treatment for malignant pleural mesothelioma 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 malignant pleural mesothelioma 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 malignant pleural mesothelioma. 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 malignant pleural mesothelioma. These characteristics have been evaluated in a series of translational analyses.

Atreus study is a phase II multicentre study conducted in patients with unresectable malignant pleural mesothelioma with epithelioid subtype previously treated with pemetrexed plus platinum-based chemotherapy, or patients with biphasic and sarcomatoid histotypes who are either chemo naive or previously treated with pemetrexed plus platinum-based chemotherapy. The study shall enrol 79 patients, of which 62 with epithelioid subtype malignant pleural mesothelioma in progression notwithstanding a previous course of treatment with pemetrexed and platinum derivatives and 17 with sarcomatoid or biphasic malignant pleural mesothelioma irrespective of treatment history. The Sponsor of the study is the IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri”.

The primary objective of the study is to assess the activity of trabectedin in patients with epithelial malignant pleural mesothelioma 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 biomarkers of malignant pleural mesothelioma, shall be evaluated.

The study started enrolling patients in July 2013. Notwithstanding the rarity of this tumour, 64 patients have already been included in the four active centres. In parallel to the initiation of the research activities, an amendment to the protocol, was submitted and approved by the single opinion ethics committee in May 2014. The amended translational part of the study 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. The amendment foresees also an increase in the number of centers involved in the study. In consideration of the anti-inflammatory activity of trabectedin a translational analysis of 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 two open studies on this disease:

**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 this 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, started the accrual. 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, 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
The study with bevacizumab 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. Duration Study finished enrollment on April 2013, 3759 patients have been randomized; the planned follow-up for the achievement of the required 940 events will end in 2016. 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 oxaliplatin 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. Results of this study about toxicity question were published in Scientific Reports: Sci Rep. 2014 Nov 5;4:6828.

**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**

COMETS is 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. Study accrual started on September 2009. 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. This is an event driven study. The study will continue until approximately 101 events have occurred in the sample of 108 patients enrolled.

**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 four 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

This 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. The study has reached the number of events required according to the protocol and the final statistical analysis is being performed.

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 9 patients have been recruited.

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

B490 is a multicentre, randomised, phase II-B, non-inferiority clinical trial, which started accrual in June 2012.

The objective is to assess 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. Up to date 108 patients have been randomized.

This study includes two substudies of translational research on cancer tissue samples and on blood/plasma samples for analysis of prognostic markers and predictive of response.

MUCOSITIS DUE TO CHEMO-RADIOThERAPY – Double-blind, randomised parallel trial comparing morphine mouthwashes to placebo mouthwashes.
The study, which is sponsored by A.O. Santa Maria di Terni, is supported by AIFA. Study population is represented by head and neck opioid naïve cancer patients receiving chemo-radiotherapy 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 is 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 are to evaluate:
- mean intensity of pain during the entire period of study and number of days spent with a level of pain intensity $\geq 4$, 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.

Study activation is being completed.

**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 promotor-dependent fashion.

**TRAVELL** - 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 year period. This study is aimed at confirming the activity of trabectedin as second/further line treatment in retroperitoneal leiomyosarcoma and well 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 the following: - 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 Translation 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. Authorization procedures have been completed in march 6th 2014. To date 6 patients out of 95 have been enrolled.

Renal carcinoma
Renal cell cancer accounts for 100,000 new diagnoses yearly in Europe and up to 25,000 deaths each year. In Italy it affects approximately 12,000 patients per year. The average age of onset is 62 years and over 80% of patients are over 50 years old when the disease is diagnosed. Kidney cancer is twice as prevalent in men as it is in women. It is regularly increasing in industrialized countries, probably due to the improvement in diagnostic techniques but also because of the ageing of the populations, the increase in the number of subjects with arterial hypertension, obesity and/or who smoke. About 30% of the patients present with metastatic renal cell cancer at the time of diagnosis. The 5-year survival rate for patients with metastatic renal cell cancer is less than 10%. Metastatic renal cell cancer is usually resistant to chemotherapeutic agents. Even though interferon alfa-2a and interleukin-2 had been standard therapies for patients with metastatic renal cell cancer the results were rather moderate with response rates less than 20% and significant toxicity. At the moment the 1st line targeted treatment for patients with good or intermediate prognosis seems to be dominated by vascular endothelial growth factor receptor tyrosine kinase inhibitors (sunitinib, sorafenib, pazopanib) but also the monoclonal antibody bevacizumab in combination with interferon. After first-line treatment with VEGF-targeted therapy, two drugs have shown substantially improved progression free survival and can be recommended: Everolimus and Axitinib. One clinical trial are open on this disease. ORCHIDEE (Outcome-related factors in patients with metastatic renal cell carcinoma treated with Everolimus after failure of a first-line treatment with VEGF inhibitor)

This is a multicentre, prospective, single arm, phase IV study, that will involve approximately 20 sites in Italy. The study is sponsored by IRCCS – Istituto di Ricerche Farmacologiche “Mario Negri” in Milan and the Principal Investigator is Dr. Giacomo Cartení, AORN “Antonio Cardarelli”, Naples. The primary objective of the study is to identify factors predictive of favourable outcome, in terms of survival without an unfavourable event, in patients treated with Everolimus as second line treatment for metastatic renal cell cancer after failure of a first-line treatment with a VEGF inhibitor. Everolimus is a derivative of rapamycin that acts as a signal transduction inhibitor. The target of this class of agents is mammalian target of rapamycin (mTOR), a protein kinase that regulate cellular proliferation, motility and survival.
Everolimus has been investigated in many clinical trials that involved patients with different solid and non-solid tumour types. It is approved for the treatment of patients with advanced renal cell carcinoma. 200 patients are required for the study in order to ensure the achievement of 150 events within the study conduct period. The study is currently in activation, the start of patients enrollment is expected for the first months of 2015.

**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 radio-surgery, 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.

Two clinical trials are open on this disease.

**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.

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

On November, 2013 started the recruitment for an Italian, multicenter, single arm phase II trial on the efficacy of Oratataxel in recurrent glioblastoma. The study Sponsor is IRCCS – Istituto di Ricerche Farmacologiche “Mario Negri” in Milan and the Principal Investigator is Dr. Antonio Silvani, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan.
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. The study primary objective is to evaluate the efficacy of Ortataxel in terms of progression free survival at 6 months from the registration. To date 20 patients have been enrolled. Two analysis will be performed: the first one after the enrollment of 33 patients and only if the Ortataxel activity will be considered as of therapeutic interest, the study will continue until 67 patients will be registered.

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. In fact, 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 (ENGT) 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 out of the target size of 686. During 2014 monitoring visits to all Italian sites were done. Data collection will continue all over 2015 and beyond. Preliminary analyses of toxicity and quality of life will be presented at the ASCO meeting to be held in Chicago. The final overall survival analysis is scheduled in 2016-2017 depending on the rate of event occurrence.

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 2014, the number sites activated in Italy was 25 and 139 patients suffering from this rare disease have been enrolled into the study out of a calculated final sample size of 150-160 patients. Recruitment should close at the end of 2015. During 2014 a total of 60 tissue samples have been collected and the expected translational data of putative prognostic/predictive markers will be analysed in 2015.

During 2014 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 and part of 2013, this trial was kept “on hold” due to the worldwide shortage of PLD. Recruitment restarted in January 2014 and is currently active in Italy; Germany, Spain, the Netherlands, Finland, Switzerland, Belgium; in the first six-month period of 2015 further sites will be opened in Austria, Denmark, Norway and UK. As of December 2014 79 patients have been randomized into the trial.

During 2014, MaNGO, in partnership with the other onco-gynecologic Italian group named MITO, completed the activation of 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. MaNGO included three patients during 2014.
In the first 2014 quarter the recruitment of patient into the MITO16a/MaNGO Ov2a fase IV study was closed as the target sample size of 400 patient was fully reached. The MaNGO group is in charge of the centralized collection of blood and plasma samples for both the phase III and phase IV MITO/MaNGO studies above mentioned. The samples repository will be at several laboratories’ disposal for translational studies.

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 was implemented in 5 site of the MaNGO’s network
As of December 2014, only twenty patients have been included into the trial. Three new sites will be soon be opened in 2015 in order to speed up the recruitment.

During 2014, MaNGO’s Technical-Scientific Committee met four-monthly while MaNGO affiliates were conveyed at the 11° General Assembly that was held in May.

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.

The accrual of the study was stopped at September 2014 and the statistically analysis started. At the beginning of 2015 a paper on basilar data will be prepared. During 2015 and 2016 it will be made the subpopulation analysis and new papers will be prepared.

During 2013, it 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).

At the end of 2014 about 70 patients were recruited, less than expected. To evaluate the enrollment process and the difficulties of the participating centers, an investigator’s meeting will be organized at the beginning of 2015.

During 2013 it was 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 2013 it was organized the first advisory board meeting to discuss the protocol and at the end of 2014 three centers were activated and about 20 patients recruited. The other centers will be activated at the beginning of 2015; the study will probably closed at the end of 2015 or at the beginning of 2016.

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.
At December 2014 a systematic review and pooled analysis about the undertreatment of cancer pain was published on J Clinical Oncology.

The educational activities, mainly in collaboration with University of Milan, will go on in 2015. Since six years the lessons of the clinical module related to the “Master in Palliative Care” were held inside our Institute. In parallel, in the last two years also lessons on pain to the students of internal medicine and geriatric specialty school has been kept.

Finally, there are active collaborations with the European network for research in the field of palliative care (EAPC Research Network), with AIOM (Italian Association of Medical Oncology), and with SIMI (Italian Society of Internal Medicine) with the purpose to produce some specific scientific publications.

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 by 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).

The enrollment of 430 patients in 28 centers is expected.

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 392 patients have been enrolled.

Medical device trials (urinary catheter)

ESCALE - A multicentre, randomised, controlled trial to evaluate the efficacy and cost-effectiveness of silver alloy-coated urinary catheters versus conventional catheters in spinal cord injured patients.

The clinical trial is sponsored by the “Biomedical Research Institute Sant Pau (IIB Sant Pau)” in Barcelona that has entrusted to our laboratory the management, coordination and monitoring activities for Italy. It is a Multi-centre trial involving 10 centres in Spain. The extension phase was planned to involve other European countries such as Netherlands, Turkey and Portugal in addition to Italy. The expected number of patients to be enrolled is 742.
The Primary Objective is to compare the incidence of catheter-associated UTIs due to the use of antiseptic silver alloy-coated urinary catheters to that of conventional urinary catheters used in Spinal cord injury patients that need a permanent urinary catheter for bladder drainage. The study will include adult subjects (18 years or older) requiring permanent urethral catheterisation for bladder drainage due to a traumatic or medical spinal cord injury. The first approval of a participating centre in Spain was obtained in February 2012. The first patient has been enrolled in November 2012. Six centres were expected to be involved in Italy; the first approval of a participating centre in Italy was obtained in July 2014.

Non-oncological diseases

Studies in ophthalmology

Glaucoma is an ocular disease typically causes by an increase in pressure inside the eye. According to the World Health Organization, glaucoma affects about 55 million people worldwide and is a leading cause of visual impairment after cataract; in Italy it is estimated that about one million people will be affected, but it is estimated that half of the patients not to be aware of (undiagnosed would be approximately half a million). Blindness and low vision caused by glaucoma can be prevented as long as the disease is diagnosed and treated promptly.

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:

Multicenter, observational study on quality of life in patients with glaucoma in Italy. Multicenter, observational Italian study on Quality of Life in patients with glaucoma. The project includes a substudy in a longitudinal cohort of patients at first diagnosis of glaucoma followed prospectively for one year with a 6 and 12 month follow-up visits.

The study is encouraged by “Associazione Italiana Studio del Glaucoma (AISG)” and the Principal Investigator is Professor Luciano Quaranta, Chief of the Glaucoma Unit at A.O. Spedali Civili di Brescia. The population consists of patients aged ≥ 18 years with instrumental diagnosis of primary open-angle glaucoma.

The evaluation of quality of life was performed through questionnaires validated in Italian, the National Eye Institute Visual Function Questionnaire -NEI-VFQ-25 and the Glaucoma Symptom Scale - GSS, filled out by patients.

The study enrolled a total of 3226 patients in 21 Italian centers, 224 patients at first diagnosis which will be followed prospectively for one year after baseline.

Abstracts with preliminary study results has been presented at international ophthalmology convention (ARVO, European Glaucoma Society Congress) and the first paper with the results of the study was submitted to specialized journal.

In 2014 the data collection of the patients first diagnosis prospectively followed was continued and during 2015 analysis and drafting of the first articles will be expected.

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

Multicenter 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 study is aimed 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 the “Agenzia Italiana del Farmaco” (AIFA) - Bando per la ricerca Indipendente and sponsored by A.O. Spedali Civili di Brescia. The Principal Investigator is Professor Luciano Quaranta, Chief of the Glaucoma Unit at A.O. Spedali Civili di Brescia.

First patient was enrolled in July 2009 and in January 2014 the recruitment phase ended. A total of 37 patients was enrolled. In 2014 the data collection of the patients was continued and during 2015 analysis and drafting of the first articles will be expected on the baseline characteristics of the patients.

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 Operating 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 2014, 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 comply with international standards the data management and oversight of clinical data. This activity has been development in order to satisfy the quality standards required by ECRIN (European Clinical Research Infrastructures Network) to act as their Data Management Centre and collaborating with clinical trials coordinated by this network.

Course of Clinical Monitor
In 2014 the Laboratory had established a Competition Announcement to select the admissions to the "Course of Clinical Monitor" with the aim to train certified Clinical Monitor, fully autonomous in carrying out monitoring activities, as fixed in Annex 1 of the Ministerial Decree of 15th July 1997.

In 2014, Clinical Monitor in training were involved in all laboratory activities, alternating monitoring visits conducted independently, monitoring visits conducted with a certified Clinical Monitor. This Course will last eighteen months and will end on July 2015 with a final examination and the achievement of the certification according to the Ministerial Decree of 15th November 2011.

Monitoring
The laboratory carries out monitoring activity for clinical trials sponsored by Institute and by other public institutions, pharmaceutical industries 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.

On December 2014, the collaboration for monitoring activities with the european collaborative group “Swiss Group for Clinical Cancer Research” (SAKK) had finished. This project was an international phase II trial with rituximab or rituximab plus lenalidomide monotherapy for patients with follicular lymphoma.

During 2014, with the same group, the Laboratory has defined the monitoring activities for another international study, single-arm phase I / II which aims to evaluate treatment with Nelfinavir and Lenalidomide / Dexamethasone in patients with multiple myeloma after failure of therapy with lenalidomide. The laboratory has ended the monitoring activities for another 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.

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 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.

Statistical analysis

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.
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.

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:


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:

Chiara Chiabrando, Head of the Analytical Biochemistry Laboratory since 1997, Unit Head 1987-97, Researcher 1978-87, Research fellow 1975-78 at the Mario Negri Institute.


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 Coordinator (ASMS).

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

Development of mass spectrometry imaging protocols for the spatial distribution description in different tissues of drugs and metabolites.

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, steroids, hormones, pharmaceuticals, drugs of abuse, pesticides, surfactants, plasticizers, antioxidants, mycotoxins).

Selected Publications

Doctoral Degree in Biological Sciences (University of Insubria, Varese, 2000). Postdoctoral Degree in Environmental Analysis, Management and Protection of Biodiversity (University of Insubria, 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, alcohol, nicotine) in the population producing wastewater. Recently, the exposure to pesticide was studied by measuring urinary metabolites in urban wastewater. Monitoring occurrence and fate of several classes of emerging contaminants in the environment and evaluation of their biological and environmental effects.

Selected publications:

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

Selected publications
5. Methods for environmental toxic compounds.


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 Industrial 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


Doctoral Degree in Biological Sciences (University of Milan, 1982), Postgraduate degree in Pharmacological Research, Mario Negri Institute (1986), Postdoctoral fellow at the Massachusetts Institute of Technology, Cambridge, MA (1987-92 at the Mario Negri Institute.

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 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

Using mass spectrometry-based metabolomics, we discovered the presence of fragile points in the metabolic network of different KRAS mutants in lung cancer that might have an impact on the response to anticancer treatments.

Different plasma amino acids patterns related to the amyotrophic lateral sclerosis (ALS) age of onset were found, providing insight into possibly aberrant biochemical pathways that might unlock key pathological pathways.

Nanostructured-initiators for matrix-free, surface-based mass spectrometry imaging allowed the spatial description of anticancer drugs in tumour tissue. of antitumor drugs in tissues.

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 bioaccumulative, 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-wide.

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 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

Fondazione Filarete, Milano
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 Vallecamonica-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"
INTERNATIONAL COLLABORATIONS

Proteomics Platform at ParcCientífic de Barcelona, University of Barcelona, Barcellona, Spagna
Custom Software & Electronics (CSE), Barcellona, Spagna
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
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European Chemicals Agency, ECHA, Helsinki, Finlandia
European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Lisbona, Portogallo
Faculté de Médecine et de Pharmacie, Université de Mons-Hainaut, Mons, Belgio
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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 (IDAIA-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
Interuniversitaires Forcuhngstitut fuer Agrarbiotechnologie, Tulln, Austria
Istituto di Chimica di São Carlos, Università di São 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, Lituania
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 Universitaet 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


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

Enrico Davoli
16th International Workshop on Quantitative Structure-Activity Relationships in Environmental and Health Sciences (QSAR2014), June 16-20, 2014, Milano, Italy
inREACH National Seminar I, Milano, 5 December 2014
Workshop: The role of in silico tools in supporting the application of the substitution principle, Milano, 10-11 December 2014

CONFERENCE AND WORKSHOP CONTRIBUTIONS

MSC Conference, Geneve, Switzerland workshop: Towards Open Access Mass Spectral Libraries. Steve Stein and Enrico Davoli
A nano-PALDI approach for absolute quantification of anticancer drugs in tumor tissues. ASMS 2014, Baltimora, US.
Meeting European Project FP7 ShockOmics, 2 October 2014, Barcelona, Spain
American Association for Cancer Research. April 5-9, 2014. San Diego, CA, USA. Poster presentation
16th International Workshop on Quantitative Structure-Activity Relationships in Environmental and Health Sciences (QSAR2014), June 16-20, 2014, Milano, Italy
inREACH National Seminar I, Milano, 5 December 2014
Workshop: The role of in silico tools in supporting the application of the substitution principle, Milano, 10-11 December 2014


ITN SEWPROF Project Supervisory Board 9 April 2014, Nieuwegein, The Netherlands,
ITN SEWPROF Project Training Course “Analytical techniques for biomarker analysis’’ 10-11 April 2014, Nieuwegein, The Netherlands
First Management Committee Meeting COST Action ES1307 “Sewage biomarker analysis for community health assessment”, 14 April 2014, Brussel, Belgio.


Seminario “diffusione, prevenzione ed indagini diagnostiche delle sostanze d’abuso. Milano 11 November 2014

EU workshop on the development of a strategic approach to pollution of water by pharmaceutical substances – 11 September 2014

COST Action ES1307, WG meeting e second Management Committee, 27-29 October, Malta.

- ASMS 2014, Baltimora, US.
- Meeting European Project FP7 ShockOmics, October 2014, Barcelona, Spain
- American Association for Cancer Research. April 5-9, 2014. San Diego, CA, USA.

**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.
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, ShockOmics)
Federcimica, Milano.
Fondazione CARIPLO, Milano
Fondazione “AQUALAB”
Fondazione Italo Monzino, Milano
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.

SCIENTIFIC PUBLICATIONS (2014)


GINI G, FRANCHI AM, MANGANARO A, GOLBAMAKI A, BENFENATI. ToxRead: A tool to assist in read across and its use to assess mutagenicity of chemicals. SAR and QSAR in Environmental Research, 2014, 25: 999-1011


GADALETA D, PIZZO F, LOMBARDO A, CAROTTI A, ESCHER S, NICOLOTTO O, BENFENATI E. A k-NN algorithm for predicting the oral sub-chronic toxicity in the rat. ALTEX 2014; 31: 423-432


Riva F, ZUCCATO E, CASTIGLIONI S. Prioritization and analysis of pharmaceuticals for human usecontaminating the aquatic ecosystem in Italy. J Pharm Biomed Anal 2014; E-pub:

CASTIGLIONI S, BORSOTTI A, RIVA F, ZUCCATO E. Illicit drug consumption estimated by wastewater analysis in different districts of Milan: A case study. Drug Alcohol Rev 2014; E-pub:


RESEARCH ACTIVITIES

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 secreome 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 Neonicotinoids insecticides on the developing Central Nervous System
During the latest years, we have characterized the effects of environmental contaminants (PBDE and methylmercury) on neuronal cell primary cultures and in a mouse model of prenatal exposure to the contaminants. We are now studying the alterations induced by a class of neuro-active insecticides chemically similar to nicotine (Neonicotinoids) on the same experimental models. 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. Platforms have been made available for in silico models and read across: www.vega-qsar.eu; www.toxgate.eu.

**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**

The laboratory operates both in the environmental and biomedical fields. Mass spectrometry based methodologies and instrumentation are developed to analyze trace and ultra-trace analysis of pollutants, to perform on-site analysis, transportable instrumentation or unattended, real-time, environmental monitoring. In the biomedical field, methodologies are developed for specific biological problems and on mass spectrometry imaging instrumentation and applications.

**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. The major objective is the spatial distribution description in different tissues (e.g tumour tissues) of drugs (e.g. anticancer drugs) and of metabolites revealing differences of drug penetration and/or metabolic activities.
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.

Protein and Gene Biomarkers Unit
The Unit focuses on the study of fundamental biological processes using mass spectrometry-based metabolomics and proteomics strategies. The main objective is to establish metabolomics and proteomics as tools for biomarkers discovery and elucidation of unknown mechanisms associated with disease, especially neurodegeneration, cardiovascular disease and cancer.
Ongoing projects focus on the characterization of proteomics profiling and RARalpha interactome network for stratified therapy in breast cancer.

Proteomics 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).

Metabolomics Analysis
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.
On-going research efforts are toward the metabolomics profiling for novel biomarkers discovery in experimental models (in-vitro, in-vivo) and clinical settings related to (i) acute heart failure induced by shock (EU-FP7 ShockOmics grant); (ii) neurodegenerative diseases progression. Moreover investigations are ongoing on tumor cell metabolism and its association with oncogene’s expression. Metabolic abnormalities in non-small cell lung cancer cells with activated KRAS oncogene are studied in relation to their response to anticancer treatments.

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**

**Wastewater-Based Epidemiology**

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.

This approach has been extended to evaluate alcohol and nicotine consumption in a population and to assess human exposure to pesticides by measuring human urinary metabolites of pesticides in urban wastewater. 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 addition the activity of the unit also focused on food safety. During 2014 studies on toxicological risk due to a contamination of freon 11 in ground waters and to acetaldehyde levels in alcoholic and non-alcoholic beverages have been carried out. In addiction the Unit also performed an investigation concerning the effect of palm oil on blood lipid related markers of cardiovascular diseases by a systematic review and meta-analysis. At the moment, a study about dietary sugars and risk of obesity, diabetes, and cardiovascular diseases is on-going.

**Unit of Analytical Instrumentation**

**Development and application of analytical methods for compounds of biological and environmental interest**

The research activities of the unit 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 Orbitrap) with conventional and nano ElectroSpray sources. Substances of interest include: proteins, peptides, steroids, hormones, pharmaceuticals, drugs of abuse, other environmental and food contaminants (pesticides, perfluorinated compounds, surfactants, plasticizers, antioxidants, mycotoxins) and small polymers (MW < 5000 Da).
# DEPARTMENT OF NEUROSCIENCE

## STAFF

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<tr>
<th>Laboratory</th>
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<tbody>
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<td>Gianluigi FORLONI, Biol.Sci.D.</td>
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<td>Diego ALBANI, Biol Sci. D.</td>
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<tr>
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Laboratory of Molecular Neurobiology
   Head                           Caterina BENDOTTI, Pharm.D.

Laboratory of Neurochemistry and Behavior
   Head                           Roberto William INVERNIZZI, Biol. Sci D

Pharmacology of Cognitive Behavior Unit
   Head                           Mirjana CARLI, Ph.D.

Laboratory of Neurological Disorders
   Head                           Ettore BEGHI, M.D.

Laboratory of Prion Neurobiology
   Head                           Roberto CHIESA, Biol. Sci. D

Laboratory of Quality Assessment of Geriatric Services Unit
   Head                           Alessandro NOBILI, M.D.
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 board 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 250 peer-reviewed scientific articles and about 30 reviews or book chapters.

Selected publications


Selected publications in the last five years

Tiziana Borsello got her Degree in Biological Science at the University of Torino 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 Institute of Neurobiology, 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 the 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 Neuropharmacology 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 span the areas of behavioural neuroscience and psychopharmacology. They mainly focuses on experimental animal models and their translational application to complex human disorders such as drug abuse, anxiety and depression. Author and co-author of several peer-review articles, author of communications in international meetings, he is member of the Society for Neuroscience, European Behavioural Pharmacological Society, Italian Society for Neuroscience and Italian Society of Neuropsychopharmacology.

Selected publications

Barbara D’Avanzo obtained her master in philosophy at the University of Milan in 1989. Her main field of interest is epidemiologic 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. She e has implemented a monitoring system of suicide attempts and self-harm episodes in various areas

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 held a Telethon Scientist position (Dulbecco Telethon Institute, Telethon Foundation) until December 2013. Since 2009 is University Professor in Pharmacology at the University of Milan. In 2011 Dr. Chiesa obtained a Ph.D. in Neuroscience and obtained his Bruno Ceccarelli Prize (2000) for research in neuroscience. He is member of editorial boards of PloS ONE and Biochemical Journal.

Selected publications

of Italy, in the framework of suicide mortality monitoring and suicide prevention study and implementation, and is also working on issues related to suicide-orientated 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. Activity of education and training in the mental health services and mental health literacy to the organizations active in the community. 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 received an Honours Doctoral Degree in Biological Sciences from the University of Milan, Italy I 1977 and subsequently a PhD degree in Neuropharmacology from the Mario Negri Institute of Milan. Having been awarded a European Community fellowship for "Advanced Professional Training", she worked as a post-doc at INSERM U 171, Université Claude Bernard, Lyon, France on the neurochemistry of sleep. Presently she is the Head of the Laboratory of Inflammation and Nervous System Diseases, Mario Negri Institute Milan, Italy. Scientific activity, Long standing experience on experimental models of brain diseases, including Alzheimer’s disease, epilepsy, stroke and trauma. Presently her main scientific interests include the study of the pathogenesis of cerebral ischemia and traumatic brain injury and the identification of molecular mechanisms and novel protective strategies, with particular focus on inflammation and immune system contribution to CNS conditions. She has demonstrated the pathogenetic role of the complement system in acute brain injury and the neuroprotective effects of complement inhibitors. She held more than 100 lectures in Italy, United States and Australia. She is the Author of more than 140 scientific papers on peer-reviewed international journals. Official H index: 40. Editorial activity. Board memberships: Senior Editor of Stroke, American Heart Association, 2007-2010; Intensive Care Medicine experimental, Senior Editor, SRN Vascular Medicine; The Open Pathology Journal; Frontiers in Immunology: Frontiers in Molecular Innate Immunity.

Selected recent publications

Roberto W. Invernizzi got his Biological Sciences degree at the Università Statale di Milano in 1986. He spent short periods at the Department of Pharmacology of the Karolinska Institutet, Stockholm (1988) and Niho University, Tokyo (1995) where he consolidated his knowledge on the intracerebral microdialysis technique in the rat. In 1996 he was appointed Head of the Intracerebral Microdialysis Unit and from 2006 Head of the Laboratory of Neurochemistry and Behavior of the I.R.F. “Mario Negri”. Scientific Interests: Biological bases of psychotropic drugs action and role of brain neurotransmitters and circuits in cognitive symptoms of neuropsychiatric diseases. Recently, the laboratory focused on the development of experimental models of Rett syndrome (RTT) in mice with the aim of studying the underlying pathogenic mechanisms and identifying biological targets to develop novel therapeutics. He is member of the Società Italiana di Neuroscienze, and Società Italiana di Farmacologia, AIRETT Research Team, editorial board of the J. Neurochemistry and reviewer for several International journals in the field of pharmacology and neurochemistry. He published more than 80 scientific articles in peer-reviewed journals and some book chapters.

Selected publications

- Carli M and Invernizzi RW. Serotoninergic and dopaminergic modulation of cortico-striatal circuit in executive and attention deficits induced by NMDA receptor hypofunction in the five-choice serial reaction time task. Frontiers in Neural Circuits 8: 58. doi: 10.3389/fncir.2014.00058
- Carli M, Calcagno E, Mainini E, Amt J, Invernizzi RW. Sertindole restores attentional performance and suppresses glutamate release induced by the NMDA receptor antagonist CPP. Psychopharmacology (Berl). 2011;214(3):625-37
- Baviere 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**


**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 theMario 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 pharmacoresistance. 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 Biology in 1996 (110/110 cum laude), and attended the course of Specialist in Pharmacological Research at the Mario Negri where he operates since 2002, after a 3-year period of post-doctoral carried out in the laboratory of Prof. Renato Dulbecco at CNR-ITBA Milan. He became head of the Unit of Genetics of Neurodegenerative Disorders in 2011. His current interests include the biological bases of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD) and frontotemporal dementia (FTLD), with particular attention to genetic aspects and oxidative stress. Dr. Albani is actively involved in research projects concerning topics of pharmacogenomics of AD, the development of biomaterials for drug delivery and modulation of neuronal enzymes of the family of sirtuins (SIRT1 and SIRT2) as a possible new strategy against AD and PD. He is currently a member of the Editorial Board of the Journal of Alzheimer's Disease and Associated Editor of Frontiers.

Selected publications

In 2005 she obtained the PhD from the Open University (UK). She is member of The Society of Neuroscience and of European Behavioural Pharmacology Society (EBPS) and peer reviewer for several international journals in the field of neuropsychopharmacology and behavioural neuroscience.

Selected publications

- Carli M and Invernizzi RW. Serotonergic and dopaminergic modulation of cortico-striatal circuit in executive and attention deficits induced by NMDA receptor hypofunction in the 5-choice serial reaction time task. Frontiers in Neural Circuits 8: 58. doi: 10.3389/fncir.2014.00058
- 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 corticostratial inputs. *Neuropsychopharmacology*. 2013;38:701-14.
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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


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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 in Institute 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


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

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

Pietro Vegliansene got a degree in Chemistry and Pharmaceutical Technologies in 2000 at the University of Milan. In 2005 he got a degree as Pharmacological Research Specialist at the Department of Neuroscience (Laboratory of Molecular Neurobiology) Istituto di Ricerche Farmacologiche Mario Negri of Milan. In 2007 he got his PhD at the Open University (UK). Since January 2014 he is Head of Acute Spinal Cord Injury and Neuroregeneration unit at the IRFMN. In addition, Pietro Vegliansene was referee for several scientific journals (Brain, Journal of Controlled Release, Cell Biology and Toxicology, ACS Nano and British Journal of Pharmaceutical Research)

Selected publications:


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 Post-doctoral 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 Anesthesia and Critical Care Medicine,

Selected publications


ACTIVITIES

The Department of Neuroscience is formed by twelve Laboratories; the activities of research are dedicated to the study of neurological and psychiatric diseases, evaluated by the biological point of view, clinical and epidemiological aspects and the quality of care. In the Department, activities like drug information service, preparation of clinical trial protocols and epidemiological studies are developed not only in the neurological field. Traditionally, part of the Department is devoted to the development of experimental models for the pharmacological, neurochemical and pathogenetic studies in Alzheimer, Parkinson or prion's diseases, epilepsy, depression and cognitive impairment. More recently, consolidated expertise were established in the studies of amyotrophic lateral sclerosis (ALS) pathogenesis, cerebral stroke, trauma and drug abuse. Some of these disorders, like epilepsy, ALS and Alzheimer's disease, are investigated from the clinical and epidemiological points of view to evaluate drug and care efficacy. Genetic investigations and the use of biomaterial tools are the last acquired expertise. The activities of the Department are aimed to the 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 treatment with functionalized liposomes with ApoE peptide to facilitate the blood brain barrier passage and phosphatidic acid with anti-amyloidogenic activity, in murine models of Alzheimer's disease reduces the brain amyloid burden and the presence of amyloid oligomers with positive consequence on cognitive behavior.
Bexarotene, a retinoic acid receptor X antagonist, proposed as anti-dementia drug, in our hands did not show any efficacy to improve the condition of Alzheimer’s disease animal models confirming recent evidence. Since the clinical efficacy of bexarotene in AD is under investigation, these negative data need to be considered.

The intracerebral application of synthetic β amyloid 1-40 e 1-42 in oligomeric form is associated with a cognitive damage, the effect 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 with some differences in the biological mechanisms to that caused by β amiloid 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 Aβ peptide with transmembrane sequence TAT (TAT 1-6 A2V) including the mutation that in homozogesis is associated to the Alzhiemr’s disease antagonized the toxic effect induced by β amyloid in vitro and in vivo models.

It has been shown in animal models of Alzheimer’s disease 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 Aβ 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 sensibile 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.

We found that expression of the prion protein (PrP) inhibits the function of voltage-gated calcium channels (VGGC) because it interferes with the GPI-anchoring of the VGGC α2δ subunit. This study reveals a new mechanism governing membrane expression of GPI-anchored proteins based on competition for rate-limiting components.

We found that ganglioside GM1 interacts with the prion protein and facilitates its conversion into a misfolded isoform. This data suggest that GM1 may play a role in prion pathology.

In a prospective population-based study in the oldest old (Monzino 80-plus Study), over a 10-year follow-up rate of mortality was significantly higher among the oldest old with dementia than among the oldest old without. Risk of mortality was significantly increased also in each 5-year age stratum from age 80-84 to age 100+. Even in the oldest-old and at the extreme end of the life span dementia shortens survival.
In the same prospective population-based study (Monzino 80-plus Study), the risk of dementia in oldest old women was not associated with age at menopause. This lack of association is consistent with previous studies in younger populations of women.

In the large population of centenarians included in the The Monzino 80-plus Study there was no significant association between education and dementia, however too few centenarians had at least a high school degree. The addition of a cohort with a higher education like that of the Centenaria Trieste [CaT] Study can thus provide sufficient power and variability to test the association between education and dementia.

In a prospective ambulatory population of consecutive patients with mild cognitive impairment or subjective memory complaints seen at the Memory Clinic of the Ospedali Regionali of Mendrisio and Lugano, Switzerland (The Canton Ticino Study), a worse performance on a test of balance (the Tinetti Balance and Gait assessment tool, a performance test which evaluates the subject ability to perform specific motor tasks) at initial visit was associated with an increased risk of dementia incidence.

During 2014 the randomization of mild-to-moderate AD patients to the EU-FP7 NILVAD Study was started. At the end of November 2014 some 390 patients have been randomized all over Europe, 47 of whom in Italy.

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 Lomabrdy 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 polypharamacy (+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.

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 2009, 8,899 subjects with bipolar disorder had at least one contact with mental health services, with a treated annual prevalence rate of 1.1‰. More than 80% of patients were treated in community settings. Rates of patients receiving structured psychosocial treatments were very limited, ranging from 0.7% to 6.1%.

The age class more represented in the services for substance dependence of Lombardy was 35-44 years, mainly for cocaine abuse and then heroin and THC abuse. The treated prevalence of alcohol, MDMA and amphetamine abuse was higher in the more urbanized areas, in agreement with what found by the waste water analyses, and alcohol and MDMA abuse in the areas with higher socioeconomic conditions.

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. The diversity of ALS phenotype, incidence and outcome of ALS can be explained by the ethnic origin of the patients.

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.

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.

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. Educational campaigns may improve knowledge but are unlikely to affect negative attitudes against epilepsy.

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

Epilepsy has a negative impact on the families of affected children and adolescents even though the latter tend to underestimate to what extent the disease interferes with the activities of daily life.

If adjusted, administrative data are a cost-effective instrument to monitor epilepsy frequency. 1/6 of patients with active epilepsy in the general population have drug-resistant epilepsy and about 1/10 patients with newly diagnosed epilepsy will develop drug-resistant epilepsy.

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

We observed that the faster course of ALS in mice with mutation SOD1 depends not on the greater loss of motoneurons but to their greater dysfunction that leads to a massive and earlier denervation of skeletal muscles compared to mice with slower disease progression. This was also highlighted in the study of the role of the role of TNFR2. These results emphasize that the ALS is a multisystemic disorder and therefore in order to obtain an effective therapeutic result is important to consider not only the protection of the motoneurons but also interventions targeted to other districts involved in the disease as the nerves, 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.

We demonstrated the crucial involvement of specific pro-inflammatory cytokines in seizure mechanisms using rodent models of epilepsy, thus describing a new pathological mechanism, i.e. neuroinflammation, which may be relevant for human epilepsy. This discovery highlights novel targets for developing antiepileptogenic and disease modifying therapies translatable to the clinical setting.

We demonstrated that membrane-bound drug transporters 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 *in vitro* and *in vivo* injury.

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

Deletion of tryptophan hydroxylase2, the gene coding for the enzyme responsible for the biosynthesis of brain serotonin, enhances amphetamine-induced hypermotility in the mouse by a mechanism independently from amphetamine’s effect on striatal dopamine release.

The stimulation of trace amines associated receptor1 (TAAR1) and serotonin 5-HT7 receptors counteracts attention deficits in a rat model of cognitive deficits of schizophrenia.

Mecp2 mutant mice, an experimental model of Rett syndrome, show progressive impairment of motor functions and altered brain glutamate metabolism.

Forced ventilation with argon attenuates brain anaerobic metabolism in a rat model of cardiac arrest and resuscitation.

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 αCaMKII 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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EVENT ORGANIZATION

12a Giornata di studio sulla malattia di Alzheimer:
La fragilità nel malato di demenza
Invecchiamento cerebrale 22 marzo 2014, Ateneo Veneto, Venezia (Lucca)

Satellite Event of the 9th FENS FORUM of Neuroscience held 3-4 July 2014 in Milano-Italy on MND, Molecular and cellular basis of selective vulnerability.(Bendotti)

50° Congresso AINPeNC - 40° AIRIC Verbania 5-7 giugno 2014 (Forlioni)

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**LAY PRESS SELECTION (2014)**


**RESEARCH ACTIVITIES**

**Laboratory 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 spettroscopical. The strumental parameters (ROI, T2, DTI) have been harmonized with the partners developing similar approaches in humans. The analysis in PDAPP mice has been interrupted due to inconsistency of the phenotype. The main result was the progressive reduction with aging of striatal volume and the entherinal cortex thickness. Also the hippocampal volume was smaller in both double and transgenic mice but in TASTPM (double) the shrinkage of this area was evident from the first months of life while in triple transgenic mice was progressive with age. The reduction of striatal volume and entherinal cortex thickness has translational meaning since it was found also in the familial form of AD. However during 2014 were performed treatments with anti-amyloidogenic drugs from 9 to 12 months of transgenic mice life without affect on the MRI structural parameters

Nanoparticles in experimental models of Alzheimer’s disease

One of the main problems that need to be addressed in the therapeutic approaches to central nervous system disorders is the passage of blood brain barrier (BBB) of the drugs and substances potentially active. In the last few years has been stressed the possibility that nanoparticles might represent a good vehicle to translate the drugs within the brain. As part of an European Consortium coordinates by Department of Biochemistry of the Bicocca University in Milan we had the possibility to test various types of nanoparticles in experimental model of Alzheimer’s disease. In collaboration with our Department of Biochemistry and the Bicocca University, liposome nanoparticles were functionalized with an ApoE peptidergic fragment and phosphatidic acid that in vitro was able to exert anti-amyloidogenic activity. Transgenic mice expressing human mutated amyloid precursor protein (APP) alone or in combination with PS1 (APP/PS1) were treated intravenously with functionalized liposomes every other day for three weeks. The treatment reduced the sizes of cerebral amyloid plaques and the content of amyloid oligomers, these effects were associated with recovery of cognitive performance determined with appropriate tests

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 we developed some in vivo models to analyze the neuronal dysfunction induced by Aβ1–42 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. More recently it has been investigated the biological mechanisms responsible of the cognitive decline and the role inflammation in this deficit

Sirtuins and neurodegeneration

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 fibrillogenic 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 oligomer.

Spinal injury and regeneration
The mission of Spinal Acute Trauma and Regeneration Unit is to develop therapies to contrast the degenerative events associated to acute spinal injury using nanomaterials to a controlled release of drugs and cells. recently in collaboration with the Department of Chemistry, Material and Chemical Engineering "Giulio Natta" at the Polytechnic University of Milan, have been tested polymeric nanoparticles poly-ε-caprolactone and polyethylene glycol to vehicular drugs interfering with the secondary damage after spinal trauma. These nanoparticles are particularly interesting because they apparently interact exclusively with macrophages and microglia cells. In the lab the toxicological aspects have been verified in vitro before to load them with minocycline a tetracycline with anti-inflammatory effect. After in vitro investigations to optimize the proportion minocycline/nanoparticles, the nanoparticles have been implanted in a murine model.
of spinal injury. The microglial selectivity has been confirmed in vivo and apparently also the functional aspects have been improved with the treatment.

**Laboratory of Cell Death and Neuroprotection**

**AD and APP gene mutations**

APP gene was the first to have been found mutated in an inherited form of AD. In 2009, the group of Prof. Tagliavini has identified a novel mutation in the APP gene in an Italian family that causes disease only in the case of homozygosity. This mutation consists of the substitution of an alanine with a valine at position 673 of the APP (A673V), corresponding to position 2 of the peptide Aβ. *In vitro* studies showed that the mutation A673V (A2V) moves the processing of APP towards the amyloidogenic pathway by increasing production of Aβ. Also, biochemical analysis of synthetic peptides of β-amyloid 1-40 with A2V mutation showed an aggregation kinetics faster than WT. On the contrary the equimolar co-incubation of two species leads to a block of oligomerization, mimicking the heterozygote state. These observations have important implications for the development of a potential new treatment for the familial and sporadic forms of AD, based on modified Aβ peptides. In this regard, short cell-permeable synthetic peptides were generated and they were able to mimic the anti-aggregating effect of the Aβ with the mutation A2V on the WT Aβ. These peptides were made by first six amino acids of mutated Aβ, necessary to maintain the ability to prevent the formation of oligomers and amyloid fibrils *in vitro*. The aminoacidic residues were conjugated with the TAT sequence of the HIV virus to allow the delivery through cell membranes. We tested the peptide D-TAT 1-6 A2V *in vitro* and *in vivo*. *In vitro* experiments showed that the peptide alone is not toxic and when it is administered in combination with the Aβ WT it is able to prevent alterations in PSD and the decrease in the number of dendritic spines. We obtained the same results also in a preliminary study *in vivo* in a mouse model of AD. These results suggest that D-TAT 1-6 A2V peptide could represent a promising strategy to block the progression of AD.

**JNK’s role in Rett syndrome**

Rett syndrome (RTT) is a progressive neurodevelopmental disorder with an incidence of 1 in 10000. RTT is caused by heterozygous mutations in the X-linked MECP2 encoding methyl-CpG-binding protein-2, a transcription factor. MeCP2 regulates activity-dependent synaptic maturation and maintenance. Studies in RTT mice established MeCP2 as a critical mediator of synaptic scaling up and raise the possibility that some of the neurological defects of Rett arise from a disruption of homeostatic plasticity. Rett syndrome appears as a synaptopathy. Analysis of dendritic morphology showed a significant region-specific reduction in number and length of dendrites. The main topic of this project is the signalling pathways that underlies untimely synaptic dysfunction in Rett Syndrome studying the biochemical changes of the PSD region using different MeCP2 mouse models: 1) MeCP +/-, heterozygous mutation founded in RTT females; 2) MeCP2 Knockout, where the gene is completely knockout in order to study its role in synaptopathy. In particular we will focus on JNK’s role, a key player in excitatory synaptic dysfunction, as well as an important link with MeCP2, as demonstrated by our System Biology data. Furthermore we will validate cell-permeable peptide D-JNKI1 for the treatment of this pathology and analyze the signalling pathways that underlie synaptic dysfunction in Rett Syndrome.

**JNK’s role in pre-synaptic vesicles mobilization and glutamate release from axonal ending.**

Even if a growing number of papers showed JNK kinase’s role in many neurodegenerative processes, such as early AD phases associated dendritic spines loss, its function in the pre-synaptic compartment is still unknown. At this purpose we initially confirmed JNK presence in the axonal ending and then studied its ability to modulate neurotransmitter release. It’s commonly known in fact, that many other kinases are able to phosphorylate pre-synaptic machinery proteins and induce an increase in vesicles fusion at the active zone. We proved that glutamatergic NMDA autoreceptors stimulation provokes a significant increase in pre-synaptic JNK activation and that glutamate release after stimulation is slightly reduced by JNK specific inhibitor (DJNKI) administration. After an aminoacidic-sequence screening we discovered that SNARE proteins, constitutive elements of synaptic vesicles docking and fusion complex, contain possible JNK
binding domains (JBD). Among these we proved that syntaxin-1 and 2, internal membrane t-SNAREs, preferentially interact with JNK in the neurotransmitter release process. We will proceed evaluating JNK’s ability to phosphorylate syntaxin-1 and 2; phosphorylation domain individuation will open the road to the design of new molecules able to displace this interaction and modulate glutamate release. Excessive glutamate release indeed, is associated to many neurodegenerative processes such as ischemia, epilepsy, AD and psychiatric disorders; for this reason the development of new compounds, aimed at blocking this phenomenon, is a promising research field, full of interesting therapeutic potentialities.

**Neuroprotective effect of new cell-permeable MKK7 inhibitor peptides in cerebral ischemia models.**

JNK MAP kinase activation is involved in the excitotoxic and inflammatory events related to early phases of cerebral ischemia. Previous works from our laboratory proved that only MKK7, one of the two JNK’s upstream activators (MKK7 and MKK4), is activated after an ischemic event. We therefore postulated that MKK7 is the only responsible of JNK’s pathological activation, while MKK4 has a homeostatic maintenance function. We hence chose JNK-MKK7 interaction as target for the development of a new therapeutic compound, in order to avoid interference with JNK’s physiological activation and risk of side effects. Modelling design preliminary studies brought us to synthesize a selective MKK7 inhibitor, based on Cell-Permeable Peptides technology, which allows therapeutic peptide to cross cell membranes thanks to its linkage to TAT peptide. Inhibitor molecule has been modelled on MKK7 binding domain site on Gadd45β, a member of NFkB pathway, which specifically blocks MKK7 catalytic activity without interfering with MKK4’s activation. We synthesized two peptides: GADD45β(69-86) which contains only the binding region, and GADD45β(60-86) which also contains a region essential for MKK7 inactivation. We firstly proved with *in vitro* experiments that the two peptides have no toxic effects on neurons and that their administration has a significant neuroprotective effect in two *in vitro* models of ischemia (NMDA treatment and Oxygen-Glucose Deprivation). Neuroprotective effect is specifically due to MKK7 inhibition, while MKK4 activation shows no significant reduction. Our promising *in vitro* results have then been reproduced in two *in vivo* models of cerebral ischemia and we proved that peptides administration, at different timepoints before and after lesion, induces a slighty reduction of ischemic volume. Evaluating *in vivo* signalling we confirmed that peptides administration significantly reduce MKK7 activation, without interfering with MKK4. We particularly observed that, 3 hours as well as 6 hours after ischemia, inhibitory effect causes a reduction in JNK’s activation, responsible for the neuroprotective effect of the peptides.

**Alzheimer's disease and eye neurodegeneration**

Alzheimer’s disease leads to eye neurodegeneration at early stages: optical coherence tomography (OCT), a non-invasive test, shows in AD patients a thinning of the retinal nerve fiber layer (RNFL), formed directly from the optic nerve and therefore particularly sensitive to neurodegeneration. This evidence was also observed in CRND8 mice model used in our laboratory for AD: preliminary data show the presence in eye total homogenate of toxic proteins such as APP, P-APP and P-TAU and APP processing towards amyloidogenic pathway with Aβ1-42 accumulation. In humans OCT is a powerful fast non-invasive diagnostic tool: it allows AD early diagnosis, because RNFL thinning is already present in early stages of the disease. Our interest is to investigate, through biochemical and morphological techniques, which eye area is affected by the presence of the toxic proteins that determine the disease, as well as to test cell-permeable peptides on AD mouse model in order to discover the molecular mechanisms of the eye neurodegeneration and to inhibit ocular pathological processes.

**Molecular mechanism in synaptopathy**

The dysfunction of excitatory synapses is the first event of toxicity that characterizes several neurodegenerative diseases, including Alzheimer's disease. In AD patients there is a decrease in the number of dendritic spines that correlates with cognitive deficits. Soluble forms of oligomeric Aβ are responsible of synaptopathy and interfere with glutamatergic transmission by reducing the levels of glutamate receptors in the postsynaptic compartment and favoring the collapse of dendritic spines. We generated an *in vitro* model...
to study the synaptic degeneration and we identify potential protective molecules. To prevent synaptopathy represents a new strategy for the development of effective therapies for AD treatment, currently incurable. In this model we studied signal transduction pathways activated by toxic stimulus and we demonstrated that exposure to beta amyloid oligomers strongly activates the JNK kinase at the synaptic level. D-JNKI1 protected neurons from the events of synaptic dysfunction, preventing the loss of dendritic spines and the reduction of postsynaptic receptors induced by Ab toxicity. Therefore D-JNKI1 is a promising molecule for the treatment of Alzheimer's disease. We therefore explored the mechanisms by which JNK exerts its function at the synaptic level of toxicity and we demonstrated that it acts on two postsynaptic targets: caspase-3 and PSD-95. JNK promotes the activation of caspase-3 in the postsynaptic compartment, which regulates the internalization of AMPA receptors from the membrane. JNK also interacts with PSD-95 at postsynaptic level and phosphorylates it at Ser320 or Thr321. PSD-95 phosphorylation results in the removal of the protein from the synaptic compartment. Being PSD-95 a scaffold protein that maintains the organization of dendritic spine recruiting glutamate receptors, its removal from the PSD causes internalization of the receptors and the loss of dendritic spines. To confirm this hypothesis, we synthesized a cell-permeable peptide, drawn on the PSD-95 portion involved in the interaction with JNK (309-REPRRIVI-316 in the PDZ domain) and we demonstrated that this peptide, by inhibiting the binding between JNK and PSD-95, is able to stabilize PSD-95 on the postsynaptic membrane as in control conditions as in pathological conditions, preventing spines degeneration induced by Aβ oligomers. The obtained results are the basis for the development of new molecules able to prevent the synaptic dysfunction.

Laboratory of Experimental Neurology

Role of neuroinflammation in epilepsy
In the last 15 years our laboratory carried out pioneer studies on the pathogenic role of neuroinflammation in epilepsy (Vezzani et al, Nature Neurol Rev, 2011), hence our research group is leading investigations related to pro-inflammatory molecules such as interleukin (IL)-1 and High Mobility Group Box 1 (HMGB1). Using experimental models of epilepsy in rats and mice, we demonstrated that epileptogenic injuries evoke neuroinflammatory processes in brain areas involved in seizure generation and spread. These processes include the release of IL-1beta and HMGB1 from glial cells and neurons, and these molecules contribute to seizure generation by decreasing excitability threshold in epileptogenic regions. Then, recurrent seizures per se trigger further neuroinflammation, thereby perpetuating a vicious pathologic cycle. We are now studying the anticonvulsive effects of specific anti-inflammatory treatments for developing novel clinical therapies for pharmacoristant epilepsies. We are also addressing the possibility of arresting epilepsy development after an inciting event, or modify the disease course after its onset, using specific anti-inflammatory drugs either alone or in combination. Finally, we are unraveling the molecular mechanisms mediating the pro-ictogenic activity of some neuroinflammatory mediators, and their involvement in cell loss and epilepsy co-morbidities. This project is part of a EU-FP7 framework programme sponsored study named EPITARGET (2013-2018).

Role of Toll-like receptor signaling in epilepsy and neurological sequelae
Infection and fever, which are concomitant with increased levels of pro-inflammatory molecules in the periphery and 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 the activation of innate immunity and inflammation following either infections or epileptogenic brain injuries (i.e. sterile neuroinflammation). We recently described that the activation of the HMGB1- TLR4 axis lowers excitability threshold, thereby promoting seizure generation (Maroso et al, Nature Med, 2010). We are now studying the role played by TLR3 activation since they sense viral infections (a risk factor for seizures in the pediatric population) and can be activated by endogenous molecules, such as genetic material released by damaged cells. We are studying the molecular mechanisms activated by TLR3 in neurons and astrocytes, and their impact on seizure threshold and cognitive deficits using in vivo murine models and in vitro cell cultures.
Characterization and validation of epilepsy biomarkers
The development of novel anti-epileptogenic therapies requires the development of non invasive biomarkers of epileptogenesis that could help to identify patients at risk of developing the disease, monitor the disease progression after its diagnosis, and possibly predict the therapeutic response to novel drugs. Our studies focus on the identification and validation of biomarkers in experimental models of epilepsy that could be measurable in blood, or with imaging techniques such as MRI or MR spectroscopy. This project is developed in particular within the Unit of Pathophysiology of glio-neuronal communication.

Epigenetic control of neuroinflammation in epilepsy: the role of microRNAs
microRNAs (miRNAs) have a key role in post-transcriptional gene regulation of several biological processes in the brain. Specific miRNAs represent a new class of modulators of the inflammatory response in the brain. Our studies focus on specific inflammation-related miRNAs shown to be upregulated in epileptogenic foci both in experimental models as well as in humans. The overall goal is to implement their endogenous levels by ad hoc pharmacological approaches for improving their control of neuroinflammation. This is expected to prevent the deleterious effects of neuroinflammation, thereby mediating anti-ictogenic and anti-epileptogenic actions.

Boosting the resolution of neuroinflammation in epilepsy
A key role of the brain immune response to pathogens or injuries is to activate homeostatic programmes in immunocompetent cells for tissue defense or repair. This task is achieved by inducing the 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 lipid mediators and proteins, and is instrumental to switch off inflammation before it becomes detrimental for tissue. If this mechanism fails then inflammation perpetuates, thereby resulting in 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 epileptogenic injuries is inefficiently controlled by pro-resolving endogenous molecules and their cognate G-protein coupled receptors, thus resulting in persistent neuroinflammation. Using experimental models of seizures and post-injury epilepsy, we are studying the role of key pro-resolving molecules such as resolvins and lipoxins, as well as annexins, governing the post-injury inflammatory response. The overarching goal is to implement the brain pro-resolving mechanisms of inflammation for developing new therapeutic strategies.

Time-lapse single-cell Ca^{2+} imaging as a read-out of pathophysiological cell activation
This project investigates the neuromodulatory activity of pro-inflammatory mediators and the astrocytic cell response to them by analyzing changes in intracellular Ca^{2+} signals. These studies use time-lapse single-cell Ca^{2+} imaging in primary cell cultures from wild-type and mutant mice with impaired cytokine signaling.

Laboratory of Geriatric Neuropsychiatry
Prospective population study on the dementias in the oldest-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. We have also investigated in this population of oldest old the cross-sectional association between reduced kidney function (estimated as glomerular filtration rate using the MDRD formula) and cognitive performance (assessed with different neuropsychologic tests).

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 study 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 partecipate in the study are being interviewed about past and present life styles and 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 activity level 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 and proteomics determinations.

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.
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 programme 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. In addition we have studied the prescription frequency of pain killer drugs to oncologic terminally patients, in particular analgesics and oppioids, and investigated the prescription trends between 2001 and 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 showed that C1-INH, an endogenous inhibitor of the complement system currently used in humans as replacement therapy for angioedema, protects against brain injury with a wide therapeutic window. Our data strongly suggested that this remarkable property of C1-INH was due to its ability to bind mannose-binding lectin (MBL), a key protein of the complement lectin pathway. Consistently we later showed that MBL pharmacological inhibition reduces functional and anatomical damage with a wide time window of efficacy (up to 18-24h) in experimental mouse models of ischemia. Ongoing studies show that MBL is deposited on the ischemic endothelium eliciting its toxic effect. Our present aim is to clarify the pathogenic mechanism driven by MBL on the ischemic endothelium focussing on the interplay between the lectin pathway and the ischemia-induced pro-coagulant endothelium. MBL presence has been demonstrated also after TBI, in human and mouse injured brain where its deletion is protective. Ongoing studies evaluate whether MBL inhibition could represent a new therapeutic strategy also for TBI.

In patients we have recently shown that the activation of lectin pathway reflects the severity of brain injury after subarachnoid hemorrhage, a stroke subtype due to aneurismal rupture. Ongoing studies are aimed at assessing the lectin pathway activation in different stroke subtypes, longitudinally, in relation to injury severity, progression and occurrence of complications and to identify genetic factors contributing to interindivdual differences.
Morphology-phenotype-function relationship in microglia after 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 or 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. We have explored the ability of microglia and recruited macrophages (M/M), to affect neuronal function and promote neurotoxicity through the expression of several harmful components such as inflammatory cytokines, proteases, reactive oxygen and nitrogen species as well as through the interaction with other inflammatory systems such as the complement system. On the other hand we have also documented that these cells possess protective qualities and may promote neurogenesis and lesion repair. These different activation states are characterized by a specific pattern of morphological changes and acquisition of phenotypic markers, whose expression depends on the temporal evolution of the brain lesion. Our ongoing studies are aimed at getting insight on previously unexplored aspects of M/M phenotype changes induced by acute brain injury, namely, the morphology and dynamics of activated cells, the presence of specific phenotype markers, whether they are expressed at distinct phases or locations within the lesion, whether they co-label with some complement factors. A deeper knowledge of the M/M features will allow to properly manipulate the inflammatory response to promote a protective environment for therapeutic purposes.

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

Biological events occur in a physical space within a specific time frame and require the action of solid objects such as blood vessels and cells, which interact in a complex network. Information on spatial motility, time-dependent dynamics and tissue integrity (e.g. simultaneous presence of all the cell types involved in the biological effect) is lost with conventional biochemical, biomolecular or histological techniques. In neurobiology in vivo two-photon microscopy offers a new way to tackle previously unexplored mechanisms in physiological or pathological conditions, thanks to its ability to provide a three-dimensional, high-resolution representation of the brain over time in living animals. We have developed in vivo two-photon microscopy protocols to image and quantify brain dynamics after cerebral ischemia. We measured the vascular changes that ischemia induces such as the drop in blood flow velocity, the occurrence of vessel leakage and the changes in vessel architecture. We used these parameters to study the vascular effects of protective manipulations. We have also visualized immune cells that activate in the ischemic territory and measured their dynamic behaviour. Using a mouse model with fluorescent microglia we have visualized in vivo microglia motility, ramification dynamics and morphological changes in steady-state and after injury. All these parameters are important to define microglia functions, so as to develop new strategies for manipulating the inflammatory response with a therapeutic perspective.

Understanding the mechanisms involved in the stem cell induced protection

We have provided evidence that mesenchymal stromal cells (MSC) stimulate protective and restorative processes through the secretion of bioactive factors, indicating the potential for a cell free approach. We are now investigating the efficacy of MSC derived secretome to understand whether it may represent a therapeutic strategy instead of cell therapy. Specifically we aim at: i) capturing the MSC-derived key effectors that induce protection after acute brain injury; ii) performing the synthetic reconstruction of the identified neuro-protective cocktail; iii) providing mechanistic insight onto how MSC derivatives affect systemic and brain cell populations.
Definition of a successful protocol to be translated to the clinical setting
We have shown the MSC efficacy after an immunological mismatched transplant (human MSC in injured animals). These data provide evidence of the possibility of an allogenic transplant in which donor and host are different individuals, allowing a rapid treatment after acute brain injury. These results are an important step towards translation to clinical practice. We are now analyzing the peculiar features of MSC isolated from different sources (bone marrow, cord blood, amnion, chorionic villi, adipose tissue). As stromal cells, they hold common traits, but they also posses peculiarities of their niche that could make a specific MSC source more or less prone to fulfil specific clinical needs. Finally, epidemiological data show a pick of stroke and TBI incidence in the elderly. We are now studying the MSC efficacy in the aged mice to provide evidence of efficacy in this specific population.

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.
We have recently demonstrated a malfunction of the mechanisms of protein degradation as an important factor for the rapid worsening of the disease (Marino et al. Neurobiology of Aging, 36, 492e504). Of considerable interest is the fact that in face of a similar loss of the motoneurons between the two murine models of ALS, those with the more severe disease show a level of muscle denervation much greater indicating an important role of the peripheral nervous system in the progression of the disease. In addition, the magnetic resonance analysis of the two models of ALS, and in particular, the analysis of DTI, has revealed that the axonal dysfunction in the spinal cord at the symptom onset may be predictive of a more severe disease (manuscript under review to PlosOne). As a result of these observations we are now trying to understand what are the molecular mechanisms responsible for the different axonal dysfunction between the two murine ALS models in order to slow down the progression of the pathology.
In collaboration with Dr. Malaspina from Queen Mary, University of London we are also examining various tissues isolated from the two ALS mouse models with a system of proteome analysis very sensitive and innovative in order to identify the molecular signals in the blood, which can be indicators of disease prognosis as well as potential therapeutic targets. (Project funded by MNDA U. K. and EUROMOTOR FP7 program)

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.
The results of this study, currently under revision in the J. Neurochemistry, demonstrate that the receptors TNFR2 but not the TNFR1 mediate the toxic response of the TNFalpha on motoneuron. However, the neuroprotective effect produced by the lack or the inhibition of TNFR2 receptor does not result in an improvement in the course of the disease. This may be due to the fact that mice SOD1G93A lacking the TNFR2 show a marked axonal dysfunction as demonstrated by an accumulation of TDP43 phosphorylated and a reduction of acetylated tubulin in their sciatic nerve. In addition, the absence of TNFR2 could inhibit
the activation of lymphocytes T reg that play a protective action on neuron and muscles. Then, if the inhibition of TNFR2 on the one hand, can play a protective action on motor neurone degeneration, on the other hand prevents protective cells of the immune system to act. This study therefore emphasizes the importance of considering not only the protection of the motoneurons but also interventions targeted to other districts involved in the disease as the nerves, muscles and the immune system for an effective therapeutic action.

We are currently addressing this aspect in 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 early increase of CCL2/MCP-1 expression as found in the spinal cord of SOD1G93A mice and presumably in ALS patients, can attract monocytes and lymphocytes from the blood able to exert a potential protective effect on damaged motoneuron. The results obtained so far show that an early and progressive increase of the expression of CCL2 in the spinal cord of SOD1G93A mice is accompanied by the activation of the microglia but not the recruitment of monocytes and lymphocytes at the early phase of the disease. It is unclear yet whether this mechanism is responsible for the heavy infiltration of lymphocytes T CD8 and CD4 cells observed in symptomatic mice. We are now exploiting the axis CCL2/CCR2 in order to facilitate the recruitment of protective Treg cells in the spinal cord of the SOD1G93A mice and assess the impact on the progression of the disease, providing the scientific basis for the potential therapeutic intervention. 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 Humanitas Foundation for the research of Milan and the Fondazione Salvatore Maugeri IRCCS, Scientific Institute in Milan.

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 MHC1 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 serotonin 1A (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.

**Laboratory Prion Neurobiology**

**Prion diseases**

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).

Alteration of intracellular trafficking in synaptic dysfunction in genetic prion diseases

Synaptic dysfunction is an important cause of neurological symptoms in prion diseases, a class of clinically heterogeneous neurodegenerative disorders caused by misfolding of the cellular prion protein (PrP). Experimental data suggest that accumulation of misfolded PrP in the endoplasmic reticulum may be crucial in synaptic failure, possibly because of the activation of the translational repression pathway of the unfolded protein response. We report that this pathway is not operative in mouse models of genetic prion disease. Building on our recent finding that intracellular retention of mutant PrP impairs the secretory trafficking of calcium channels essential for synaptic function, we propose a model of pathogenicity in which intracellular retention of misfolded PrP results in loss of function or gain of toxicity of proteins important for synaptic function, such as neurotransmitter receptors and signaling complexes.

Prion protein and voltage-gated calcium channels

We previously found (Senatore et al., Neuron 2012) that PrP interacts with the α2δ subunit of voltage-gated calcium channels (VGCCs), suggesting a possible role of PrP in the channel function. We discovered that expression of PrP inhibits the function of VGCCs because it interferes with the GPI-anchoring of the VGCC α2δ subunit. This study reveals a new mechanism governing membrane expression of GPI-anchored proteins based on competition for rate-limiting components.

Ganglioside and prion protein misfolding

We found that a cell membrane glicolipid, ganglioside GM1, interacts with the prion protein and facilitates its conversion into a misfolded isoform. This data suggest that GM1 may play a role in prion pathology.

Laboratory of Neurochemistry and Behavior

Role of brain serotonin in the effects of psychostimulants: studies in tryptophan hydroxylase2 ko mice

Serotonin (5-HT) is involved in the regulation of many brain functions and in the mechanism of action of various psychotropic drugs including psychostimulants, which motor effects are mainly dependent on the activity of meso-limbic and meso-striatal dopaminergic systems. It is well known that brain serotonergic neurons influence the effects of psychostimulants but their precise contribute remains to be clarified. About
ten years ago tryptophan hydroxylase2 (Tph2) was identified as the gene coding for the enzyme responsible for the hydroxylation of tryptophan to form 5-hydroxytryptophan (5-HTP) in the brain, an essential step in the biosynthesis of 5-HT. The generation of Tph2 ko mice permitted to reconsider the role of brain 5-HT in the psychostimulant effect of amphetamine. Amphetamine-induced motor activity is strongly enhanced in Tph2 ko mice and the administration of 5-HTP restores the normal response to amphetamine. Lack of brain 5-HT does not affect the normal development of 5-HT neurons or the synaptic availability of striatal dopamine in response to amphetamine. The results indicate a significant role of 5-HT in the individual response to amphetamine’s effects.

Experimental models of Rett Syndrome: role of brain glutamate and cholesterol metabolism

Rett syndrome (RTT) is a serious pathology classified as part of the autistic spectrum disorders mainly affecting females during neonatal development. RTT is caused by mutations of the X-linked methyl-CpG-binding protein2 (Mecp2) gene, a regulator of gene transcription. No disease modifying therapies are available at the moment and the pathogenic mechanisms downstream gene mutation are not known. In the last two years, colonies of Mecp2 mutant mice carrying gene truncation (Mecp2-308) or deletion (Mecp2 ko) have been established at our animal facility with the aim of identifying common biological targets in the two experimental models for the development of novel therapeutic strategies. Both genotypes show progressive deterioration of motor functions associated to reduced brain levels of glutamate, glutamine, taurine and GABA in Mecp2-308 mutants. Ongoing studies aim to assess the presence of these alterations in the ko mice. Studies in collaboration with the University of Siena are under way to establish the role of cholesterol metabolism in the RTT and the potential benefit of statins.

Development of pharmacological therapies for cognitive deficits of schizophrenia in the rat: experimental studies on the role of brain trace amines associated receptor1 (TAAR1) and serotonin 5-HT7 receptors in the rat

Cognitive deficits occurring in various neuropsychiatric diseases such as schizophrenia may be highly disabling. The development of novel pharmacological therapies to counteract cognitive deficits of schizophrenia is therefore crucial. We have established an experimental model of cognitive deficit of schizophrenia based on the administration of an antagonist of the N-methyl-D-aspartate (NMDA) receptor to produce deficit of attention in the 5-choice serial reaction time task in rodents, which require to sustain spatial attention to detect a brief visual stimulus. Using this model we highlighted the role of glutamatergic neurotransmission of the prefrontal cortex and the cortico-striatal circuits in attention and identified several neuroactive molecules able to counteract attention deficits. Based on previous data showing that the serotonergic receptor subtype 5-HT7 and the trace amine associated receptor TAAR1 interact with glutamatergic mechanisms, during the last year we have studied the effect of selective agents for these receptors and found that the stimulation of TAAR1 receptors with a selective agonist counteracted the attention deficits. Studies on the role of 5-HT7 receptor are under way and preliminary results show that their stimulation attenuate attention deficits.

Role of brain glutamate, glutamine taurine and GABA and anaerobic metabolism in the neuroprotective effects of argon in an experimental model of cardiac arrest and resuscitation in the rat (in collaboration with the Cardiovascular Dep. of the I.R.F. “M. Negri”)

In Europe, hundreds of thousands people every year are affected by cardiac arrest. Among the survivors, the quality of life depends on the recovery from neurological deficit following ischemia. Thus, novel therapies that limit ischemic damage and preserve brain are needed. Xenon and helium show neuroprotective effects. Unfortunately, they have suppressive effects on hemodynamic and are quite rare and expensive. Therefore other noble gases such as argon, which is more abundant and cost-effective could be a valid alternative. In collaboration with the colleagues of the Cardiovascular Department of our institute, we studied the effects of argon in an experimental model of cardiac arrest and resuscitation in the rat on neuroactive aminoacids and energy metabolism (lactate/pyruvate ratio) in the hippocampus, a brain region particularly sensitive to ischemic damage. Cardiac arrest and resuscitation induced a transient and strong increase of extracellular
levels of taurine and GABA and a slight reduction of glutamate and glutamine. These changes were not affected by forced ventilation with argon (70% in air), which in contrast was effective in reducing the lactate/pyruvate ratio that, as expected, rose after cardiac arrest and resuscitation. These preliminary results suggest that argon may have neuroprotective effects by suppressing the alteration of anaerobic 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 Lombard registry. Information obtained from patients enrolled in the Lombard 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 svc. 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 Lombardy. 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 Lombardy, 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
Pharmacoepidemiology
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. In collaboration with the Health Directorate of Lombardy Region, several Local Health Units and hospitals, a collaborative study has been set up aimed 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.

A. PHARMACOEPIDEMIOLOGICAL STUDY USING ADMINISTRATIVE DATABASE
- Prevalence and appropriateness of antidepressant use in elderly. 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 SSRI: paroxetine (2.2%) and sertraline (1.9%).

- Antipsychotics prescription and cerebrovascular events in Italian older persons. 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 cerebrovascular events (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 acetylcholinesterase inhibitors (AChEI) co-
prescription on CVEs (OR=0.46; 95% CI=0.23-0.92). In conclusion, 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.** 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.

- **Electrocardiographic monitoring for new prescriptions of quetiapine co-prescribed with acetylcholinesterase inhibitors or memantine from 2005 to 2009.** A population study on community-dwelling older people in Italy. Overall 2,623 community-dwelling older people started therapy with quetiapine, co-prescribed with acetylcholinesterase inhibitors AChEIs or memantine from 2005 to 2009. At least one electrocardiographic ECG was performed in 714 cases (27%) in the 6 months before-and in 398 cases (15%) within 3 months after-the starting of this prescription. ECG monitoring was performed both before and after starting quetiapine in only 160 cases (6%). At multivariable analyses, number of drugs taken, beta-blocker and antiarrhythmic drug use were found to be independent correlates of ECG monitoring whereas female sex was associated with a lower probability of receiving an ECG within 3 months after the initiation of quetiapine (odds ratio 0.78, 95 % CI 0.62-0.98). In conclusion, this study showed that ECG monitoring for new prescriptions of quetiapine in older people suffering from behavioural and psychological symptoms in dementia was actually performed infrequently, independently of the age of drug users, especially in women. Our results support the need for greater awareness within the medical community of the importance of such ECG monitoring.

B. DRUG UTILIZATION STUDIES

- **Within region differences in outpatient antibiotic prescription.** 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.

- **Antipsychotic use in a sample of Italian Alzheimer Special Care Units.** In institutionalised patients with dementia in northern Italian Alzheimer’s special care units (ASCU), 60% 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.

- **Comparison of Health Care Resource Utilization by Immigrants Versus Native Elderly People.** For each immigrant (an older people born out of Italy), one person born in Lombardy (native) was randomly selected and matched by age, sex and general practitioner. The 25,508 immigrants selected were less prescribed with at least one drug (OR 0.72, 95% CI 0.67-0.76) and had a lesser use of health care services (OR 0.79, 95% CI 0.75-0.84) than natives. No statistically significant differences were found for hospital admission rates (OR 0.99, 95% CI 0.99-1.04). A lower rate of health care resource
utilization was observed in elderly immigrants who had been living in the host region for as many as 10 years.

c. EPIFARM-ELDERLY PROJECT

- Drug utilisation in elderly patients. All prescription for elderly aged 65 years or older (n=1 767 239), reimbursed by the National Health Service (NHS) 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. 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).

- Pattern of Cholinesterase Inhibitors Use in Alzheimer’s disease: Results of the EPIFARM-Elderly Project. The rate of elderly who received at least one prescription of cholinesterase inhibitors (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 Alzheimer’s disease (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 (ChEIs): the EPIFARM-Elderly Project. 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.

- Geographical differences in the prevalence of chronic polypharmacy in elderly people. 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: p=0.08, p=0.0032; 2005: p=0.11, p=0.001; 2010: p=0.18, p=0.0001), but not with mortality.

- One-year outcome changes of community-dwelling elderly people exposed to chronic polypharmacy: a comparison between data from 2001 and 2009 of the EPIFARM-Elderly Project. Among community-dwelling elderly people, the prevalence of those with incident chronic polypharmacy was 22,822/1,567,575 (1.46%) in 2001, and 51,471/1,800,257 (2.86%) in 2009. Both among elderly with chronic polypharmacy and among controls, the outcomes occurred with a lower frequency in 2009 than in 2001. In univariable regression analyses, chronic polypharmacy was associated with a higher risk of hospitalization (HR 3.21, 95% CI 3.14-3.29), of institutionalization (OR 1.88, 95% CI 1.76-2.01) and of death (HR 2.27, 95% CI 2.24-2.30). Chronic polypharmacy remained an independent predictor of adverse outcomes also after adjusting for index year, sex, age class, and number of drugs. In univariable and multivariable analyses index year, sex, age class, and number of drugs were also statistically
significantly associated with the outcomes. In particular, the year 2001, an older age and a higher number of drugs were associated with a higher risk of events; men were more frequently hospitalized or more frequently died, but were less frequently institutionalized, compared with women.

d. STUDY ON DRUG-DRUG INTERACTIONS IN ELDERLY POPULATION

- Drug interactions in elderly patients. 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.

- 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.

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

e. STUDY ON EPILEPSY

- Validation of healthcare administrative data for the 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.

f. NETWORK ANALYSIS

- The Drug Prescription Network: a system-level view of drug co-prescription in community-dwelling elderly people. Networks are well suited to display and analyze complex systems that consist of numerous and interlinked elements. This study was aimed i) to generate a series of drug prescription
networks (DPNs) displaying co-prescription in community-dwelling elderly people; ii) to analyse DPN structure and organization; iii) to compare various DPNs in order to unveil possible differences in drug co-prescription patterns across time and space. Data were extracted from the administrative prescription database of the Lombardy Region, Northern Italy, in 2000 and 2010. DPNs were generated, in which each node represents a drug chemical subclass, while each edge linking two nodes represents the co-prescription of the corresponding drugs to the same patient. At a global level, the DPN was a very dense and highly clustered network, while at the local level it was organized into anatomically homogeneous modules. In addition, the DPN was assortative by class, as similar nodes (representing drugs with the same anatomic, therapeutic and pharmacologic annotation) connected to each other more frequently than expected, which indicates that similar drugs are often co-prescribed. Finally, temporal changes in the co-prescription of specific drug subgroups (for instance, proton pump inhibitors) translated into topological changes of the DPN and its modules. Complementing more traditional pharmacoepidemiology methods, the DPN-based method allows appreciating (and representing) general trends in the co-prescription of a specific drug (e.g., its emergence as a heavily co-prescribed hub) in comparison with other drugs.

**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.** 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.

- **The ELICADHE-AIFA Project.**

Effect of an integrated e-learning intervention, focused on “Comprehensive Geriatric Assessment” to improve the quality of drug prescribing in hospitalized elderly patients. With the aim to evaluate whether an integrated e-learning program of medical education, focused on teaching and implementing Comprehensive Geriatric Assessment (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 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.
- **FARMAGOOD-BIOSIMILARS PROJECT**

The FARMAGOOD-BIOSIMILARS Project is a collaborative study between the IRCCS-Istituto di Ricerche Farmacologiche Mario Negri (IRFMN) and the Health Directorate of the Lombardy Region aimed to improve the appropriate use of biosimilars through several interventions shared with physicians. These intervention will be aimed:

- to promote the appropriateness of care pathways and rationalize the requirements of biological medicinal products "originator" and "biosimilars";
- monitor the benefit-risk profile of the use of these drugs in clinical practice (real life utilization);
- to save and free up resources in the pharmaceutical and healthcare spending.

The project is carried out in collaboration between the Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico of Milan (where the Service SM-VAP will be realized) and will be divided into two phases:

1. Organization of a multidisciplinary team of internists, geriatricians, hospital pharmacists, nurses and general practitioners (GPs), who will constitute the operational staff of the service. Planning and standardization of procedures of the evaluation process in accordance with the methodologies of Evidence Based Medicine.
2. Feasibility study and assessment of the impact of the service.

The feasibility study will involve a sample of five hospital wards (internal medicine and geriatrics) and 500 patients aged ≥ 65 years, with multimorbidity (at least two chronic conditions) and in polytherapy (treated with 5 or more drugs). The evaluation service will proceed to the review of therapeutic profiles and send to the clinicians a report with suggestions of how to improve the appropriateness of drug prescription and how to manage in each patient the drug-related problems emerged during the evaluation. If these counselling will be shared and accepted by the clinician and the patient, the adjustments-variations in the therapeutic profile will be implemented. At 1 and 3 months there will be a follow-up to verify the degree of persistence of the new therapeutic regimen.

**Study on multimorbidity and polypharmacy in hospitalized elderly patients**

**The REPOSI Registry**

The REGistro Politerapie SImi (REPOSI) study is a collaborative effort between the Italian Society of Internal Medicine (SIMI-Società Italiani 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 polypharamacy (+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:

- Among 1155 patients eligible for the analyses, elderly treated with drugs for the treatment of gastro-oesophageal reflux disease (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).

- 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.

- 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.

- After multiajustment, 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.

- 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).

- 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.

- 19% 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.

- There was a dose-response relation between total Anticholinergic Cognitive Burden (ACB) score and cognitive impairment. Patients identified by the Anticholinergic Risk Scale (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.

- Multivariate analysis 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). 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.
- 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).

- 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.

- 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.

- 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.

- Only 38.8% of patients with a diagnosis of pneumonia received an empirical antibiotic regimen adherent to guidelines. However, no significant association was found between adherence to guidelines and outcomes. Having HAP, older age and higher CIRS Severity Index were the main factors associated with in-hospital mortality.

- The main gender differences in-patients included in the REPOSI registry were: polypharmacy (> 5 drugs) was more frequent in men both at hospital admission and discharge. Neuropsychiatric drugs were significantly more prescribed in women (p<0.0001); at admission men were more likely to be on antiplatelets (41.7% vs 36.7%; p=0.0029), ACE-inhibitors (28.7% vs 24.7%; p=0.0072 ) and statins (22.9% vs 18.3%; p=0.0088). At discharge, antiplatelets (43.7% vs 37.3%; p=0.0003) and statins (25.2% vs 19.6%; p=0.0001) continued to be prescribed more often in men, while women were given beta blockers more often than men (21.8% vs 18.9%; p=0.0340). Proton pump inhibitors were the most prescribed drugs regardless of gender.

- Women were older than men, more often widow and living alone or in nursing homes. Disease distribution showed that malignancy, diabetes, coronary artery disease, chronic kidney disease and chronic obstructive pulmonary disease were more frequent in men, but hypertension, osteoarthritis, anemia and depression were more frequent in women. Severity and comorbidity indexes according to the Cumulative Illness Rating Scale (CIRS-s and CIRS-c) were higher in men, while cognitive impairment evaluated by the Short Blessed Test (SBT), mood disorders by the Geriatric Depression Scale (GDS) and disability in daily life measured by Barthel Index (BI) were worse in women. In-hospital and 3-month mortality rates were higher in men.

Assessment of quality of services for elderly 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 Alzheimr 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 ChEs 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 ChEs; 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®)**

([https://clinicalweb.marionegri.it/intercheckweb/](https://clinicalweb.marionegri.it/intercheckweb/))

- 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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Head Enrico NICOLIS

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Head Gianni TOGNONI, M.D.

Nursing Research Unit
Head Paola DI GIULIO, R.N., MSc
CURRICULA VITAE

Roberto Latini got his Medical Doctor degree in 1978 at the University of Milan.

Education
1970-1978 University of Milan School of Medicine, degree in Medicine
1981-1983 Merck Sharp & Dohme International Fellow in Clinical Pharmacology. Cardiology Fellow, Stanford University Medical Center, California, USA

Main fields of activity
Mechanisms of cardiac damage following ischemia, with focus on neurohumoral activation. Use of stem cells for cardiac repair. Biohumoral investigations within large scale clinical trials in heart failure and atrial fibrillation.

Positions
From Mar 2013 Director of the Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy
From 1991 Member of the Steering Committees of the randomized clinical trial: ALOFT, ValHeFT, GISSI-HF, GISSI-AF, CandHeart, CYCLE, ICOS-ONE.
From 1999-2009 Visiting Professor Dept of Medicine, New York Medical College, Valhalla, NY, USA
1981-1983 Cardiology Fellow (Dr. R. E. Kates, Laboratory) Stanford University Medical Center, CA, USA
1976-1981 Member of the Sub-Group RM5s for Drugs (Community Bureau of Reference, Commission of the European Communities)
1973-1990 Fellow at the Laboratory of Clinical Pharmacology of the Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

Selected publications

Simona Barlera got her degree in Political Science, area Statistics at the “Università degli Studi di Milano” in Milano in 1992, followed by a master in Medical Statistics at the London School of Hygiene and Tropical Medicine, “University of London” in 1998.

Education and training
1987-1992 Degree in Political Sciences, course of studies Statistics, Università degli Studi di Milano, Milano (Italy)
1993-1995 Post-degree Specialization in Pharmacological Research. School of Specialization in Pharmacological Research of Lombardy Region, Milan
1997-1998 Master of Science in Medical Statistics at the London School of Hygiene and Tropical Medicine, University of London, London.
1998-1999 Visiting Scientist in the Department of Statistical Genetics, Wellcome Trust Centre for Human Genetics, University of Oxford (UK).

Main fields of activity
Methodology of Clinical Trials in the cardiovascular field. Preparation and viewing of research protocols, planning and conduct of statistical analyses and the reporting of findings on scientific journals.
Genetic epidemiology: genome-wide strategies (linkage analysis) to identify susceptibility genes in coronary artery disease; case-control studies in order to identify candidate genes involved in the cardiovascular pathology.

Position Held
2014 Member of the organizing committee for the Master of Science in Clinical Research, Department of Pathophysiology and Transplantation, University of Milan.
2014  Member of the Scientific Committee for the Consensus Conference on Closing of Abdomen Complex (ACOI)

2013  Member of the Scientific Committee for the Consensus Conference on Laparoscopic cholecystectomy (ACOI)

2012  Member of the Scientific Committee for the Consensus Conference on Surgery of Rectal Cancer (ACOI)

from Oct 2006  Head of the Laboratory of Medical Statistics, Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

1999 - 2006  Head of the Medical Statistics Unit, Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

1992-1997  Researcher in the Unit of Applied Statistics and Information Technology, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

**Selected publications**


**Maria Grazia Franzosi**

got her Biological Science degree in 1972 at the University of Milan.

**Education**

1972  Doctoral degree in Biological Sciences, University of Milan, Italy

1978  Postdoctoral degree in Pharmacological Research, Istituto di Ricerche Farmacologiche "Mario Negri" di Milano, Italy

**Main fields of activity**


**Position**

from 2002 to Feb 2013  Director of the Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

from 2005  Member of the Coordinating Committee of Master course in Clinical Research - University of Milano

from 2004  Member of Steering Committee, Studio GISSI-AF Study, Milano, Italy

from 2001  Member of Steering Committee, Studio GISSI-HF Study, Milano, Italy

from 1998  Member of Steering Committee of the PROCARDIS Research Programme - A genome-wide strategy to identify susceptibility loci in precocious coronary artery disease - University of Oxford, UK

from 1996  Member of "Antithrombotic Triallist's Collaboration", Oxford, UK

from 1996  Member of Steering Committee e National Coordinator for Italy of the Organization to Assess Strategies for Ischemic Syndromes (OASIS-2, OASIS-4 CURE, Michelangelo OASIS-5 e OASIS 6, CURRENT OASIS-7, FUTURA OASIS-8), INTER-HEART, ACTIVE, RE-LY, ACTIVE, RE-LY, AVERROES, RE-LY Registry, RIVAL, MANAGE, Population Health Research Institute, McMaster University, Hamilton, Canada

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 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

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, Rijksuniversity of Limburg, Maastricht, The Netherlands (Prof. C. Hemker);
1998-1999 “Visiting Scientist” at the Cardiovascular Research Unit, Hammersmith Hospital, London, UK (Prof. A. Maseri)
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; hemato-oncology.  

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 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 Associated professor at the Turin University. Coordinator of the Editorial Board of “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
Morphological and structural analysis of cells and tissue by optical, confocal and transmission electron microscopy, focusing on the mechanism of internalization and intracellular localization of nanoparticles for therapeutic use.

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 Consorzio di 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 Bjorn Nicolas 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 everyday 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 sumbission 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
   - SHOCKOMICS

2. European projects (Horizon 2020- PHC-17-2014 - Call: “Comparing the effectiveness of existing health care interventions in the elderly”):
   - SECURE

3. 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**

In 1000 patients with severe sepsis or septic shock, a new circulating marker has been studied, CD14-ST, also called presepsin. Presepsin proved to be strongly associated to clinical response and patients’ outcome. Good relationships have been shown.

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 enrollment 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 manoeuvers improves the recovery of neurologic functions and reduces histological injury in the brain and in the heart.

One of the causes of the low survival of patients resuscitated from cardiac arrest is the lack of knowledge in the mechanisms involved in organ damage after cardiac arrest. We used experimental models of cardiac arrest and resuscitation in rats and in pigs to study these mechanisms. We have found that, after cardiac arrest and resuscitation, the catabolism of the essential aminoacid tryptophan is importantly activated through the kynurenine pathway. Tryptophan is an aminoacid introduced in our body through the diet and is important for the synthesis of serotonin and vitamins of the B group. The catabolism of tryptophan into kynurenine observed in our experiments has also been confirmed in a small cohort of 7 patients resuscitated from cardiac arrest. Further analyses have shown that the activation of this process was significantly correlated with the severity of cardiac dysfunction, brain damage, cognitive impairment and survival, in all the studied species. We evaluated the kynurenine pathway on a large cohort of resuscitated patients enrolled in the FINNRESUSCI study, one of the most comprehensive European biobank. This biobank collects the plasma samples from 245 patients resuscitated from cardiac arrest in Finland, and hospitalized in one of the 21 intensive care units in the country. FINNRESUSCI also collects data about the rescue time, therapy, intra-hospital outcome, and 1 year survival with neurological recovery. Through this analysis we confirmed on a large cohort of patients the data previously shown in the animals and on a smaller number of patients.

The degradation of tryptophan was activated within a few hours after resuscitation, and depended on the duration of cardiac arrest, the type of cardiac arrest and the necessity of the use of vasopressors for resuscitation, such as adrenaline. Furthermore the activation of the degradation of tryptophan was directly related to the severity of cardiac dysfunction and hemodynamic alterations observed after resuscitation.

Finally, we demonstrated that the extent of degradation of tryptophan was a predictive factor of early death in ICU and long-term outcome.

The early use of a defibrillator is, along with chest compressions, the only intervention able to restore spontaneous circulation and to improve survival in victims of cardiac arrest caused by ventricular fibrillation.
However, timing of defibrillation in relationship to chest compression is a subject of major interest because it is difficult to determine the priority of intervention once rescuer arrives at the cardiac arrest scene, namely chest compression or defibrillation first. Indeed, there are no parameters available that allow the rescuer to determine whether it is more effective to perform chest compression or electrical defibrillation as initial treatment. The real-time analysis of the electric waveform of ventricular fibrillation seems to be an useful and non-invasive way to set a strategy for successful resuscitation. In particular, the algorithm that enables the spectral analysis of the ventricular fibrillation waveform, generates a value, called AMSA, that represents one of the most accurate predictor of the success of defibrillation. The study was performed with the collaboration of all the 118 systems in Lombardia Region on cardiac arrest patients resuscitated from 2008 to 2010. The study was supported by funds from the competitive grant application “Ricerca Indipendente della Regione Lombardia”. The algorithm of defibrillation has been studied and tested on more than 1600 patients. In patients with low AMSA values, the probability of unsuccessful defibrillation was predicted with high sensitivity and specificity, thus indicating an advantage for non interruption of chest compressions. Instead a high value of AMSA was indicative of a successful defibrillation (with a positive predictive value greater than 80%), thus indicating the best time to stop chest compressions and to deliver the electric shock. In conclusion we validated AMSA as an accurate predictor of defibrillation success and as a valid tool to guide cardiopulmonary resuscitation.

The Department has contributed to the 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.

The results of the FOCUS study (Fixed Dose Combination Drug for Secondary Prevention), funded by the European Commission and coordinated by the Centro Nacional de Investigaciones Cardiovascular of Madrid, have been published in the J Am Coll Cardiol 2014; 64: 2071-2082. The study conducted in collaboration with Spain, Italy, Argentina, Brazil and Paraguay had the aims to:

a) know the reasons hindering proper use of drugs in different setting and countries (phase I);
b) assess whether the administration in a single pill (polypill) of drugs recommended for secondary cardiovascular prevention (aspirin 100 mg, simvastatin 40 mg, ramipril 2.5, 5.0, or 10 mg) would improve adherence to treatment compared with three drugs administered separately (phase II).

Overall in the phase I of the study have been included 2118 patients with myocardial infarction and 695 have been also involved in the phase II.

The study shows that: a) identify the reasons (clinical and socio-economic) of poor adherence to therapies is useful to implement strategies in order to improve the continuity of care; b) the use of the polypill increased the adherence compared to the same drugs taken separately and can be useful to improve access to care even in countries where economic resources are scarce.

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
CDI - Centro Diagnostico Italiano Spa, Milano
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 Tuoi 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 Anestesiologia e Rianimazione, IRCCS Ospedale Maggiore Policlinico, Mangiagalli, Regina Elena, Milano
Istituto Auxologico Italiano IRCCS 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
Italian Resuscitation Council, Bologna
IRCCS Fondazione Ca’ Granda Ospedale Maggiore Policlinico, Milano
Laboratorio di Endocrinologia, Ospedale Luigi Sacco, Milano
PoliMi 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 Neurorianimazione, 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 Bari, Aldo Moro, Dipartimento di Scienze Biomediche e Oncologia Umana
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 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 Palermo, Scuola di Specializzazione in Anestesia e Rinimazione
Università degli Studi di Parma, Dipartimento di Scienze Biomediche, Biotecnologiche e Traslazionali
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, Spain
CTSU (Clinical Trial Service Unit) /ISIS (International Studies on Infarct Survival), Oxford, UK
Department of Cardiology, Italian Hospital of Buenos Aires, Argentina
Department of Communications Engineering, Universidad de Pais Vasco, Bilbao, Spain
Department of Epidemiology, Harvard School of Public Health, Boston, USA
Department of Intensive Care, Erasme Hospital, Brussels, Belgium
DSAN SUPSI (Scuola Universitaria Professioni Sanitarie), Lugano, Switzerland
ECLA (Estudios Cardiologicos de Latino-America)
ECRIN (European Clinical Research Infrastructures Network)
ERC (European Resuscitation Council), Basic Life Support Working group
Helsingborg Hospital, Sweden
ILCOR (International Liaison Committee on Resuscitation), Task Force for Cardiopulmonary Resuscitation 2015 International Guidelines
Institut Lorrain du Coeur et des Vaisseaux Louis Mathieu, Vandoeuvre-les-Nancy, France
Karolinska Institutet, Stockholm, Sweden
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 of Oslo, Division of Medicine, Akershus University Hospital, Norway
University Medical Center, Groningen, The Netherlands
University Medical Center, Maastricht, The Netherlands
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

NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP

Comitato Etico della Provincia di Trento
Comitato Ordinatore del Corso Master di 1° Livello in Ricerca Clinica, Università degli Studi di Milano
Comitato Scientifico IRC - Italian Resuscitation Council, Bologna
Comitato Scientifico ACOI - Associazione Chirurghi Ospedalieri Italiani, Roma
Consiglio di Amministrazione Consorzio MIA (Microscopy and Image Analysis), Monza
ERC (European Resuscitation Council), Basic Life Support Working group
Gruppo di Studio SIAARTI - Società Italiana Anestesia Analgesia Rianimazione Terapia Intensiva
ILCOR (International Liaison Committee on Resuscitation), Task Force for Cardiopulmonary Resuscitation 2015 International Guidelines
Società Italiana Terapia Intensiva SITI

EVENT ORGANIZATION

Investigator's Meeting - Riunione sullo stato di avanzamento dello studio BeTACTIC - Best Therapy After Cardiac Transplantation, the Italian Challenge
13/03/14, Aula E, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

Investigator's Meeting - Riunione per la presentazione dei risultati della fase ospedaliera dello studio CYCLE (Ciclosporina A nell’infarto miocardico acuto riperfuso)
30/05/14, Sala Giacomo Binda - Fortezza da Basso, Firenze

Investigator's Meeting - Riunione di aggiornamento delle novità emerse in letteratura, stato di avanzamento dello Studio FALCO (Sorveglianza dei pazienti con Fibrillazione Atriale in Lombardia trattati con Anticoagulanti Oral)
19/06/14, Aula E, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

Seminar - William Harris: What's left of n-3PUFA in cardiovascular prevention?
10/09/14, Aula Guasti, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

Seminar - Hans-Peter Brunner La Rocca: Role of circulating biomarkers in guiding the management of patients with heart failure
10/10/14, Aula Guasti, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

Workshop - Ecografia nel Piccolo Animale. Presentazione ed utilizzo della nuova piattaforma d’imaging VEVO®3100
22-23/10/14, Aula Poster e Stabulario, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano
(Evento organizzato in collaborazione con il Dipartimento di Oncologia)

Investigator's Meeting - Riunione sullo stato di avanzamento dello studio ICOS-ONE (International CardiOncology Society-ONE Trial)
30/10/14, Aula Pinta, DIBIT, Ospedale San Raffaele, Milano
MASTER di I° Livello in Ricerca Clinica dell’Università degli Studi di Milano, Facoltà di Medicina e Chirurgia (Anno Accademico 2014-2015)

METODOLOGIA DELLA RICERCA CLINICA ED ELEMENTI DI STATISTICA
17/11/14 Introduzione al corso. Il disegno dello studio in epidiemiologia. Il disegno degli studi clinici
18/11/14 Corso di introduzione alla statistica medica. Elementi di statistica descrittiva
19/11/14 Inferenza statistica-1: stima e intervalli di confidenza. Esercitazione di inferenza statistica-1
20/11/14 Metodi statistici per l'analisi dell'outcome. Le principali misure di rischio. Monitoraggio degli studi clinici profit & report delle reazioni avverse

L'ORGANIZZAZIONE DI UNO STUDIO CLINICO: ASPETTI REGOLATORI IN ACCORDO CON LE REGOLE DI BUONA PRATICA CLINICA
24/11/14 Gestione della ricerca clinica in Azienda. Lo studio clinico: il protocollo, il consenso informato e la comunicazione con le autorità competenti
25/11/14 Legislazione sulla sperimentazione clinica e ruolo dei Comitati Etici-1. La farmacovigilanza degli studi no profit: nuove direttive e prospettive future
26/11/14 Gestione della ricerca clinica in un IRCCS. Monitoraggio negli studi no-profit
27/11/14 Legislazione sulla sperimentazione clinica e ruolo dei Comitati Etici-2. La dimensione del campione negli studi clinici

GLI STRUMENTI PER LA RACCOLTA E L’INTERPRETAZIONE DEI DATI
01/12/14 Systematic review and meta-analysis. Meta-ricerca. La valutazione del rischio di bias nei trial clinici randomizzati
02/12/14 Trasparenza nei clinical trial. Trial di non-inferiorità
03/12/14 La ricerca bibliografica oggi. Internet e le nuove tecnologie per l’aggiornamento del medico-scientifico

LA RICERCA CLINICA NELLE VARI AREE TERAPEUTICHE
03/12/14 Ricerca in sanità pubblica
04/12/14 Ricerca in medicina generale. Problemi aperti nella scoperta e nello sviluppo di farmaci
09/12/14 Uso clinico dei biomarker in oncologia. Gestione della complessità clinico-terapeutica del paziente anziano ospedalizzato. Le interazioni tra farmaci
11/12/14 Reazioni avverse e farmacovigilanza. Il "discorso etico": dalla linearità dei buoni principi alla provocazione del reale

ELEMENTI DI STATISTICA AVANZATA
15/12/14 Inferenza statistica-2: Test statistici. Farmaci equivalenti
16/12/14 Dalla preclinica alla clinica: sviluppo di nuovi farmaci cardiovascolari. Ricerca clinica nel campo dell'epilessia. Ricerca clinica nell'ictus
17/12/14 Analisi della sopravvivenza. Esercitazione di inferenza statistica-2

IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Milano

CONFERENCE AND WORKSHOP CONTRIBUTIONS

Azienda Ospedaliera Papa Giovanni XXIII di Bergamo - Dipartimento Cardiovascolare Clinico e di Ricerca. 02/04/14, Sala Riunioni, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy
- Infiammazione e malattie cardiovascolari: insufficienza cardiaca e fibrillazione atriale

ANMCO Associazione Nazionale Medici Cardiologi Ospedalieri - ANMCO Lombardia, Fondazione per il tuo Cuore ONLUS - Heart Care Foundation. 7° UTIC Lombarde Convention (Session: La gestione intraospedaliera dell’arresto cardiocircolatorio), 04-05/04/14, Antico Borgo La Muratella, Cologno al Serio, Bergamo, Italy
- Ipotermia: incertezze e lati oscuri

Transatlantic Heart Failure Biomarker Working Group. Ninth Annual Meeting. Biomarkers for innovative medicine in heart failure - Biomarker and clinical decision making in CV disease: focus on heart failure. 26-27/04/14, Cannes, France
- Revisiting clinical applications of high sensitivity troponin in CHF (Session: Novel approaches to personalize heart failure care)
- Potential novel heart failure biomarkers (Session: Utilization of emerging biomarkers in clinical heart failure care)

SIARED Società Italiana di Anestesia Rianimazione Emergenza e Dolore. 10° Congresso Nazionale SIARED. Appropriatezza delle cure e risorse disponibili. 12-14/05/14, Sheraton Hotel, Catania, Italy
- Defibrillatori e defibrillazione: update

ERC European Resuscitation Council. Resuscitation 2014. The pathway to new guidelines. 15-17/05/14, Bilbao, Spain
- Reduction in carotid blood flow after epinephrine during CPR in a porcine model of cardiac arrest is probably related to an increased vascular bed resistance
- Predicting defibrillation success
- Increased rate of bystander-initiated CPR during the initial 3 months after the week of cardiac arrest awareness “viva!” in two Italian cities
- Early activation of the kynurenine pathway predicts early death and long-term outcome in patients resuscitated from out-of-hospital cardiac arrest
- Relationship between plasma high-sensistive cardiac troponin T and infarct size in a porcine model of acute myocardial infarction and cardiac arrest and resuscitation
- European restart a heart day initiatives: are they worthwhile?

ESICM European Society of Intensive Care Medicine. Cardiac arrest. From CPR to recovery. ESICM Regional Conference. 22-23/05/14, Zagreb, Croazia
- Defibrillation update
- VF analysis

SIAARTI Società Italiana di Anestesia Analgesia Rianimazione e Terapia Intensiva. SMART - Organizing and Scientific Committee. 25° SMART Simposio Mostra Anestesia, Rianimazione e Terapia Intensiva. 28-30/05/14, MiCo-Milano Congressi Ala Nord, Milano, Italy
- Improving the chances of successful defibrillation: the AMSA

ANMCO Associazione Nazionale Medici Cardiologi Ospedalieri - Fondazione per il tuo Cuore ONLUS - Heart Care Foundation. 45° Congresso Nazionale di Cardiologia ANMCO. 30/05/14, Fortezza da Basso, Firenze, Italy
- Presentazione dei risultati dello Studio CYCLE. End point primario ed eveni clinici durante la fase ospedaliera

EAS European Atherosclerosis Society. 82nd EAS Congress. 31/05-03/06/14, Madrid, Spain
- Characterization of ANP genetic variant rs5068 in chronic heart failure patients

Centro Studi 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 2013-2014. Modulo 5, 13/06/14, Hotel Athenaeum, Firenze, Italy
- Biomarkers in cardiologia

ADA American Diabetes Association. 74th Scientific Session, ADA 13-17/06/14, San Francisco, USA
- Prospective pilot study on microangiopathy in diabetic foot ulcer
- Marvin E. Levin Award from the American Diabetes Association's Interest Group on Foot Care

Università dell’Insubria, Università degli Studi di Brescia. MASTER di II° Livello in Elettrofisiologia ed Elettrostimolazione Cardiaca, V^ Edizione, 25/06/14, Ospedale di Circolo e Fondazione Macchi, Varese, Italy
- Elementi di farmacocinetica-1
- Elementi di farmacocinetica-2 - Dosi ripetute
- Circulating presepsin (soluble CD14 subtype) in patients with severe sepsis and septic shock. Data from the Albumin Italian Outcome Sepsis (ALBIOS) Study

- Efficacy of aspirin in people with diabetes: an individual participant meta-analysis of 26 randomised trials

- GLORIA-AF - Study protocol presentation

- Myocardial Injury after Noncardiac Surgery: un problema multidisciplinare

- Biomarker panels already tested in atrial fibrillation studies, OPERA and GISSI-AF

- Exploratory marker studies (PREDICTOR - GISSI-AF)

- Prevenzione della cardiotoxicità da antracicline: lo studio ICOS-ONE

- Saranno famosi? Neuregulina 1 ricombinante umana nella insufficienza cardiaca cronica

- VIVA! 2013 e 2014. Il primo anello della catena della sopravvivenza

- Defibrillatori intelligenti

- Take home message

- IRC - 20° Anniversario, 10-11/10/14, Castel dell’Ovo, Napoli, Italy

- Relationship between duration of untreated cardiac arrest and neurological dysfuction and injury in a porcine model of cardiac arrest and CPR

- Severity of postresuscitation myocardial dysfunction is dependent on the duration of untreated cardiac arrest

- Defibrillatori intelligenti

- Take home message

- VIVA! 2013 and 2014. Il primo anello della catena della sopravvivenza

- Defibrillatori intelligenti

- Take home message

- VIVA! 2013 and 2014. Il primo anello della catena della sopravvivenza
Amplitude spectrum area to guide defibrillation: a conclusive validation of 1,617 ventricular fibrillation patients
- Effect of cyclosporine A on infarct size reduction in reperfused acute myocardial infarction treated with primary angioplasty
- N-terminal probrain natriuretic peptide is a strong predictor of long-term mortality in patients with severe sepsis and septic shock. Data from the Albumin Italian outcome sepsis study
- Abnormal left ventricular midwall fractional shortening and elevated circulating biomarkers predict high mortality in elderly individuals in the general population
- Histopathology of the atrium and cardiac post-operative atrial fibrillation
- Predictive value of plasma copeptin and free cortisol on admission and at 48 hours in patients resuscitated from out-of-hospital cardiac arrest
- Circulating cardiac and inflammatory biomarkers to predict post-operative atrial fibrillation in the OPERA trial

Scuola di Specializzazione in Anestesia, Rianimazione e Terapia Intensiva - Università Vita-Salute San Raffaele, Milano, 24/11/14, Aula San Raffaele, Milano, Italy
- Arresto cardiaco e defibrillazione: cosa prevedo e cosa vorrei studiare per le Linee Guida 2020

Centro Studi ANMCO Associazione Nazionale Medici Cardiologi Ospedalieri - Fondazione per il tuo Cuore ONLUS - Heart Care Foundation. Incontro dei Ricercatori dello Studio VAR, Congresso SICCH 29/11/14, Hotel Ergife, Roma, Italy
- Studio VAR - Core Lab Genetico: stato avanzamento lavori e risultatati preliminari

SIC Società Italiana di Cardiologia. 75° Congresso Nazionale SIC, 13-15/12/14 Rome Cavalieri, Roma, Italy
- Fattori di rischio metabolici e rischio di fibrillazione atriale

GRANTS AND CONTRACTS

SCIENTIFIC PUBLICATIONS (2014)
Prevalence and determinants of diabetes mellitus in a representative sample of Italian adults
Epidemiology Biostatistics Public Health 2014; 11: e9980-1-e9980-8

The cardiokine secreted Frizzled-related protein 3, a modulator of Wnt signalling, in clinical and experimental heart failure
J Intern Med 2014; 275: 621-630
Multifunctional liposomes reduce brain β-amyloid burden and ameliorate memory impairment in Alzheimer’s disease mouse models
J Neurosci 2014; 34: 14022-14031

No evidence for genome-wide interactions on plasma fibrinogen by smoking, alcohol consumption and body mass index: results from meta-analyses of 80,607 subjects.

Changes in prescribing patterns and clinical outcomes in elderly diabetic patients in 2000 and 2010: analysis of a large Italian population-based study
Eur J Clin Pharmacol 2014; 70: 965-974

Sex differences in cardiovascular outcomes, pharmacological treatments and indicators of care in patients with newly diagnosed diabetes: Analyses on administrative database

CREACTIVE A European endeavor to improve outcome of patients with Traumatic Brain Injury. GiViTI-PROSAFE-CREACTIVE collaboration
Brain Injury Professional Journal 2014; 11: 24-27

In vivo fate of avidin-nucleic acid nanoassemblies as multifunctional diagnostic tools
ACS Nano 2014; 8: 175-187

Albumin replacement in patients with severe sepsis or septic shock

The Anp genetic variant Rs5068 and circulating levels of natriuretic peptides in patients with chronic heart failure
Int J Cardiol 2014; 176: 1249-1251

A polypill strategy to improve adherence. Results from the FOCUS Project
J Am Coll Cardiol 2014; 64: 2071-2082

Chen B, Yu T, Ristagno G, Quan W, Li Y
Average current is better than peak current as therapeutic dosage for biphasic waveforms in a ventricular fibrillation pig model of cardiac arrest
Resuscitation 2014; 85: 1399-1404

Expression of A2V-mutated Aβ in Caenorhabditis elegans results in oligomer formation and toxicity
Neurobiol Dis 2014; 62: 521-532

*Caenorhabditis elegans* based assay recognizes immunoglobulin light chains causing heart amyloidosis

*BLOOD* 2014; 123: 3543-3552


Investigating heart-specific toxicity of amyloidogenic immunoglobulin light chains: a lesson from *C. elegans*

*Worm* 2014; 3: e965590


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* 2014; 31: 569-578


Biomarkers of activation of renin-angiotensin-aldosterone system in heart failure: how useful, how feasible?

*Clin Chim Acta* 2014; Epub


Integrated multiphase method for in vitro quantitative assessment of cellular uptake for polymer nanoparticles

*Nanotechnology* 2014; 25: 045102


Ranolazine ameliorates postresuscitation electrical instability and myocardial dysfunction and improves survival with good neurological recovery in a rat model of cardiac arrest

*Heart Rhythm* 2014; 11: 1641-1647

Gurrieri C, Ristagno G, Gullo A

Resuscitation science: from the beginning to the present day. Chapter 1


Heart 'omics' in AGING (HOMAGE): design, research objectives and characteristics of the common database


Latini R, Masson S

Circulating cardiac biomarkers and outcome. Chapter 20


Patterns of alcohol consumption and myocardial infarction risk. Observations from 52 countries in the INTERHEART case-control study

*Circulation* 2014; 130: 390-398


Presepsin (soluble CD14 subtype) and procalcitonin levels for mortality prediction in sepsis: data from the Albumin Italian Outcome Sepsis trial

*Crit Care* 2014; 18: R6


Elevated risk of death and major cardiovascular events in subjects with newly diagnosed diabetes: Findings from an administrative database

*Nutr Metab Cardiovasc Dis* 2014; 24: 263-270

Network of Nurses of GISSI-HF, Di Giulio P

Should patients perception of health status be integrated in the prognostic assessment of heart failure patients? A prospective study

*Qual Life Res* 2014; 23: 49-56
Nobili A, Pasina L, Latini R
Beta-adrenoceptor antagonists and antianginal drugs. Chapter 18
In: Side Effects of Drugs. Annual 35. Elsevier, Amsterdam 2014; 351-357

Nobili A, Pasina L, Latini R
Beta-adrenoceptor antagonists and antianginal drugs. Chapter 18

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 2014; 19: 267-284

Clinical competence in the surgery of rectal cancer: the Italian Consensus Conference
Int J Colorectal Dis 2014; 29: 863-875

Pileggi S, Barlera S, Nicolis E, Crociati L, Pietri S, Specchia C, Franzosi MG
Association of ADIPOQ variants and heart failure in an Italian population
Ther Adv Cardiovasc Dis 2014; 8: 89–96

Sunitinib prevents cachexia and prolongs survival of mice bearing renal cancer by restraining STAT3 and MuRF-1 activation in muscle
Oncotarget 2014; E-pub

Ristagno G
Transthoracic impedance waveform during cardiopulmonary resuscitation: On size does not fit all!
Resuscitation 2014; 85: 579-580

Ristagno G
Mechanical versus manual CPR. Chapter 8

Ristagno G, Fumagalli F
Amplitude spectrum area to predict the success of defibrillation. Chapter 6

Postresuscitation treatment with argon improves early neurological recovery in a porcine model of cardiac arrest
Shock 2014: 41: 72-78

Early activation of the kynurenine pathway predicts early death and long-term outcome in patients resuscitated from out-of-hospital cardiac arrest
J Am Heart Assoc 2014; 3: e001094

Ristagno G, Li Y
Letter by Ristagno and Li regarding Article "Waveform analysis-guided treatment versus a standard shock-first protocol for the treatment of out-of-hospital cardiac arrest presenting in ventricular fibrillation: results of an international randomized, controlled trial"
Circulation 2014; 129: e648

Ristagno G, Pellis T, Li Y
Cardiac arrest and cardiopulmonary resuscitation: Starting from basic science and bioengineering research to improve resuscitation outcome
Biomed Res Int 2014; Article ID 737542

The "Italian Registry of Cardiac Arrest - RIAC", a National achievement to portray the Italian reality and to contribute to the wider European vision by "EuReCa"
Resuscitation 2014; 85: e193-e194
Ranolazine prevents INaL enhancement and blunts myocardial remodelling in a model of pulmonary hypertension

Santonocito C, Sanfilippo F, Ristagno G, Gullo A
Resuscitation and ethics: how to deal with the “do not resuscitate order”? Chapter 22
In: Gullo A, Ristagno G (eds) Resuscitation. Springer-Verlag Italia, Milano 2014; 229-234

Relationship between post-cardiac arrest myocardial oxidative stress and myocardial dysfunction in the rat
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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 neuropathic patients have been enrolled in the study and the histopathological analysis are in progress.

Preclinical and clinical studies in cardiac arrest and cardiopulmonary resuscitation
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 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. More specifically, new interventions of blockade of this route are a topic of interest of current studies. Experimental studies in the pig directed to investigate new treatments to improve outcome of acute heart failure (i.e., by infusion of serelaxin) are also ongoing in our labs. Finally, the consequences of hemorrhagic shock on myocardial function in the pig are investigated, 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.

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). Based of the encouraging results, a prospective interventional study is under planning.

**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. A new marker of sepsis, sCD14-ST or presepsin, has been shown to be strongly associated with the host response and prognosis. Its changes over time are also related to the appropriateness of antibiotic therapy. There is an ongoing programme of evaluation of markers related to innate immunity (PTX3, in collaboration with the Istituto Clinico Humanitas at Rozzano), cardiac function, coagulation and fibrinolysis (in collaboration with the University of Bari), immunoglobulins (in collaboration with the University of Brussels), neuropeptides (in collaboration with the University of Oslo) and renal function. We are also participating to a new phase of biomarkers discovery, using proteomics and metabolomics, within the frame of the EU FP7 ShockOmics project.

**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-
The association between left ventricular mass and two cardiac markers (troponin and natriuretic peptide) has been described (Masson et al, J Inter Med 2013; 273: 306-317). We have published a manuscript on two markers related to bone mineral and the cross-talk between the kidney and the heart (Fibroblast Growth Factor-23 and Vitamin D; Masson et al, J Inter Med 2015; 277: 318-330). We are currently assessing circulating markers of ventricular hypertrophy and atrial fibrillation.

**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; 308: 2001-2011). The circulating cardiac (natriuretic peptide or troponin) or inflammatory markers do not seem to predict accurately post-operative incident atrial fibrillation (Masson et al, Eur J Clin Invest 2015; 45: 170-178). In collaboration with the Universities of Parma and Boston (Harvard Medical School), we are currently evaluating several histomorphological parameters (collagen deposition, myocytolysis, myocyte dimension) in relation to post-operative atrial fibrillation. The distribution pattern of connexin-43 (a protein member of the gap junction between myocytes) in the atrial tissue seems to predict post-operative incident 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. Patient enrollment has been concluded on date 30th April 2014 in 31 centers. A total of 473 patients from 31 sites entered into the study. 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 2014, 227 patients have been enrolled in 21 centers; enrollment should be concluded by the end of April 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. CREATIVE - 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 CREATIVE, 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. The protocols for biological samples collection are ready. The materials for sample collection has been distributed to the clinical centers during the kick-off meeting of the subproject.

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 aims and methods used for this project have been published (Jacobs et al, J Biomed Res 2014). The laboratory is also involved in a proof-of-concept clinical study that will evaluate the efficacy of heart failure therapy based on the knowledge of omics-derived biomarkers. The first samples of clinical data from a cohort of elderly subjects have been made available to the HOMAGE consortium. The protocol and operative procedures of the clinical study have been finalized.

**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 on myocardial function 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 late 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.

**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 multcenter 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 to 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 Centers 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.

Studio GISSI Outliers

CAPIRE - Coronary Atherosclerosis in outlier subjects: Protective and Individual Risk factor Evaluation

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).
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 behavior 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.

Risk and Prevention Study (R&P)

R & P, a study on the optimization of cardiovascular prevention in high-risk patients conducted at national level by general practitioners (GPs), had two main objectives: one epidemiological and one experimental. The goal of the epidemiological study was to evaluate the effectiveness of a personalized approach for the implementation of preventive strategies, while the experimental hypothesis was to assess, through a randomized double-blind controlled clinical trial, the efficacy of a daily treatment with n-3 PUFA in reducing the incidence of cardiovascular events, fatal or nonfatal, in a population at high risk. All patients were followed for a mean of five years.

Update of the study

The study was completed in 2011 and 12,521 patients were randomised by a network of 860 GPs. Key findings of the study, published in the New Engl J Med 2013; 368: 1800-1808, show that the addition of n-3 PUFA in high-risk individuals - already treated at best - is not effective in further reducing major fatal and nonfatal complications. Data of the epidemiological study will be soon published: the analysis confirm that a personalized approach, through the use of a checklist for the assessment of the single patient risk profile and the shared decision on treatment priorities, can better support the management and the adherence to preventive approaches in subjects at high cardiovascular risk.

FARMAGOOD Project

Farmagood is a collaborative project in which the IRCCS - Institute for Pharmacological Research Mario Negri (IRFMN) makes available to the Regional Health Service its scientific expertise in the field of pharmacology with the aim of:

- Plan activities aimed at integrating components of innovation and training to address critical issues in regional prescriptive;
- Develop new methods to involve health professionals, patients and citizens in programs of rationalization of the use of drugs in at-risk populations and in specific areas of care and treatment;
- Initiate independent comparative clinical studies, systematic reviews and meta-analysis to answer specific questions of public health priority.

The first phase of this project will be addressed to the issue of the appropriateness of use of biological medicinal products covered by a patent ("originators") and non-patent biologics ("biosimilars") with the implementation of the Project FARMAGOOD - biosimilars.

A number of years since their introduction on the market, the drugs "biosimilars" (somatropin, erythropoietin and filgrastim) remain an unappealing prospective for Italian clinicians (unlike those of other European countries) with consequent impact on potential savings achievable by the NHS.
The next entry into the market of new "biosimilars" - infliximab and in the short-term of some very expensive drugs (cetuximab, trastuzumab, rituximab, insulin glargine) is an urgent problem to start with information of prescribers and patients on the use of biosimilars.

The project FARMAGOOD - biosimilars, consistent with the objectives in terms of Regional prescription appropriateness and rationalization of resources is proposed to build a path agreed and shared with the various operators in the Regional Health Service a number of activities/interventions to:
- Promote the appropriateness of care pathways and rationalizing requirements of biological medicinal products "originator" and "biosimilars";
- Monitor the benefit-risk profile for the use of these drugs in clinical practice (real life utilization);
- Savings and free up resources in the pharmaceutical and healthcare spending.

The project is run in cooperation with the Laboratory for Assessment of quality of care and services for the elderly and the Centre for Health Economics A. and A. Valenti (CESAV).

The health care and economic burden of subjects at high cardiovascular risks in the Lombardy Region

The study is part of a collaboration between the IRCCS-IRFMN and the Lombardy Region (Agreement EPIFARM 2013-2016) which has the overall objective to implement models for the evaluation and control of health care resources in subjects at high cardiovascular risk, through the use of administrative databases. These databases include data of the resident population (encrypted) of the region of Lombardy (2000-2012) relating to: demographic data, drug prescriptions, laboratory test and medical exams and hospital discharges. The objective of the work is to link the information of these databases to identify the pathways of management of subjects at high cardiovascular risk comparing with the recommendations and evidence, in order to promote the appropriate use of health care and economic resources.

In the year 2014 the following analyses were performed:
- Case-control study to assess whether diabetes is an independent risk factor for hospital discharge due to atrial fibrillation (AF) and to estimate the risk of stroke and mortality in diabetic patients compared with non-diabetic subjects (controls) according to presence or absence of AF. Both cohorts were followed for nine years, from 2002 to 2010.
  The results obtained showed, for the first time in a large unselected population, that diabetes is an independent risk factor for AF development. Diabetes and the AF are both independent risk factors for stroke and mortality in diabetic patients and the presence of AF is an additional risk factor for stroke and mortality.

  The project is run in cooperation with the Laboratory of Cardiovascular Clinical Pharmacology.

- Epidemiologic trend of ischemic stroke from 2002 to 2010: prevalence, incidence, recurrence (re-hospitalizations for ischemic stroke) and total mortality in patients with a new diagnosis of ischemic stroke. Trends of the recommended drug prescriptions for secondary prevention of ischemic stroke (blood pressure lowering, anti-platelets, anticoagulants and lipid-lowering drugs) were also analyzed.
  During the observation period 43 352 patients newly diagnosed with ischemic stroke were identified and the data showed a significant reduction in the incidence, recurrence of stroke and mortality and an increase in the percentage of patients receiving the recommended drugs was also observed.

  The project is run in cooperation with the Laboratory for Assessment of quality of care and services for the elderly.

“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, 1282 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 meta-analyses 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 (ISCIHI), FERRER and the Federación Argentina de Cardiología (FAC).

Two countries in Europe (Spain and Italy) and three in South America (Argentina, Brazil e Paraguay) are involved in the study.

The study was divided 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 simvastatin (40 mg), acetylsalicylic acid (100 mg) and ramipril (2.5, 5.0 and 10.0 mg) 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.

The study ended in June 2014 and the results have been published in the J Am Coll Cardiol 2014; 64: 2071-2082. The main results of the study showed that the adherence is significantly higher in patients treated with polypill (FDC) compared with those taking the three drugs separately. No difference was observed between the two groups in term of risk profile and adverse events.

**SECURE study (Secondary prEvention of CardiovascUlaR disease in the Elderly trial)**

The SECURE study is funded by the European Commission through the Horizon 2020 - PHC-17-2014 - Call titled "Comparing the effectiveness of existing health care interventions in the elderly".

In the aging population, the complexity of the treatments and the access to health care, may compromise the adherence to treatments, increasing the number of clinical events. In particular, in cardiovascular prevention, the polypill strategy (with the aim of simplify adherence and improve the control of risk factors), may reduce the risk of death and hospitalizations.

The main objective of the SECURE study is to evaluate, through a randomized multicenter open trial, the efficacy of a polypill containing aspirin (100 mg), atorvastatin (20 or 40 mg), ramipril (2.5; 5.0 o10mg)
compared with drugs taken separately on the prevention of cardiovascular events (cardiovascular death, hospitalization for myocardial infarction, stroke and revascularization procedures), in an elderly population (aged ≥ 65 years) with myocardial infarction.

Secondary objectives of the study are: adherence to treatment, lipid profile and blood pressure reduction, safety / tolerability and cost of the polypill pharmacological strategy.

After the FOCUS study, in which adherence was the primary end point, the SECURE study will assess the efficacy of the polypill on clinical endpoints. The study will be conducted by a consortium of European partners (Spain, Italy, France, Germany, Czech Republic, Poland and Hungary) coordinated by the Centre de Investigaciones Cardiovascular (CNIC) in Madrid.

The study will involve departments of cardiology which will include 3200 patients in 2 years (600 in Italy). The follow-up study will be 24 months.

"Il Sale è meglio averlo in Zucca" project
The project originates 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 its taste by substituting some foods with other adding up spices and aromatic plants or utilizing salt substitutes. The second phase of the project, conducted in collaboration with the Laboratory of Toxicology and Nutrition Institute Mario Negri and Elior (a leader company of collective catering), had the practical aim to collect data on simple actions to reduce salt content in diet without jeopardize its palatability in order to produce a manual for the cooks of Elior. Based on the experience gained during the first phase of the pilot project and after some meetings in a sample kitchen, guidelines for cooking food with less salt and a "low-sodium" cookbook were finalized.

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). Characteristics 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 foorable that these characteristics 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).

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

Forty six centers joined the study so far: 21 Cardiological unit, 14 Centers for the control of anticoagulant therapy, and 11 Internal Medicine/Geriatric units and the number of patients included is 415.

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 ≥180mmHg are actually under treatment) or at high cardiovascular risk (82%)
- Blood pressure control is improved (patients with systolic blood pressure levels ≥ 180mmHg 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, atrial fibrillation and cardiac surgery 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. At present, the superiority trial BeTACTIC is randomizing patients undergone heart transplantation. Recently, The CYCLE study has recruited 410 patients with reperfused acute myocardial infarction and it is in the final data collection phase for patients with follow-up at 12 months.

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.

FINNRESUSCI study recruited patients receiving cardiopulmonary resuscitation and it is evaluating the prognostic value of some biomarkers such as tryptophan, PTX3 and kinurenine to understand their relationship and involvement in the damage post-resuscitation. Main results regarding the large trial concerning cardiovascular prevention, Risk & Prevention study (Rischio & Prevenzione) which included more than 12500 patients have been published on the New England Journal of Medicine.

The epidemiological history of this population is under evaluation according to several statistical analyses with the following main aims: a) to evaluate how the control of modifiable risk factors (smoking, sedentary lifestyle, dietary habits, weight, blood pressure, glycemia, cholesterol) influence the improvement of the overall patient risk profile; b) create a risk chart to assess the outcome of comorbidity in patients with diabetes mellitus, very common disease in this population.

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, on Discriminations Indices and Restricted Cubic Splines (RSC) analysis).

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 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 ten 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. Analyses of the results on subgroups of patients are ongoing.
DEPARTMENT OF MOLECULAR BIOCHEMISTRY AND PHARMACOLOGY

STAFF

Head                     Mario SALMONA, Food Technology D, Ph.D.

Laboratory of Biochemistry and Protein Chemistry
   Head                     Mario SALMONA, Food Technology D, Ph.D.

Human Pathology in Model Organisms Unit

Laboratory of Molecular Biology
   Head                     Enrico GARATTINI, M.D.

Pharmacogenomics Unit
   Head                     Maddalena FRATELLI, Biol.Sci.D.

Gene Structure and Regulation Unit
   Head                     Mineko TERAO, Bioch.D., Ph.D.

Laboratory of Pharmacodynamics and Pharmacokinetics
   Head                     Marco GOBBI, Pharm.D.

Laboratory of Translational Proteomics
   Head                     Valentina BONETTO, Chem.Pharm.D.

Laboratory of Systems Biology
   Head                     Gianfranco BAZZONI, M.D.
Laboratory of Signal Transduction

Head Ester ZITO, Chem.Pharm.D., Ph.D. Genetics
Valentina Bonetto has received a degree in Pharmaceutical Chemistry and Technology from 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 42 publications from 1994 to 2013, in peer-reviewed journals. She is reviewer for scientific journals in the field of Proteomics 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 Responsible of 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) amyloidosic protein and new therapeutic strategies for corresponding diseases (eg Alzheimer’s disease, prion diseases and peripheral amyloidosis); ii) development and application of new analytical assays to study drugs, proteins, nanoparticles, therapeutic antibodies and endogenous biomarkers; iii) nanoparticles for diagnostic and therapeutic purposes. These research fields are investigated by a close integration of
pharmacodynamic (e.g. biomolecular interactions, mainly using surface plasmon resonance) and pharmacokinetic 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 130 scientific publications on peer-reviewed international journals. First or last author in 60 of them.

Reviewer for international scientific journals operating in the Neuroscience/Neuropharmacology, Biochemistry, Nanotechnology fields.

Selected publications


Ester Zito obtained her degree in CTF (Farmaceutical Chemistry) in 2001 and the PhD title in genetics in 2007. She joined the laboratory of Prof. David Ron as post-doc, supported by a Long Term EMBO Fellowship and a Marie Curie IRG (International Reintegration Grant). She focused on the study of the ER (Endoplasmic Reticulum) redox homeostasis and has remained in that area since.

From June 2013 the Laboratory of Signal transduction is directed by Ester Zito as Telethon assistant scientist supported by a DTI (Dulbecco Telethon Institute) career award.

Principali pubblicazioni


Luisa Diomede is a Chemico-Biological Analysis Doctor (University “Carlo Bo”, Urbino, Italy) 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.

Coauthor in more than 60 scientific publications on international journals. Reviewer “ad hoc” for International journals.

1985-1991 Research Assistant, Laboratory of Enzymology, at “Mario Negri” Institute for Pharmacological Research, Milan


1992-2010 Senior Scientist, Laboratory of Biochemistry and Protein Chemistry.

2005- now Member of Quality Assurance Committee of “ Mario Negri” Institute for Pharmacological Research, Milan.

2011-now Head of “Human Pathologies in Model Organisms” Unit.

Principali pubblicazioni

Maddalena Fratelli got her degree in Biological Sciences at the University of Pisa and at the Scuola Normale Superiore in 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 1983-1987 Postdoctoral Associate of the Institute for Cancer Research, Philadelphia, USA From 1987 Visiting Scientist of Mario Negri Institute From 1998 Head of the Unit of Gene Structure and Regulation, Mario Negri Institute

Selected publications

ACTIVITIES

The Department comprises six 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 synthesis Aβ1-40/42 peptides.
Synthesis and chemo-physical characterization of peptides deduced from prion protein sequence.
Identification of molecular mechanisms leading to the formation of soluble toxic oligomers.
Characterization of the elongation kinetics of Aβ peptides by Surface Plasmon Resonance (SPR).
Characterization of the ability of Aβ oligomers to bind to prion protein.
A newly developed study using Surface Plasmon Resonance-based epitope scanning indicates structural differences in brain-derived aggregated mutant prion proteins related to genetic prion diseases.
Role of mutations in tau protein in the pathogenetic mechanisms underlying frontal temporal demetia.
Effect of A2V mutation on the in vitro and in vivo formation of Aβ1-40/42 toxic oligomers.
Generation of new transgenic C. elegans strains pan-neuronally expressing wild-type or A2V-mutated human Aβ1-40.
Recognition of soluble oligomers by a new immunoassay based on SPR and evaluation of oligomers toxicity by a new behavioral test on C. elegans.
Doxycycline persistently accumulates in the brain of patients with Creutzfeldt-Jakob disease chronically treated with the drug.
Generation of a C. elegans model for the investigation of the mechanisms underlying immunoglobulin light chain amyloidosis toxicity.
Identification of tetracyclines as potential therapeutic agents for central and systemic amyloidosis.
Determination of plasma levels of doxycycline in dialysed patients suffering from dialysis related amyloidosis after a chronic treatment with the drug, that resulted in effectively reducing articular disability.
Role of SEPN1 mutations in causing congenital myopathies affecting the Endoplasmic reticulum redox homeostasis.
Role of ascorbic acid in counteracting phenotypic changes associated to congenital myopathies related to the SEPN1 deficiency.
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 disease and motor neuron death in a mouse 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.
Identification and characterization of novel retinoid-based pharmacological combinations for the treatment of acute myelogenous leukemia.
Development of new strategies based on retinoic acid for the stratified therapy of breast.
Molecular cloning and characterization of the cDNAs and genes of four novel members of the mammalian molybdo-flavoprotein family. Definition of a novel gene cluster on human chromosome 2 and mouse chromosome 1.

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 neuro-protective 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.

Development of a new Surface Plasmon Resonance-based immunoassay for rapid, reproducible and sensitive quantification of pentraxin-3 in human plasma.

Sub-cellular distribution studies of nanoparticles.

In vivo tissue distribution of nanoparticles.

Development of new protocols to evaluate, by Surface Plasmon Resonance, the formation of protein corona on the nanoparticles surface.

Development of Surface Plasmon Resonance protocols to evaluate the interaction between nanoparticles and their putative targets.

**NATIONAL COLLABORATIONS**

Advanced Biology Center, Genoa
Fondazione Maugeri, Milan
Fondazione IRCCS Istituto Nazionale Neurologico "C. Besta", Milan
Fondo Edo Tempia, Biella
IFOM Fondazione Istituto FIRC di Oncologia Molecolare, Milan
IRCCS Fondazione "Istituto C. Mondino", Laboratorio di Neurobiologia Sperimentale, Pavia
IRCCS Multimedica, Polo Scientifico e Tecnologico, Milan
Istituto di Biomedicina e Immunologia Molecolare CNR, Palermo
Istituto di Chimica del Riconoscimento Molecolare, Consiglio Nazionale delle Ricerche, Milan
Istituto Clinico Humanitas, Milan
Istituto di Neuroscienze C.N.R., Pisa
Istituto G. Gaslini, Genoa
Istituto Nazionale dei Tumori, Milano
Istituto Nazionale dei Tumori, Naples
Istituto Oncologico Europeo, Milan
Istituto Regina Elena, Rome
Istituto Toscano Tumori, Florence
Ospedale Maggiore Policlinico, Milan
Ospedale Maggiore Policlinico. Istituto di Clinica Neurologica, Milan
Ospedale Niguarda, Centro Clinico Nemo, Milan
Ospedale S. Gerardo, Monza
Ospedale S. Maria Nuova, Reggio Emilia
Ospedale San Matteo, Pavia
TIGEM, Telethon Institute of Genetics and Medicine, Naples
Università degli Studi di Ferrara, Dip. Medicina Sperimentale e Diagnostica, Ferrara
Università degli Studi di Messina, Dip. Farmaco-Chimico, Messina
Università degli Studi di Milano, Dip. Chimica Biochimica e Biotecnologie per la Medicina, Milan
Università di Catania, Dip. Scienze Farmaceutiche, Catania
Università di Genova, Dip. Scienze Farmaceutiche, 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 Research University, Cleveland, OH, USA
Dept. de Quimica-Fisica de Macromoleculas Biologicas, CSIC, Madrid, Spain
ETH, Zurich, Switzerland
Group of C. elegans New Investigators in Europe
IBSN CNRS, Marseille, France
Imperial College London, UK
Indiana University, Indianapolis, USA
Institut de Genetique et Biologie Moleculaire et Cellulaire, Strasbourg, France
Institute for Behavioral Genetics, University of Colorado, USA
Institute Pasteur, Paris, France
John Innes Centre, Norwich, UK
Keio University, Tokyo, Japan
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
Tel Aviv University, Tel Aviv, Israel
University College, Dublin, Ireland
Universidad Nova, Lisbon, Portugal
Université Paris, France
University of Cambridge, UK
University of Cardiff, UK
University of Glasgow, UK
University of Gottingen, Germany
University of London, Royal Veterinary College, UK
University of Muenster, Germany
Vanderbilt University, Nashville, USA
Waring-Webb Institute, University of Colorado, Denver, USA
Weizmann Institut, Rehovot, Israel

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: “The Essential Protein Engineering Summit (PEGS)”, “Applications of Surface Plasmon Resonance for Studying Amyloidogenic Peptides/Proteins”, 5-9 May, Boston, USA

Congress: “ERC-Congress 2014”, “Early activation of the kynurenine pathway predicts early death and long-term outcome in patients resuscitated from out-of-hospital cardiac arrest”, 15-17 May, Bilbao, Spain

Conference: “7th International Conference on Complement of Therapeutics”, “Inhibition of mannose binding lectin is protective in experimental traumatic brain injury”, 6-11 June, Olympia, Greece

Congress: “FENS”, “Cyclophilin A governs TDP-43 function and assembly in hnRNP complexes”, 5-9 July, Milan, Italy


Conference: “V National AriSLA Conference, Fondazione Cariplo”, “Extracellular cyclophilin A as a possible therapeutic target for amyotrophic lateral sclerosis”, 26 September, Milan, Italy

Conference: “ScientificaMente ASC”, “Esempi sulla malattia di Alzheimer”, 27 November, Cislago, Varese, Italy
Congress: “Brain Ischemia and Stroke - BIS14”, “A novel assay to predict mannose binding lectin deposition on the activated endothelium, a key pathogenic event in acute brain injury”, 10-12 December, Rome, Italy

**GRANTS AND CONTRACTS**

Agenzia Italiana del Farmaco, Rome, Italy
Associazione Italiana Ricerca sul Cancro (AIRC), Milan, Italy
Banca Intesa SanPaolo, Milan, Italy
Centro Europeo di Nanomedicina (CEN), Milan, Italy
Comunità Europea (EU), Bruxelles, Belgium
Consiglio Nazionale delle Ricerche (CNR), Milan, Italy
Dipartimento Politiche Antidroga, Presidenza del Consiglio dei Ministri, Rome, 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, Milan-Paris, France
Indena S.p.A., Milan, Italy
Istituto Nazionale Neurologico "C. Besta", Milan, Italy
Ministero della Salute, Rome, Italy
Ministero dell'Istruzione, Università e Ricerca Scientifica (MIUR), Rome, Italy
Perfetti-Van Melle, Lainate (Mi), Italy
Telethon, Milan, Italy

**SCIENTIFIC PUBLICATIONS (2014)**

Rejuvenation Res. 2014 E-pub

SEPN1, an endoplasmic reticulum-localized selenoprotein linked to skeletal muscle pathology, counteracts hyper-oxidation by means of redox-regulating SERCA2 pump activity
Hum Mol Genet 2014 E-pub

Expression of A2V-mutated Aβ in C. elegans results in oligomers formation and toxicity
Neurobiol Dis 2014 62: 521-532

Markoutsa E, Papadia K, Giannou A, Spella M, Cagnotto A, Salmona M, Stathopoulos G T, Antimisiaris S G
Mono and dually decorated nanoliposomes for brain targeting, in vitro and in vivo studies
Pharm Res 2014 31: 1275-1289

Neuroprotective effects of the Sigma-1 receptor (S1R) agonist PRE-084, in a mouse model of motor neuron disease not linked to SOD1 mutation
Memantine prevents reference and working memory impairment caused by sleep deprivation in both young and aged Octodon degus
Neuropharmacology 2014 85: 206-214

Cimini S, Rizzardini M, Biella G, Cantoni L
Hypoxia causes autophagic stress and derangement of metabolic adaptation in a cell model of amyotrophic lateral sclerosis
J Neurochem 2014 129: 413-425

Different mutations at V363 MAPT codon are associated with atypical clinical phenotypes and show unusual structural and functional features
Neurobiol Aging 2014 35: 408-417

Bana L, Minniti S, Salvati E, Sesana S, Zambelli V, Cagnotto A, Orlando A, Cazzaniga E, Zwart R, Scheper W, Masserini M, Re F
Liposomes bi-functionalized with phosphatidic acid and an ApoE-derived peptide affect A? aggregation features and cross the blood-brain-barrier: Implications for therapy of Alzheimer disease
Nanomedicine 2014 10: 1583-1590

A Caenorhabditis elegans-based assay recognizes immunoglobulin light chains causing heart amyloidosis
Blood 2014 123: 3543-3552

Doxycycline in Creutzfeldt-Jakob disease: a phase 2, randomised, double-blind, placebo-controlled trial
Lancet Neurol 2014 13: 150-158

In vivo fate of Avidin-Nucleic Acid Nanoassemblies as multifunctional diagnostic tools
ACS Nano 2014 8: 175-187

Integrated multiplatform method for in vitro quantitative assessment of cellular uptake for fluorescent polymer nanoparticles
Nanotechnology 2014 25: 045102

Messa M, Colombo L, Del Favero E, Cantu' L, Stoilova T, Cagnotto A, Rossi A, Morbin M, Di Fede G, Tagliavini F, Salmona M
The peculiar role of the A2V mutation in Amyloid-β (Aβ)1-42 molecular assembly
J Biol Chem 2014 289: 24143-24152

Monomeric Aβ1-42 and RAGE: key players in neuronal differentiation
Neurobiol Aging 2014 35: 1301-1308

Merlo S, Sironi E, Colombo L, Cardona F, Martorana A M, Salmona M, La Ferla B, Airoldi C
Cis-glyco-fused benzopyran compounds as hit compounds for the development of therapeutic and diagnostic tools against neurodegenerative diseases
Chempluschem 2014 79: 835-843
Blood protein coating of gold nanoparticles as potential tool for organi targeting
Biomaterials 2014 35: 3455-3466

Retinoids and breast cancer: from basic studies to the clinic and back again
Cancer Treat Rev 2014 40: 739-749

A possible role of transglutaminase 2 in the nucleus of INS-1E and of cells of human pancreatic islets
J Proteomics 2014 96: 314-327

Canovi M, Lucchetti J, Stravalaci M, Valentino S, Bottazzi B, Salmona M, Bastone A, Gobbi M
A new surface plasmon resonance-based immunoassay for rapid, reproducible and sensitive quantification of pentraxin-3 in human plasma
Sensors 2014 14: 10864-10875

Sironi E, Colombo L, Lompo A, Messa M, Bonanomi M, Regonesi M E, Salmona M, Airoldi C
Natural compounds against neurodegenerative diseases: molecular characterization of the interaction of catechins from green tea with a?1-42, PrP106-126 and ataxin-3 oligomers
Chemistry 2014 20: 13793-13800

Synthesis and evaluation of a 18F-curcumin derivate for b-amyloid plaque imaging
Bioorg Med Chem 2014 22: 2753-2762

Biodistribution of PEGylated PCL-based nanoparticles in C57BL/6 mice bearing B16/F10 melanoma
Nanotechnology 2014 25: 335706

An integrated approach for the systematic evaluation of polymeric nanoparticles in healthy and diseased organisms

In vivo PET imaging of beta-amyloid deposition in mouse models of Alzheimer's disease with a high specific activity PET imaging agent [18F]flutemetamol

Role of lipid rafts and GM1 in the segregation and processing of prion protein

Davoli E, Scip A, Cecchi M, Cimini S, Carrà A, Salmona M, Borsello T
Determination of tissue levels of a neuroprotectant drug: The cell permeable JNK inhibitor peptide
J Pharmacol Toxicol Methods 2014 70: 55-61

Ranolazine ameliorates postresuscitation electrical instability and myocardial dysfunction and improves survival with good neurological recovery in a rat model of cardiac arrest
Soil quality in the Lomellina area using in vitro models and ecotoxicological assays
Environ Res 2014 133: 220-231

Targeting dopamine D3 and serotonin 5-HT1A and 5-HT2A receptors for developing effective antipsychotics: synthesis, biological characterization, and behavioral studies
J Med Chem 2014 57: 9578-9597

Genevini P, Papiani G, Ruggiano A, Cantoni L, Navone F, Borgese N
Amyotrophic lateral sclerosis-linked mutant VAPB inclusions do not interfere with protein degradation pathways or intracellular transport in a cultured cell model

Investigating heart-specific toxicity of amyloidogenic immunoglobulin light chains: A lesson from C. elegans
Worm 2014 3: e965590

Early activation of the kynurenine pathway predicts early death and long-term outcome in patients resuscitated from out-of-hospital cardiac arrest
J Am Heart Assoc 2014 3: e001094

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J Neurosci 2014 34: 14022-14031
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 present absence of an effective therapy. The severity of the effects 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. With the aim to develop simple models that enable monitoring of the conformational changes that give rise to fibril deposition, we have designed and developed a variety of synthetic peptides. These peptides are deduced from the primary sequence of human amyloidogenic proteins in their wild-type or mutated forms. In addition, synthetic peptides with different chemo-physical properties and biological effects have been employed to elucidate the biochemical and molecular mechanisms underlying the toxicity of different molecular protein assemblies.

In collaboration with the Istituto Neurologico “Carlo Besta” of Milan we have identified a mutated form of -amyloid (A2V) that displays amazing biological features since it binds to wild-type -amyloid and inhibits amyloid formation and the onset of the disease. This observation paves the way for new therapeutic perspectives, for both 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 A peptides containing the same mutation and we have evaluated its importance in the aggregation. Similar studies have been carried out using amyloidogenic proteins responsible of peripheral amyloidosis. In particular, in collaboration with the Centro per la cura delle Amiloidosi Sistemiche of S. Matteo, Pavia, we have carried out molecular studies to unveil the mechanisms responsible for the cardiotoxicity of light chain immunoglobulins.

The nematode Caenorhabditis elegans to investigate 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 an experimental model since it offers the unique opportunity to analyze the genetic and molecular functions of human disease-related genes in vivo. Using this nematode, it is possible to correlate the phenotype of the transgene with the degeneration, by examining the protein expression and its aggregation into the oligomeric or fibrillar forms. Different transgenic strains, constitutively or temperature-dependently expressing various fragments of human 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 of these mutations. The expression of these peptides results in the appearance of specific phenotypes, such as the progressive paralysis of the nematodes. 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, and the kinetics 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.
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Tau protein represents another important research topic in our laboratory. In particular, in recent years, we demonstrated that mutations in tau could affect the pathogenesis of frontotemporal dementia, a heterogeneous group of neurodegenerative diseases that belongs to tauopathies. We are currently generating transgenic worms expressing different isoforms of tau. We have demonstrated that the molecular mechanisms observed in transgenic C. elegans strains expressing central amyloidogenic proteins are similar to those underlying proteins involved in systemic amyloidosis, such as immunoglobulin light chains and β2-microglobuline. These simple models, that can be used to integrate multidisciplinary approaches, comprising those that are both genomic and molecular, represent the basis for in vivo analysis of the functional activity of genes related to human amyloidosis and the design and validation of innovative therapies.

The use of nanoparticles for diagnosis and therapy

The therapeutic effectiveness of molecules with promising pharmacological activity is often hindered by problems relating to their poor bioavailability, rapid clearance, difficulty in crossing biological barriers and their risk of generating significant side-effects. The use of nanoparticles (NP) able to selectively interact with sub-cellular structures, represents an innovative approach to overcome, at least in part, these problems. To this end, the understanding of the behavior of NP in biological systems at an increasing complexity (biological fluids, cells, healthy and pathological model of human disorders) represents the starting point. Within the frame of a project granted by the Italian Association for Cancer Research (AIRC 5x1000) we have developed an integrated platform to evaluate the potentiality of polymeric NP. In collaboration with the Department of Oncology and the Polytechnic of Milan, we have investigated the sub-cellular and tissue distribution of biocompatible NP using both cellular and animal models of breast cancer (triple negative breast cancer). The results obtained have contributed to the outlining of the chemical-physical parameters required for the generation of aimed NP with a tissue-specific tropism.

In collaboration with Dr. M. Morpurgo of the University of Padua, we have developed and characterized an innovative biocompatible NP called Avidin-Nucleic Acid Nanoassemblies (ANANAS). It is composed of two proteins expressed in the egg linked to a back-bone of inactivated nucleic acids. These NP offer the advantage of being biocompatible, degradable and capable of exerting chemical residuals, that are highly tunable in terms of binding to molecules of therapeutic interest. Studies performed in biological fluids, cells, and in vivo confirmed ANANAS’ ability to transport molecules applicable for diagnosis and therapy in many research areas.

The use of NP for diagnosis has been evaluated by using the technique identified by the term "preclinical imaging" which integrates the information obtained from non-invasive imaging with histopathology and immunohistochemistry. The biodistribution of stem cells loaded with NP functionalized with fluorescent or superparamagnetic contrast agents has been evaluated by using magnetic resonance imaging and fluorescence molecular tomography. In applying these techniques, we have evaluated the distribution of human amniotic mesenchymal nucle cells containing NP leaded with Feridex or polymeric NP containing a fluorofore, in transgenic mice currently used as a model of amyotrophic lateral sclerosis (ALS).

Recently we have developed an innovative analysis employing “dual” NP containing both a paramagnetic compound and a fluorofore. If successful, this technique could serve as a diagnostic procedure that can be applicable to the clinical practice.

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 vitamin 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 amyotrophic 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 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.

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. For example, the laboratory is a partner in an integrated European 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 plasma concentrations of donepezil and memantine, either in humans and new animal models, were pharmacological effects have been evaluated in parallel. Moreover, we have been 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 Creutzfeldt-Jacob diseases (PrP disease) or peripheral amyloidosis (dialysis-related or transthyretin-related amyloidosis).

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) and with Istituto Clinico Humanitas, for studies regarding the characterization of protein interactions relevant for immune response and complement cascade activation.

Development and application of innovative analytical assays
As a partner of a multicentre project entitled “Miniaturized System for Molecular Diagnostic and Proteomic of Sepsis Based on Integration of Surface Plasmon Resonance”, in 2014 we have developed and validated an innovative surface plasmon resonance-based immunoassay for rapid, reproducible and sensitive quantification of pentraxin-3 in human plasma, a marker of inflammation.
We also contributed in a project carried out with the Cardiovascular Clinical Pharmacology lab (Drs Ristagno and Latini) and with the Department of Anesthesiology and Intensive Care Medicine of Helsinki University, in a study on 245 patients resuscitated from out-of-hospital cardiac arrest. In particular we developed and applied HPLC-MS methods to measure plasmatic
levels of the metabolites of the so-called kinurenin pathway, observing that this pathway is activated early after cardiac arrest and is associated with severity of post–cardiac arrest shock.

In 2014 a project also started regarding new psychoactive substances (NPS), i.e. synthetic molecules with unknown pharmacological and toxicological properties, but whose appearance in the market poses new threats to public health. This is a project carried out for the National Early Warning System on Drugs of the Department for Antidrug Policy, Presidency of the Council of Ministers, in collaboration with the laboratory of Experimental Psychopharmacology. The project aims at defining the pharmacological and pharmacokinetic properties of these molecules in animal models, and our laboratory is in charge of the development and application of new HPLC-MS methods to measure their plasma and brain concentrations.

**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 nitritative 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 nitrated 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 nitrated 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.
Dissecting the complex interplay between ER redox homeostasis and muscle pathophysiology

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 oxidoreductases; 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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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.

**Awards:** EEC scholarship for postgraduate training in Epidemiology (1988).

**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.

**Awards:** “Rafaelson Scholar Award”from the Collegium Internationale Neuro-Psychopharmacologicum (CINP), 16th Meeting, Munich (F.R.G.), (1988).

**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

- Santoro E. “Web 2.0 e social media in medicina: come social network, wiki e blog trasformano la comunicazione, l’assistenza e la formazione in sanità. 2° edizione. Il Pensiero Scientifico Editore, Roma 2011
- Santoro E. “Facebook, Twitter e la medicina”, Il Pensiero Scientifico Editore, Roma 2011
- Santoro E., Tinazzi A. “Clinical Trials Data Management”. In “Clinical Trials Handbook” (Wiley 2009, Edited by Gad S.C).

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, and a PhD at the, School for Nutrition, Toxicology and Metabolism (NUTRIM), Maastricht University, Maastricht, the Netherlands. 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/June 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 and other chronic conditions. In particular case-control studies on cancers of the upper respiratory and digestive sites, thyroid, breast and hormone-related cancers; 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 over 300 publications on peer-reviewed scientific Journals cited in PubMed/MEDLINE. Mean Impact Factor: 4.3. H-index: 46.

Selected publications:

Liliane Chatenoud


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. H-index: 35 (Web of Science).

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 Obesity Journal (since 2008), The Open Demography Journal (since 2009), World Journal of Gastrointestinal Oncology (since 2009), World Journal of Dermatology (since 2010), World Journal of Clinical Urology (since 2011), ISRN Public Health (since 2013).

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 210 publications on peer-reviewed scientific Journals cited in PubMed/MEDLINE. H-index: 31 (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).

Areas of interest: Case-control and occupational cohort studies on risk factors for cancer and other chronic diseases. Meta- and pooled-analysis of observational studies and of clinical trials. Analysis of the clinical and socio-economic impact of influenza and other infections in the pediatric age. Author/co-author of about 160 publications in international scientific journals. H-index: 30 (SCOPUS); 42 (Google Scholar).

Selected publications
ACTIVITIES

The Department of Epidemiology is involved in the epidemiology of several common neoplasms (including cancers of the breast, female genital tract, respiratory and digestive sites, prostate and urinary organs, sarcomas, lymphoid malignancies, etc.) and other chronic conditions, 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 the development of cancer at various anatomical sites or cardiovascular diseases and several factors, such as genetic factors (family history), selected lifestyle habits (diet, tobacco, alcohol, coffee, etc.), selected diseases (diabetes, hypertension, obesity, etc.), use of selected drugs and exogenous hormones, and exposure to various environmental substances. In particular, the Department works on the analysis of dietary correlates of cancer risk and cardiovascular diseases; 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, herbicides and other known or potential carcinogens; the study of the role of infections in the etiology of atopic diseases (“Hygiene hypothesis”); the evaluation and monitoring of human papillomavirus (HPV) in women at high risk of cervical cancer; 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. Moreover, the Department of Epidemiology collaborates in epidemiological and clinical studies in pediatrics and oncology with other Italian and European groups. 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

Through a systematic literature review and meta-analysis of epidemiological studies on the association between alcohol consumption and risk of developing cancer, we provided solid and updated scientific evidence showing that alcohol consumption leads to increased risk of developing cancer of the oral cavity, esophagus, colorectum, liver, larynx, and female breast. In addition, there is growing evidence of an increased risk of pancreatic, prostate and skin (malignant melanoma) cancer in alcohol drinkers.

A systematic review and meta-analysis showed that alcohol consumption is not related to the risk of developing leukemia, although a significantly increased risk emerged in Asian Studies and a decreased risk in American ones.

A systematic review and meta-analysis of cohort studies of workers exposed to polycyclic aromatic hydrocarbons showed an excess risk of cancers of the respiratory tract (mainly lung) and bladder in workers employed in iron and steel foundries. The meta-analysis also showed
that workers employed in the aluminum production had an increased, although not significant, risk of developing bladder cancer.

A systematic review of the literature and meta-analysis showed that alcohol consumption is positively associated with the risk of developing malignant melanoma of the skin; the effect of alcohol consumption is no longer statistically significant when taking into account the exposure to sunlight.

A systematic review of the literature and meta-analysis showed that alcohol consumption is not related to the risk of developing multiple myeloma, although a modest protection seems to emerge for moderate consumption.

An Italian case-control study showed that an appreciable proportion of pancreatic cancers could be avoided by intervention on a few selected modifiable factors, including tobacco smoking, heavy alcohol consumption, and diabetes.

An International large collaborative study within the Pancreatic Cancer Case-control Consortium (PanC4), including more than 8,000 pancreatic cancer cases, confirmed an excess risk of pancreatic cancer among diabetics and it showed that a 30% excess risk persists for more than 20 years after diabetes diagnosis. Moreover, oral antidiabetics may decrease the risk of pancreatic cancer, whereas insulin showed an inconsistent duration-risk relationship.

A systematic review and meta-analysis of the literature indicated a modest favorable effect of aspirin on prostate cancer. Inference for causality and public health implications are, however, far from conclusive given the heterogeneity of results and the lack of dose and duration-risk relationships.

An analysis of dietary patterns showed that a diet characterized by a high consumption of animal products, including several types of meat and dairy products, as well as of (refined) cereals and sugars, is positively associated with prostate cancer.

An Italian case-control study on breast cancer with information on estrogen receptor (ER) and progesterone receptor (PR), showed stronger associations with selected menstrual and reproductive factors for ER+(PR+) than for ER–(PR–) breast cancers, though in the absence of significant heterogeneity.

Low levels of income and educational attainment are associated with more than 2-fold increased risk of head and neck cancer; this association is not entirely explained by differences in the distributions of lifestyle risk factors for these cancers.

Bladder cancer risk is 3-fold higher in former smokers and more than 6-fold higher in current smokers. Bladder cancer risk steadily increases with increasing intensity (OR=8.75 for ≥25 cigarettes/day) and duration of smoking (OR=5.46 for ≥50 years). Elimination of tobacco smoking may prevent about 65% of bladder cancers.

Our study provided strong evidence of a beneficial role of the Mediterranean diet on oral cavity and pharyngeal cancer.

Long-term decreasing trends were observed for male esophageal cancer mortality in several southern and western European countries, whereas in central Europe mortality increased until the mid-1990s and started to stabilize or decline over the last years.

Considerable decreases in alcohol consumption have been reported in France, Italy, Spain, and other southern European countries over the last decades.
In a study based on data from two large Italian case-control studies on gastric cancer, through three different scores, we found a favorable effect of a high adherence to the Mediterranean diet on this neoplasm.

In a case-control study, \textit{a posteriori} derived dietary patterns characterized by high intakes of animal products increased endometrial cancer risk, the association being stronger among obese women.

\textit{A posteriori} derived dietary patterns indicated that a diet rich in animal products, starch and fat increase the risk of nasopharyngeal carcinoma.

A systematic review and meta-analysis of epidemiological studies indicated that patients with sarcoidosis have a higher risk of developing cancer.

Data from an Italian case-control study, including 760 cases and 682 controls, support a favorable role of high adherence to the Mediterranean diet on non-fatal acute myocardial infarction.

Data from our study on gastric cancer and results from our meta-analysis on 23 case-control studies and 4 cohorts showed a reduced gastric cancer risk for high consumption of allium vegetables.

Our study supported a positive association between dietary GL and the risk of cataract extraction, independently from diabetes, and a lack of association for GI.

A meta-analysis of 16 studies suggested a favorable role of high allium vegetables consumption (particularly garlic) on colorectal cancer risk. A significant inverse association emerged also for colorectal adenomatous polyps (4 studies).

A systematic review and meta-analysis of cohort studies showed an increased liver cancer risk among drinkers of 3 or more drinks per day. Moderate drinkers (less than 3 drinks) were not at increased risk.

A meta-analysis of 17 studies on over 8500 cases found no increased bladder cancer risk among personal users of hair dyes.

In a brief review, we discussed on aggregations within families of cancers at the same site and at discordant sites. These include aggregations between cancers at breast, stomach and ovary, between cancers at prostate, urinary tract and other sites, and between several tobacco-related neoplasms. Shared exposures to environmental factors within families account for some of the observed aggregations, together with heritable, and hence, genetic factors.

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.

A systematic review and meta-analysis of epidemiological studies indicated that dietary acrylamide is not related to the risk of most common cancers. A modest association for kidney cancer could not be excluded.

A synthesis of the epidemiological evidence led to conclude that high allium vegetable consumption is likely to reduce gastric cancer risk.

Smoking and being overweight before a diagnosis of pancreatic cancer may play a role in the prognosis of this disease, besides its etiology.
A large pool of case-control studies within the International Head and Neck Cancer Epidemiology (INHANCE) Consortium supports a protective effect of total folate intake on the risk of oral cavity and pharyngeal cancers.

Using data from a representative survey conducted in Italy in 2013, we found that more than 45 million Italian adults have heard about e-cigarettes, 3.5 million have tried e-cigarettes, and more than 600,000 Italians regularly use e-cigarettes. 90% of e-cigarette users did not quit smoking as a consequence of starting vaping e-cigarettes. Almost 900,000 Italian never-smokers, particularly young never-smokers, have tried this new and potentially addictive product at least once.

Despite the high percentage of smokers who smoke in private vehicles (65.5%), the majority of the Italian population (almost 80%) supports the adoption of a smoking ban in cars. Smoke-free laws should therefore be extended to private vehicles, particularly if they are carrying children.

In a survey conducted in 2013, we observed that 11% of Italian adults regularly use aspirin for the prevention of cardiovascular diseases. However, more than half of the subjects at high cardiovascular diseases risk do not regularly use it for primary or secondary prevention.

In 2010, within the PPACTE project, we conducted a representative face-to-face survey on smoking in 18 European countries (Albania, Austria, Bulgaria, Czech Republic, Croatia, England, Finland, France, Greece, Hungary, Ireland, Italy, Latvia, Poland, Portugal, Romania, Spain and Sweden) on a total of 18 056 adults. Overall, 27.2% of the participants were current smokers (30.6% of men and 24.1% of women). Smoking prevalence was highest in Bulgaria (40.9%) and Greece (38.9%) and lowest in Italy (22.0%) and Sweden (16.3%).

Using the PPACTE data, we found that in Europe 10.4% of current smokers (12.9% of men and 7.5% of women) were 'predominant' roll-your-own (RYO) users. The proportion of RYO smokers is substantial in several European countries including England (27.3%), France (16.5%) and Finland (13.6%).

PPACTE data provided information on the size of the illicit tobacco trade in Europe, using a comprehensive measure based on visual inspection of the latest purchased cigarette pack. Tax evasion is 6.5% in Europe.

Using PPACTE data, we observed that in Europe, the greater is the adoption of tobacco control policies at national level, the greater is the proportion of homes with voluntary smoking ban.

This finding was in broad agreement with results from a previous ecologic study using data from a special Eurobarometer survey conducted in 2009, showing that smoke-free legislation in workplaces and public places was not correlated with increased smoking prevalence in private venues (houses and cars).

PPACTE data also provided a baseline for evaluating future trends in public support for extreme propositions to end or drastically cut smoking: in 2010, approximately one in three adults (and one in four smokers) supported a comprehensive tobacco endgame intervention in Europe.

In Europe, 47.6% of adults are overweight or obese (54.5% in men and 40.8% in women), and 12.8% (14.0% in men and 11.5% in women) are obese. The lowest prevalence of obesity was observed in Mediterranean countries, particularly in Italy and France.

The effectiveness of trastuzumab (T) as first-line therapy for metastatic breast cancer (mBC) in women previously treated with T in the adjuvant setting was investigated. The outcome of
women receiving first-line T treatment for mBC after T failure in the adjuvant setting is comparable to that of women not receiving T for early breast cancer.

A favorable role of dietary non-enzymatic antioxidant capacity in the prevention of myocardial infarction emerged in our study, encouraging a high consumption of fruit and vegetables and a moderate consumption of wine and whole cereals.

In a large cohort of Italian women treated with trastuzumab-based adjuvant regimen for HER2-positive early breast cancer in clinical practice, the survival rate within the fourth year of follow-up (89.4%) was consistent with estimates from clinical trials. Regimens lasting < 6 months resulted in a worse prognosis.

Our study showed that the glycemic load is an important determinant of the more common ischemic — though not of the hemorrhagic — stroke, in the Greek cohort of the population-based European Prospective Investigation into Cancer and nutrition (EPIC) comprised of about 20,000 participants.

Health care administrative databases showed that breast cancer patients using trastuzumab increased between 2004 and 2009 both in Lombardy and in Palermo district. Younger breast cancer patients were more likely to receive trastuzumab than the elderly, but the difference declined over time.

In the Multicenter Italian Lung Detection (MILD) trial, plasma microRNA signatures showed predictive, diagnostic, and prognostic value for lung cancer. They could reduce the false-positive rate of low-dose computed tomography, thus improving the efficacy of lung cancer screening.

In a review we discussed the history, the health properties, the chemistry, the anticancerogenic evidences and the anticancer mechanisms of allium vegetables. Epidemiological studies suggested a favorable role of high intakes of allium vegetables, mainly garlic and onion, in the etiology of gastric cancer.

Official data for thyroid cancer from the World Health Organization (WHO) online database, for the period 1970–2012 were analyzed. The declines in thyroid cancer mortality reflect both variations in risk factor exposure and changes in the diagnosis and treatment of the disease, while the increases in the incidence are likely due to the increase in the detection of this neoplasm over the last few decades.

Cancer mortality predictions for 2014 confirm the overall favorable cancer mortality trend in the EU, translating to an overall 26% fall in men since its peak in 1988, and 20% in women, and the avoidance of over 250,000 deaths in 2014 compared with the peak rate. Notable exceptions are female lung cancer and pancreatic cancer in both sexes.

Gastric cancer incidence and mortality decreased substantially over the last decades in most countries worldwide, with differences in the trends and distribution across regions. The predictions for 2015 show that a leveling off of rates is expected in the USA and a few other countries.

NATIONAL COLLABORATIONS

Associazione Italiana di Oncologia Medica (AIOM)
Arcispedale S. Maria Nuova, Reggio Emilia
ASL di Bergamo
ASI Milano 2
Associazione Nazionale dei Medici Cardiologi Ospedalieri (ANMCO)
Azienda Ospedaliera Niguarda Ca’ Granda, Milano
Azienda Ospedaliera San Gerardo, Monza
Azienda Provinciale per i Servizi Sanitari Azienda Autonoma di Trento
Azienda ULSS n.6 Vicenza
Centro di Riferimento Oncologico, Servizio di Epidemiologia e Biostatistica, Aviano (PN)
Comune di Milano, Direzione Centrale Salute, Settore politiche per la Salute
Ente Ospedaliero Cantonale di Bellinzona
Federazione Italiana Medici Medicina Generale (FIMMG)
Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milano
Fondazione Politecnico di Milano
Fondazione SmithKline, Milano
Gruppo Italiano Studi Epidemiologici in Dermatologia GISED, Bergamo
Gruppo Italiano Documentalisti dell’Industria Farmaceutica e degli Istituti di Ricerca
Biomedica
International Centre for Pesticides and Health Risk Prevention, Milano
Istituto Auxologico Italiano, Laboratorio Sperimentale di Ricerche Endocrinologiche IRCCS, Milano
Istituto DOXA, Milano
Istituto Europeo di Oncologia, Divisione di Epidemiologia e Biostatistica, Milano
Istituto Europeo di Oncologia, Divisione Melanomi e Sarcomi Muscolo Cutanei
Istituto di Fisiologia Clinica CNR, Sezione di Milano, Milano
Istituto Internazionale di Telemedicina (IITM)
Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (INRAN), Roma
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 Ortopedico Gaetano Pini, Centro di Chirurgia Ortopedica Oncologica, Milano
Istituto per lo Studio e la Prevenzione Oncologica (ISPO), Firenze
Istituto Superiore di Sanità, Osservatorio Fumo Alcol Droga, Roma
Istituto Tumori “Fondazione Pascale”, Servizio di Epidemiologia, Napoli
Ospedali Riuniti di Bergamo
Ospedale Alessandro Manzoni, Unità di Gastroenterologia, Lecco (LC)
Ospedale Galliera di Genova
Ospedale “Luigi Sacco” Azienda Ospedaliera – Polo Universitario ok
Azienda Ospedaliera S. Gerardo, Unità Operativa di Maltitie Infettive, Monza (MB) ok
Ospedale Galliera di Genova
Policlinico di Monza, Unità Operativa di Endoscopia I, Monza (MB)
Ospedale “Luigi Sacco” Azienda Ospedaliera – Polo Universitario ok
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Prima Clinica Ostetrico Ginecologica, Mangiagalli, Milano
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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, Ferrara
Università Milano-Bicocca, Dipartimento di Statistica e Metodi Quantitativi, Milano
Università di Milano-Bicocca, I Clinica Otorinolaringoiatria, DNTB, Monza
Università degli Studi di Milano, Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Milano
Università degli Studi di Milano, Dipartimento di Scienze Cliniche e di Comunità, Milano
INTERNATIONAL COLLABORATIONS

Aichi Cancer Center Research Institute, Division of Epidemiology and Prevention and Nagoya University Graduate School of Medicine, Nagoya, Japan
Institut Català d'Oncologia (ICO), Cancer Prevention and Control Program, Tobacco Control Unit, L'Hospitalet de Llobregat, Barcelona, Spain
Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Cancer Control and Prevention Group, L'Hospitalet de Llobregat, Barcelona, 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
Harvard School of Public Health, Department of Epidemiology, Boston, USA
Harvard School of Public Health, Department of Nutrition, Boston, USA
Hellenic Health Foundation, Athens, Greece
Hôpital Necker - Enfants Malades, Centre of the Association Claude Bernard on Auto-immunes diseases, Parigi, France
Institute de Academie des Sciences, Paris, France ok
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
Society for Internet in Medicine
Spanish National Cancer Research Center, Centro Nacional de Investigaciones Oncologicas (CNIO), Madri, Spain
The Ohio State University College of Public Health, Columbus, Ohio, USA
The Tisch Cancer Institute and Institute for Translational Epidemiology, Mount Sinai School of Medicine, New York, NY, USA
Tobacco Free Research Institute, Dublino, Ireland
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
University of Porto, Faculty of Medicine, Department of Clinical Epidemiology, Preventive Medicine and Public Health. Porto, Portugal

EDITORIAL BOARD MEMBERSHIP

Advances in Therapy (Eva Negri)
BMC Public Health (Silvano Gallus, Associate Editor)
European Journal of Cancer (Cristina Bosetti)
ISRN Cardiology (Eugenio Santoro)
ISRN Public Health (Silvano Gallus)
PEER REVIEW ACTIVITIES

Acta Dermato-Venereologica; Acta Psychiatrca 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 Neurology; European Journal of Public Health; Evidence-Based Healthcare and Public Health; Food and Chemical Toxicology; Global Health Action; 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 Internet Research; 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; Oxford Economic Papers; 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; Tobacco Induced Diseases; Tumori; World Journal of Gastroenterology.

NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP

Executive Committee, International Head and Neck Cancer Epidemiology (INHANCE) consortium (Negri)

Steering Committee, Stomach cancer Pooling (StoP) Project (Pelucchi, Negri)
Member of the European Food Safety Agency (EFSA) Panel on Acrylamide in Food (Bosetti)

Member of the EFSA Panel on Isoflavones (Bosetti)

Member of the Scientific Evaluation Committee (SEC) of the ERA-NET TRANSCAN (European Research Area Network on Translational Cancer Research) (Bosetti)

Jury of the National Communication, Marketing and Information for Health - International Journalism Festival (Santoro)

Steering Committee of the portal www.familyhealth.it (Santoro)

Jury Committee Digital Awards (organized by Aboutpharma prize for the best applications in the medical and social media apps) (Santoro)

Scientific Committee of the congress TeleMediCare 2014 (Santoro)

**EVENT ORGANIZATION**

Meeting ERC. Case-control study on atopic dermatitis. Milan, 23/1/2014

Meeting Case-control study group, Istituto Mario Negri, Milan, 7/2/2014


Course “Web 2.0, social media e apps per l’aggiornamento del medico e dell’operatore sanitario”, IRCCS Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, 11/10/2104

Course “Social network, social media e apps per la comunicazione e la promozione della salute”, IRCCS Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, 19/10/2014

Workshop “Smart media e social network in medicina”, DISCO - Università Milano Bicocca, Milano, 24/1/2014

**CONFERENCE AND WORKSHOP CONTRIBUTIONS**

Giornata di studio. Facoltà di Medicina della Seconda Università di Napoli e dell’Osservatorio Permanente Giovani e Alcool. Le bevande alcoliche tra stili alimentari e conseguenze per la salute. “Alcol e tumori con focus sulle basse dosi”,”Alcol e rischio di cancro nella popolazione anziana italiana”. Napoli. 21/1/2014
Television program Fuori TG (RAI 3) as an expert on coffee and health. Milan, 12/2/2014


The ESHRE Capri Workshop. The unexpected global success of emergency contraception. Capri 29-30/8/2014

Scientific Evaluation Committee JTC 2013. Palermo, Italy. 17-18/9/2014

ESMO conference 2014; Poster discussion - Challenges in cancer screening and care: dealing with the issues of access and cost of therapy; Madrid, Spain 27/9/2014

XXIX Meeting GISED (Gruppo Italiano Studi Epidemiologici in Dermatologia). The hygiene hypothesis: revisiting the concept in an Italian matched case-control study on incident atopic dermatitis in childhood. Cremona, 2/10/2014.

Television program Elisir (RAI 3) as an expert on coffee and health. Milan. 6/10/2014.

Alcohol and cancer risk in the Italian elderly population. Alcohol, tobacco, obesity. Noto, 8/10/2014

Award Ercole Pisello, XXII edition. Bevagna, 18/10/2014

Capita Selecta in Complex Disease Analysis conference and EU COST Pancreas annual meeting. Liège, Belgium 24-26/10/2014


Workshop “Smart media e social network in medicina”, DISCO - Università Milano Bicocca. “Social media e apps nel monitoraggio e prevenzione delle malattie e nella promozione della salute”. Milano 24/1/2014

Course “La ricerca bibliografica su PubMed/Medline”, Ente Ospedaliero Cantonale di Bellinzona, Bellinzona 26/2/2014

Advanced course “Formazione su metodologia, strategie e tecniche della ricerca clinica”. Associazione Nazionale Medici Cardiologi Ospedalieri, Firenze 27/2/2014
Seminar “La letteratura scientifica per la qualità dei Servizi Sanitari”, Biblioteca Virtuale Per la salute.”Internet e social media per la medicina”. Piemonte, Centro Incontri - Regione Piemonte, Torino 28/2/2014

Course “Seminario di formazione per Ricercatori a Tempo Determinato”, Facoltà di Medicina e Chirurgia, Università degli Studi di Milano, Milano 21/3/2014

Course “Internet al servizio dell’aggiornamento del medico e dell’operatore sanitario”, Ente Ospedaliero Cantonale di Bellinzona, Bellinzona 2/4/2014


Course “La nuova comunicazione sanitaria ed il web 2.0”, Ausl della Romagna, Cesena 14/4/2014

Course “Twitter, Facebook, i nuovi social media e le apps per l’aggiornamento medico e dell’operatore sanitario”, Ente Ospedaliero Cantonale di Bellinzona, Bellinzona, 7/5/2014

Course “Social media in sanità: nuove opportunità di aggiornamento e comunicazione”, Federazione dei Medici di Medicina Generale, Milano, 16/5/2014

Course “Social media e social network per l’aggiornamento in medicina e per la promozione della salute”, Fondazione Biblioteca Biomedica Biellese, Biella 19/5/2014

Workshop “La comunicazione della medicina sul web” organizzato dal Dipartimento di Studi Umanistici nell’ambito del Master in giornalismo e comunicazione istituzionale della scienza, Università di Ferrara, Ferrara 9/5/ 2014


Course “Il web 2.0 e i social media al servizio della formazione e dell’aggiornamento del medico e dell’operatore sanitario”, Azienda Provinciale per i Servizi Sanitari Azienda Autonoma di Trento, Trento 4-5/6/2014-1-2/10/2014


Congress “Medicine 2.0 Congress”. “Social Media and Mobile Usage Among Italian Cardiologists”, Malaga 8-10/10/2014

Course “Web 2.0, social media e apps per l’aggiornamento del medico e dell’operatore sanitario “, Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, 11/10/2104

Course “Social network, social media e apps per la comunicazione e la promozione della salute “, Istituto di Ricerche Farmacologiche “Mario Negri”, Milano, 19/10/2014

Congreese “Giornata Mondiale del Diabete 2014”. “Social media, blog Internet… istruzioni per non farsi acchiappare dalla rete”. Palazzo delle Stelline, Milano 14/10/2014

Course “I social media e le apps per l’aggiornamento dell’operatore sanitario e per la promozione della salute”, Azienda ULSS n.6 Vicenza, 16/10/2014

Workshop “Digital Health. Marketing e Comunicazione digitali nell'area della Salute”, Master in Economia e Gestione della Comunicazione e dei Media, Facoltà di Economia, Università Tor Vergata.e “Il marketing e la comunicazione digitali in sanità: canali, strumenti e opportunità” Roma, 17/10/2014

Course “Digital Health Days”, Roche, Monza 20-21/10/2014

Round Table “Digital Awards”, AboutPharma Digital Awards 2014, Milano 30/10/2014


University Master in Clinical Research (1° level), Università degli Studi di Milano, 2014-2015. “Internet e le nuove tecnologie per l’aggiornamento medico-scientifico”, Milano 3/12/2014

Course “Comunicazione e Salute 2.0. FOCUS SOCIAL MEDIA DA STRUMENTO DI AGGIORNAMENTO PROFESSIONALE A MEZZO PER COMUNICARE LA SALUTE” Ospedale Galliera di Genova, Genova, 19/12/2014

GRANTS AND CONTRACTS

Associazione Italiana per la Ricerca sul Cancro (AIRC)
ASL 2 Provincia Milano
Azienda Ospedaliera S. Gerardo, Unità Operativa di Maltie Infettive, Monza (MB)
Azienda Provinciale per i Servizi Sanitari Azienda Autonoma di Trento
CEFIC/LRI
Centro di Ricerche sulla Gestione dell’Assistenza Sanitaria e Sociale (CERGAS), Università Commerciale
SCIENTIFIC PUBLICATIONS (2014)


**LAY PRESS SELECTION (2014)**


Santoro E. Smart media e social networks in medicina. Partecipasalute 18 aprile 2014; http://www.partecipasalute.it/cms_2/node/3411

Santoro E. MyHealthApps e i portali per la diffusione di apps mediche. Partecipasalute 3 aprile 2014; http://www.partecipasalute.it/cms_2/node/2907

Santoro E. Medicina, social network e smart media: come sta cambiando il nostro mondo. Avvenire medico 2014; n. 1: 9-10
Santoro E. Scambio di e-mail tra medico e malato: si o no? Recent progressi in Medicina 2014: 105: 360.

Santoro E. MyHealthApps: un nuovo portale. Ricerca & Pratica 2014 n.176 : 83

Santoro E. All'empowerment le ali del web 2.0. Sole 24 Ore Sanita 2014 n. 19 : 11

OTHER PUBLICATIONS (2014)


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, dietary indexes) of food groups and nutrients, with focus on several specific diet components (e.g., flavonoids, antioxidants).
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. History of diabetes and the metabolic syndrome in the development of various common neoplasms.
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. 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.

7. Prospective studies on factors associated with cancer risk, survival and mortality, by linking our case-control studies database with local and national (administrative) data.

8. 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, stomach, pancreas, breast and female genital tract, thyroid and lymphomas.

9. 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 CONSORTIUM**

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. Over 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, over 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 (e.g., folate intake, allium vegetables consumption) have been analyzed during 2014.

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 8000 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 mastectomy, diabetes) and reproductive factors. New analyses are on-going for selected dietary items (including acrylamide, vitamin D, coffee, …) and selected risk factors in never smokers.

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 asses 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.

**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 non-enzymatic antioxidant capacity (NEAC) from diet has also been investigated, and inverse relations were reported for colorectal, gastric and endometrial cancer risk. The goal of the project is to examine the role of flavonoids, including proanthocyanidins, and NEAC 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.

**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 favorably 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 favorable 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 favorable 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. We achieved 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 2014, 838 women were enrolled in the DHIV cohort, 521 in the DDRI, 1302 in the D1318P, 573 in the D1325G, and 1424 in the DASS, for a total of 4,658. Of these, 1350 had at least one follow-up. The follow-up will continue up to December 2015.

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.

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 viii) 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. Development phases of the project conducted during 2014 include: data collection from participating studies (completed); harmonization of the datasets using a standardized format (ongoing); conduction of two-step statistical analyses to estimate the pooled odds ratios and 95% confidence intervals for several risk factors of interest (pilot phase); conduction of subgroup and other analyses to allow the interpretation of results (pilot phase); development of the project website (completed). 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 are 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, 500 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. Further, we will analyze the survival of patients with STS included in the study in relation to their characteristics and habits before diagnosis, by linking clinical/prognostic data with epidemiological information collected through interview. 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 histopathological 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 carcinogens; 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), melanoidins 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. The issue has been considered in two television programs “Fuori TG” and “Elisir” after invitation.

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 have been conducted 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. Two new recent and detailed surveys are still ongoing on Italian diabetologists and on Italian oncologists which are members of the Italian Society of Oncologists (AIOM).

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
The Laboratory of Medical Informatics is involved in training activity on issues related to the use of social media and web 2.0 technologies in medicine. The training activity is addressed to health professionals and health communicators. 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 are also delivered using the training/educational facilities and equipment available at the Mario Negri Institute.
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.

Laboratory of Clinical Epidemiology

Head of Laboratory
Guido BERTOLINI, MD.

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 Utilization 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 Eudipharm); from May 2008 Head of the 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 Anaesthesia 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.
Livio Garattini: got his degree in Economics in March 1983 at the Bocconi University in Milan.

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.partecipasalute.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, Satolli R, Colombo C, Senatore S, Cotichini R, Da Cas R, Spila Alegiani 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 the Laboratory for Mother and Child Health, Department of Public Health.

Since January 2012 he is the head of Pharmacoepidemiology Unit of the Laboratory of Mother and Child Health.

Selected publications:

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:
• Luciani D, Bazzoni G. From networks of protein interactions to networks of functional dependencies. *BMC Systems Biology* 2012;6-44.

**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 coordinates one of the first and largest groups of collaborative research in the world, the GiViTI (Italian Group for the Evaluation of Interventions in ICU).

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

- SINPE (Società Italiana di Nutrizione Artificiale e Metabolismo)
- 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 Onlus, Pistoia
- Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano
- Fondazione Nerina e Mario Mattioli Onlus, Milano
- Fondazione per la ricerca sulla Fibrosi Cistica Onlus FFC, Verona
- Zadig agenzia di editoria scientifica, Milano

Laboratory for Mother and Child Health

- Associazione Culturale Pediatri (ACP)
- A.O. Spedali Civili di Brescia
- Centro Antiveleini –Tossicologia Clinica – Ospedali Riuniti di Bergamo
- Centro per la Salute del Bambino (CSB)
- Fondazione Emanuela Zancan Onlus
- Il Pensiero Scientifico Editore
- 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
– Istituto di Anestesia e Cure Intensive, Università di Semmelweis, Budapest, Ungheria
– 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
– Terapia Intensiva Pediatrica, Soroka University Medical Center, Beer-Sheva, Israele
– Terapia Intensiva, Heraklion University Hospital, Greta, Grecia

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 Santè 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 Guaraní Asociacion, Bolivia
– Unione Europea (UE)
– Università di Amsterdam – Universiteit Van Amsterdam, Olanda
– Università College London Hospital NHS Fondation Trust, UK
– Università di Nottingham - Derbyshire Children's Hospital, UK
– Universidad Peruana Cayetano Heredia, Perù
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; Journal of Medical Economics; The European Journal of Health Economics.

NATIONAL:
FarmacoEconomia News; Farmeconomia e Percorsi Terapeutici; L'Internista; PharmacoEconomics Italian Research Articles; Quaderni di FarmacoEconomia.

Laboratory of Clinical Epidemiology

NATIONAL:
Ricerca & Pratica;
Dedalo. Gestire i sistemi complessi in sanità.

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; 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; Health Policy; PharmacoEconomics; The European Journal of Health Economics; Epilepsia; British Medical Journal.

Laboratory of Clinical Epidemiology

INTERNATIONAL:
Annals of Internal Medicine; American Journal of Respiratory and Critical Care Medicine; BMJ Open; Critical Care Medicine; Intensive Care Medicine; PLOS ONE, Critical Care; Lancet Neurology.

NATIONAL:
Ricerca & Pratica

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**Laboratory for medical research and consumers involvement**

**INTERNATIONAL:**

**NATIONAL:**
Ricerca & Pratica

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**Laboratory for Mother and Child Health**

**INTERNATIONAL:**
Allergologia et Immunopathologia; Archives of Disease in Childhood; BMJ Open; British Journal of Clinical Pharmacology; Canadian Medical Association Journal; Clinical Infectious Diseases; Epidemiology and Psychiatric Sciences; Expert Opinion on Pharmacotherapy; Expert Review of Clinical Pharmacology; European Journal of Pediatrics; European Journal of Clinical Pharmacology; European Neuropsychopharmacology; Italian Journal of Pediatrics; Pharmacotherapy; Pediatrics; Pediatric Drugs; PLoS ONE; The New England Journal of Medicine; Thorax.

**NATIONAL:**
Medico e Bambino.

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**NATIONAL AND INTERNATIONAL COMMITTEE MEMBERSHIP**

- Laboratory of Clinical Epidemiology

- Laboratory for medical research and consumers involvement
  - Comitato Guida Slow Medicine
  - Comitato Tecnico Scientifico Associazione ACTO
  - Comitato Direttivo Attilia Pofferi Onlus
  - Comitato Direttivo Fondazione Nerina e Mario Mattioli Onlus
  - Comitato AIOM, Linee Guida psicosociali

- Laboratory for Mother and Child Health
  - Commissione tecnica per l'elaborazione, gestione e aggiornamento del Prontuario Terapeutico Regionale (P.T.R.), Regione Autonoma Valle d'Aosta.

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**EVENT ORGANIZATION**

"A. and A. Valenti" Centre for Health Economics (CESAV)
May
Congress “Economia del Farmaco Fra soluzioni tecniche e decisioni politiche” 21-22 May, Centro di Ricerche Cliniche per le Malattie Rare ALDO E CELE DACCÒ, Ranica (BG).

November
Meeting “Progetto BIOSIMILARI” 20 November, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan.

Laboratory of Clinical Epidemiology
February
Workshop “Il follow-up telefonico nel trauma cranico” 17-18 February, Centro residenziale universitario di Bertinoro, Bertinoro (FC).

March
Workshop “The CREACTIVE telephone follow-up” 5-6 March Centro residenziale universitario di Bertinoro, Bertinoro (FC).
Workshop “1° CREACTIVE Scientific Advisory Board meeting” 31 March, Centro di Ricerche Cliniche per le Malattie Rare ALDO E CELE DACCÒ, Ranica (BG).

April
Workshop “CREACTIVE pediatrico” 2 April, Centro di Ricerche Cliniche per le Malattie Rare ALDO E CELE DACCÒ, Ranica (BG).

May
Workshop “1° CREACTIVE Ethics Advisory Board meeting” 6 May, Milan
Workshop “Investigator meeting compact 2” 20 May, Centro di Ricerche Cliniche per le Malattie Rare ALDO E CELE DACCÒ, Ranica (BG).

September
Workshop “Meeting annuale Margherita Tre” 15-16 September, Centro di Ricerche Cliniche per le Malattie Rare ALDO E CELE DACCÒ, Ranica (BG).

October
Workshop “Il follow up telefonico in CREATIVE” 1th October, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan.
Workshop “CREACTIVE Country Coordinator Meeting” 21-22 October, Park Inn by Radisson Budapest

November
Congress “23° GiViTI Meeting” 5-6-7 November, il Centro Congressi Baia Flaminia Resort, Pesaro

December
Workshop “BIOBANCA e IMMAGINI: i sottostudi CREACTIVE” 17 December, Centro di Ricerche Cliniche per le Malattie Rare ALDO E CELE DACCÒ, Ranica (BG).

Laboratory for medical research and consumers involvement
May
Meeting “Il Servizio Sanitario deve o no organizzare uno screening nella popolazione con lo scopo di individuare persone sane che potrebbero avere figli malati di fibrosi cistica?” 17 May, Pistoia.

September
Meeting “Il Servizio Sanitario deve o no organizzare uno screening nella popolazione con lo scopo di individuare persone sane che potrebbero avere figli malati di fibrosi cistica?” 26-27 September, Palermo.

Laboratory for Mother and Child Health
February
1° Meeting “Zoom Approach: una strategia per valutare gli out come” 6 February, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan.

March
2° Meeting “Autismo. Un’umanità possibile” 5 March, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan.
3° Meeting “OMS: i governi non si facciano intimidire dalle case farmaceutiche” 31 March, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan.

April
4° Meeting “I disturbi dell’apprendimento: in una società disattenta” 16 April, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan.

May
5° Meeting “Abilitazione precoce nella SMA1 e valutazione degli interventi” 7 May, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan.
Congress “La valutazione di esito nella pratica clinica in situazioni di complessità per curare e prendersi cura” 17-18 May, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan.
6° Meeting “Per il superamento (abolizione) degli Ospedali Psichiatrici Giudiziari” 30 May, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milan.

CONFERENCE AND WORKSHOP CONTRIBUTIONS

"A. and A. Valenti" Centre for Health Economics (CESAV)

April
VALUTAZIONE ECONOMICA HPV. Course “HPV: un problema di sanità pubblica”. ASL AL, Regione Piemonte; Turin.
RISK-SHARING AGREEMENTS IN ITALY. 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; Ohrid, Macedonia.

May
REGISTRI DI MONITORAGGIO.
CONTRATTI D’ESITO.
CONTINUITÀ ASSISTENZIALE TERRITORIALE.
PROCESSI DI ACQUISTO REGIONALI DEI BIOSIMILARI.

June
DAI GENERICI AI BIOSIMILARI. Meeting “Il mercato del farmaco e i bisogni indotti”. Altroconsumo; Trento.

October
BIOSIMILARI: UNA CHIAVE DI LETTURA ECONOMICA.
UN’ANALISI EMPIRICA DELLE GARE D’ACQUISTO REGIONALI DI BIOSIMILARI IN ITALIA. Course “Dai farmaci equivalenti ai biosimilari di anticorpi monoclonali”. ASL Brindisi; Brindisi.
MARKET-ACCESS AGREEMENTS. Congress “Risk-sharing agreements in Poland”. Sequence HC Partners HTA Consulting KPRW; Varsavia, Polonia.

November
THE CRITICAL SUCCESS FACTORS FOR PERSONALIZED MEDICINE DATA DEVELOPMENT AND DEMONSTRATING VALUE. ISPOR 17th Annual European Congress “Personalized medicine workshop”. International Society for Pharmacoeconomics and Outcomes Research (ISPOR); Amsterdam, The Netherlands.
BIOSIMILARI: UNA CHIAVE DI LETTURA ECONOMICA.
UN’ANALISI EMPIRICA DELLE GARE D’ACQUISTO REGIONALI DI
BIOSIMILARI IN ITALIA.
SVILUPPO E PRODUZIONE DEI BIOSIMILARI: ASPETTI BIOTECNOLOGICI E
IMPLICAZIONI REGOLATORIE. Meeting “Progetto biosimilari”. CESAV,
Dipartimento di Salute Pubblica IRCCS Istituto di Ricerche Farmacologiche Mario Negri;
Milan.

December
HEALTH SYSTEM AND VALUE PRICING IN ITALY. Conference “PLC France-Italy
HLG joint meeting”. Italy HLG e PLCF; Milan.

Laboratory of Clinical Epidemiology

February
IL CONTESTO E L’EDUCAZIONE CONTINUA TRA ESPERIENZA, RICERCA
ERELAZIONE CON IL MALATO. Congress “FORMARE E FORMARSI. Alla ricerca
di una visione condivisa” 13 February, Crema.
CREACTIVE (COLLABORATIVE RESEARCH ON ACUTE TRAUMATIC BRAIN
INJURY IN INTENSIVE CARE MEDICINE IN EUROPE). Esercitazioni
INIZIALI. LA SCHEDA DI RACCOLTA DATI. LA GOSE QOLIBRI-OS IN
DETTAGLIO. Workshop: “Il follow-up telefonico nel trauma cranico” 17-18 February,
Bertinoro (FC).

March
GLI STUDI PER LA VALUTAZIONE DELLA QUALITÀ DELL'ASSISTENZA.
Course: “Formazione Monitor” 4 March, Milan.
THE PROSAFE DATABASE. Workshop: “The CREACTIVE telephone follow-up” 5-6
March, Bertinoro (FC).
CASE REPORT FORM: DIFFERENCES WITH CDES PROPOSED BY THE
TRAUMATIC BRAIN INJURY COMMON DATA ELEMENT WORKING GROUP
(VERSION 2.0). ORGANIZATION OF PATIENT FOLLOW-UP AND FOLLOW-UP
PROVIDER TRAINING. Workshop: “1° CREACTIVE Scientific Advisory Board
meeting” 31 March, Ranica (BG).

April
CREACTIVE PEDIATICO. Workshop: “CREACTIVE pediatrico” 2 April, Ranica (BG).
LA QUALITÀ DEL DATO NEI PROGETTI GIVITI. TEST D’IPOTESI.
STATISTICA DESCRITTIVA. INTERVALLI DI CONFI DENZA. Course “Formazione
Monitor” 7 April, Milan.

May
1°CREATIVE ETHICS ADVISORY BOARD MEETING. Workshop:
“1°CREATIVE Ethics Advisory Board meeting” 6 May, Milan.
PERCHÉ È STATO SOSPESO LO STUDIO? ASPETTI METODOLOGICI DEL
PROTOCOLLO. MONITORAGGIO DELLO STUDIO. Workshop: “Investigator
meeting compact 2” 20 May, Ranica (BG).
LA COMPARATIVE EFFECTIVENESS RESEARCH (CER) NEL TRAUMA
CRANICO. Congress “6° NEUROMEETING” 22 May, Naples.

June
UTILIZZO DELLA CARTELLA CLINICA ELETTRONICA MARGEHRITA TRE.
Course: “Utilizzo del software Margherita Tre” 13 June, Treviso.
CREATIVE PRESENTATION. Workshop “3rd International Traumatic Brain Injury
Research (InTBIR) Meeting” 28-29 June, San Francisco.

July
ASSESSING QUALITY OF CARE TO SEEK IMPROVEMENT. COMPARATIVE
EFFECTIVENESS RESEARCH IN TRAUMATIC BRAIN INJURY. Workshop “The
56th Annual Conference of The Israeli Society of Critical Care Medicine” 10 July, Herzliya
(Tel Aviv).
September

October
CREACTIVE (COLLABORATIVE RESEARCH ON ACUTE TRAUMATIC BRAIN INJURY IN INTENSIVE CARE MEDICINE IN EUROPE): FACCIAMO IL PUNTO! CREACTKIDS: IL FOLLOW-UP PEDIATRICO. LA SCHEDA DI RACCOLTA DATI IN PROSAFE. Workshop “Il follow up telefonico in CREACTIVE” 1th October, Milan.
IL MALATO END STAGE: OUTCOMES IN TERAPIA INTENSIVA. Workshop “Grandi insufficienze d’organo End Stage: Cure intensive, Cure ordinarie o Cure palliative?” 4 October, Sassari.
LAVORARE NELLA RICERCA SCIENTIFICA. Course: “Settimana di (in)formazione a cura dei ricercatori dell’Istituto di Ricerche Farmacologiche Mario Negri” 9 October, Noto, Siracusa.

November
LE TAPPE DEL GIVITI SULLA STRADA DI UNA MEDICINA BASATA ANCHE SULLE RELAZIONI. LA RESISTENZA AI CARBAPENEMI NELLE TERAPIE INTENSIVE ITALIANE. LE LINEE GUIDA COME STRUMENTO DI MIGLIORAMENTO DELLA PRATICA CLINICA? (DUE PUNTI DI VISTA A CONFRONTO). L’ANGOLO DELL’INFORMATICA: DATI STRUTTURALI E ALTRE NOVITÀ. ESERCITAZIONI SUI DATI PROSAFE TRAMITE L’ANALIZZATORE. L’IMPORTANZA DEL FOLLOW-UP PER IL PERSONALE, PER IL RICERCATORE, PER IL PAZIENTE. COMPACT-2: SI PARTE! Congress “23° GiViTI Meeting” 5-6-7 November, Pesaro
DECIDERE IN CONDIZIONI DI INCERTEZZA: QUALCHE SPUNTO DI METODO. Congress “Le Infezioni in Terapia Intensiva” 14 November, Naples.
CPFA – COSA ABBIAMO IMPARATO DAGLI STUDI COMPACT? Congress “Questioni aperte in Terapia Intensiva: dai Biomarcatori ai Trattamenti Depurativi per la Sepsis” 29 November, Bergamo.

December
CREACTIVE: SITUAZIONE ATTUALE. Workshop “BIOBANCA e IMMAGINI: i sottostudi CREACTIVE” 17 December, Ranica (BG).

Laboratorio of Medical Research and Consumer Involvement

February
ECRAN PROJECT: TOOLS, MATERIALS AND DISSEMINATION. Meeting “First HIV ECAB” EATG European AIDS Treatment Group; Brussels.

GENERAL OVERVIEW OF THE ACTIVITIES DONE, DISSEMINATION.
Workshop “ECRAN Project Meeting ” IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.

EMPOWERMENT DEI CITTADINI NELLA RICERCA. Congress “La ricerca oncologica per il paziente, con il paziente”. Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC); Florence.

May
PRESENTATION OF THE ECRAN PROJECT. ECRAN Project Meeting “International Clinical Trials’ Day”. ECRIN, ECRAN, ICTD e CRP; Luxembourg.

INTRODUZIONE ALLA GIORNATA. Congress “Gli amori difficili. Ricerca e comunicazione possono andare d’accordo?” Associazione Alessandro Liberati, Network Italiano Cochrane; Milan.

June
MODERATORE. Course “Comprehensive geriatric assessment nella patologia oncologica” Azienda Ospedaliera S. Maria Nuova Reggio Emilia; Reggio Emilia.

July
ASSOCIAZIONI E PAZIENTI. Course “Health Technology assessment in sanità” Società Italiana di Igiene (SITI); Milan.

September
BEYOND THE MEDICAL ASPECT: THE POINT OF VIEW OF THE PATIENT. Congress “Follow up in gynaecological malignancies” European Society of Gynaecological Oncology (ESGO); Turin.

QUALE RUOLO PER LE ASSOCIAZIONI DEI PAZIENTI. Congress “Le Associazioni dei pazienti tra dimensione eur opea, mondo mediatico, contenimento della spesa sanitaria e quotidianità” Associazione Nazionale Italiana Patologie Ipofisarie (ANPI); Verona.

October


IL PUNTO DI VISTA DI PAZIENTI E CITTADINI. 40° National Congress ANMDO “Ripensare la Sanità: ruoli strategici e responsabilità” Associazione Nazionale dei Medici delle Direzioni Ospedaliere (ANMDO); Naples.

LA VALENZA DELL’OPEN ACCESS E DELL’OPEN SCIENCE PER L’AMBITO DELLA SALUTE PUBBLICA. Seminary “Open science in open society: prospettive sul valore sociale dell’accesso aperto” Università del Piemonte Orientale UPO; Novara.

PROSPETTIVE DI COLLABORAZIONE. Meeting “Le azioni effettuate, programmate e da programmare nei confronti dei cittadini” Slow Medicine, IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.

QUALITÀ DELL’INFORMAZIONE NELLE GRANDI PATOLOGIE CRONICHE: QUALE CONTRIBUTO PROGETTUALE PER LA FONDAZIONE SMITH KLINE? Congress “I think tank FSK: fatti, riflessioni, progetti” Fondazione Smith Kline; Bologna.

November
TRIALS CLINICI E TRASPARENZA. 13° National Congress AME “Ruolo attivo dei pazienti nella ricerca e sviluppo dei farmaci: utopia o realtà?” Associazione Medici Endocrinologi (AME); Rome.

DEFINIRE LE PRIORITÀ DELLA RICERCA COINVOLGENDO CITTAĐINI E PAZIENTI. National Convention Sperimentazioni Cliniche “Nuove sfide per i Comitati Etici” Fondazione GIMBE; Bologna.


PRESENTAZIONE POSTER CITIZENS’ JURY AND DECISION MAKING ON CYSTIC FIBROSIS CARRIER SCREENING: TO SCREEN OR NOT TO SCREEN? Congress “XII Convention d’Autunno dei ricercatori in fibosi cistica” Fondazione Ricerca Fibrosi Cistica Onlus; Verona.

December

RESPONSABILITÀ CONDIVISA. National Workshop “Consumismo Sanitario Responsabilità condivisa” Ordine Provinciale dei Medici Chirurghi e degli Odontoiatri di Arezzo; Arezzo.

Laboratory for Mother and Child Health

January

TAVOLA ROTONDA DI PROSPETTIVE IN PEDIATRIA. Giornate di pediatria “Giovani”. Società Italiana di Ricerca Pediatrica; Naples.

February

BURDEN OF PSYCHIATRIC DISORDERS IN THE PAEDIATRIC POPULATION. 9° International Workshop “Child Health in Camagüey”. Camagüey Children’s Hospital Department of Provincial Health Camagüey and University of Nottingham, Camagüey Cuba.

WRITING AN ABSTRACT. Workshop “Writing Scientific Papers”. University of Havana, Havana Cuba.

March

IL PERCORSO DELL’ASSISTITO IN ETÀ EVOLUTIVA CON PROBLEMI NEUROPSICHIATRICI. Esperienze: LA RETE REGIONALE ADHD. Course. Società Italiana di Igiene Medicina Preventiva e Sanità Pubblica, Sezione Lombardia; Milan.

IMPACT OF OFF-LABEL DRUG USE IN PEDIATRICS ON DRUG SAFETY AND EFFECTIVENESS PROFILE. Annual meeting “ISPE 2014”. International Society for Pharmacoepidemiology; Rotterdam, The Netherland.

April

LA SALUTE INFANTILE NEI PAESI IN VIA DI SVILUPPO. PVS. Elective Course “Promozione della salute infantile nei paesi in Via di Sviluppo”. Università degli Studi di Milano-Bicocca, Facoltà di Medicina e Chirurgia, Scuola di Medicina; Monza (MB).


June

REAZIONI AVVERSE IN GRAVIDANZA. Master. Servizio di Epidemiologia e farmacologia Preventiva (SEFAP), Università degli Studi di Milano, Dipartimento di Scienze Farmacologiche; Milan.

AGGIORNAMENTO TERAPEUTICO IN TEMA DI WHEEZING E ASMA. Graduate School in pediatrics. Università degli Studi di Milano-Bicocca,Facoltà di Medicina e Chirurgia, Scuola di Medicina; Monza (MB).

July

L’USO DEI FARMACI PER I BAMBINI. Course “Summer Students”. IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Milan.

L’UTILIZZO OFF LABEL DEI FARMACI IN NEUROPSICHIATRIA INFANTILE: NORMATIVE, CRITERI DI IMPIEGO E CRITICITÀ. Course “L’uso di farmaci off
Label in Neuropsichiatria Infantile: normative, criticità, proposte operative”. Fondazione IRCCS Ca’Granda Ospedale Maggiore Policlinico; Milan.

September
UN MODELLO PER IL MONITORAGGIO E LA VALUTAZIONE DEI PERCORSI IN NPIA. CONTINUITÀ DELLE CURE VERSO L’ETA’ ADULTA IN REGIONE LOMBARDIA. CONDIVISIONE E MONITORAGGIO DEI PERCORSI DIAGNOSTICI E TERAPEUTICI IN NEUROPSICHIATRIA: IL PROGETTO ADHD LOMBARDIA. XXVI National Congress SINPIA. Società Italiana di Neuropsichiatria dell’Infanzia e dell’Adolescenza; Rome.
L’USO DI CGAS E CGI NEGLI UTENTI DEL REGISTRO LOMBARDO ADHD. LA SFIDA DELLA COMPLESSITA’. Congress “La valutazione di esito nella pratica clinica in situazioni di complessità per curare e prendersi cura”. Fondazione IRCCS Ca’Granda Ospedale Maggiore Policlinico, Fondazione Emanuela Zancan Onlus, la Nuova Famiglia Eugenio Medea; Milan.

October
APPROPRIATEZZA PRESCRITTIVA IN GRAVIDANZA. FARMACINETICA E ALLATTAMENTO. Congress “L’uso dei farmaci in gravidanza e allattamento”. Società Italiana di Tossicologia (SITOX); Pavia.
MAMME «LO SAI MAMMA». FARMACI, GRAVIDANZA E ALLATTAMENTO. Course “Settimana (in)formazione a cura dei ricercatori dell’IRCCS Istituto di Ricerche Farmacologiche Mario Negri”. Comune di Noto, IRCCS Istituto di Ricerche Farmacologiche Mario Negri; Noto (SR).

November
LA SALUTE INFANTILE NEI PAESI CON SCARSE RISORSE. Congress “Salute, malattie e cure nel mondo globalizzato”. MNIAA Mario Negri Institute Alumni Association, Società Svizzera Milano; Milan.
LA SALUTE DEI BAMBINI. ASPETTI EPIDEMIOLOGICI. II ed. Congress “Outdoor Education, l’educazione Sicura all’aperto”. Comune di Bologna; Bologna.
FARMACI, NEONATI E BAMBINI… DALL’OFF-LABEL ALL’“ON” EVIDENCE. III National Congress “Farmaci e Neonato: Luci, Ombre e Prospettive”. Gruppo di Studio di Farmacoterapia Neonatale (SIN); Rome.

December
FARMACI E BAMBINI. Graduate School in Pediatrics. Università degli Studi di Milano-Bicocca, Facoltà di Medicina e Chirurgia; Monza (MB).
FARMACI: LA MARCA FA LA DIFFERENZA? Course “Farmaci equivalenti e farmaci di marca a confronto”. ASS I Triestina; Trieste.
GROW OLD IN THE PUBLIC MENTAL HEALTH SYSTEM OF LOMBARDY REGION: FINDINGS FROM TWO COHORT STUDIES. European Conference “Youth Mental Health: from continuity of psychopathology to continuity of care”. IRCCS Fatebenefratelli di Brescia; Venice.

GRANTS AND CONTRACTS

"A. and A. Valenti” Centre for Health Economics (CESAV)
- Abbott
- AIFA
- Grunenthal-Prodotti Formenti
- Merck Sereno
- Sanofi Aventis
- Sanofi Pasteur MSD
- Schering Plough
- Vivisol

**Laboratory of Clinical Epidemiology**
- Bellco SpA
- Commissione Europea DG Research & Innovation
- Brahms
- CNT
- Regione Toscana
- Regione Veneto
- Astellas
- Novartis
- A.O. Como
- A.O. Lecco
- A.O. Reggio Emilia
- ASL AL
- Dedalus
- ASL TO2
- ASL TO4
- ASL 1 Sassari
- ASL 2 Olbia
- ASL 3 Genovese
- AUSL Romagna
- Azienda Sanitaria di Firenze
- Fondazione Poliambulanza di Brescia
- Ospedale Evangelico Internazionale di Genova
- IRCCS Policlinico S. Matteo di Pavia
- USL 1 Massa Carrara
- USL 7 di Siena
- Azienda USL 9 Grosseto

**Laboratory for medical research and consumers involvement**
- Associazione Italiana Sclerosi Multipla AISM/Fondazione Italiana Sclerosi Multipla FISM, Genova
- European Commission, Brussels
- Fondazione per la ricerca sulla Fibrosi Cistica FFC Onlus, Verona
- IRCCS Istituto Neurologico Carlo Besta, Milano
- Fondazione Nerina e Mario Mattioli Onlus, Milano

**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 (2014)**

"A. and A. Valenti" Centre for Health Economics (CESAV)


**Laboratory of Clinical Epidemiology**


**Laboratory for medical research and consumers involvement**

Colombo C, Mosconi P. Transparency of funding of patient groups is mandatory but is not enough. BMJ 2014;349:g6301. [IF: 17,215]


**Laboratory for Mother and Child Health**


Piovani D, Clavenna A, Bonati M, on behalf of PeFAB group. Review of Italian primary care paediatricians identifies 38 commonly prescribed drugs for children. Acta Paediatr 2014;103:e532-e537. [IF: 1,842]
Review


Letter
Clavenna A, Bonati M. In the real-life setting nebulized beclomethasone is scantily effective in preventing episodic viral wheezing. *Pediatrics* 2014;e-letter: http://pediatrics.aappublications.org/content/133/3/e505/reply#content-block. [IF: 5,297]


LAY PRESS SELECTION (2014)

"A. and A. Valenti" Centre for Health Economics (CESAV)


Laboratory of Clinical Epidemiology


Laboratory for medical research and consumers involvement


Mosconi P. Definire le priorità della ricerca coinvolgendo cittadini e pazienti. Evidence 2014;6(11):e000094
http://www.evidence.it/articoli/pdf/e1000094.pdf


Mosconi P. Informazione e strumenti innovativi per il coinvolgimento dei cittadini in sanità. Intervista rilasciata a Morfologie 2014;14:6-7.


Mosconi P. Screening mammografico: una storia senza fine. AALert luglio 2014;3-5.

Mosconi P. L’Unione europea e la ricerca clinica. AALert maggio 2014:5-6.


**Laboratory for Mother and Child Health**


Bonaccorsi A. Corruzione e farmaci stimolanti nella sindrome ADHD. R&P 179:225-228; 2014.


Bonaccorsi A. La storia di una vita dalla parte dei pazienti. R&P 2014;177:128-129.


Bonaccorsi A. Promuovere la prescrizione di medicine appropriate. R&P 2014;175:33-34.


Bonati M. Non si muore quando si deve, ma quando si può. *R&P* 2014;180:243-244.


Campi R. L’Italia non è un “paese per bambini”. *R&P* 2014;178:174-175.


Clavenna A. Lo strano derby tra bevacizumab e ranibizumab. Ma il biglietto chi lo paga? *R&P* 2014;180:252-255.


Confalonieri V. Costruire percorsi urbani di felicità. *R&P* 2014;176:82.


**Laboratory of Clinical Epidemiology**


**Laboratory for medical research and consumers involvement**


Mosconi P, Castellani C. Citizens’ jury and decision making on cystic fibrosis carrier screening: to screen or not to screen? Atti del Convegno XII Convention d’Autunno dei ricercatori in fibrosi cistica, Garda (Vr) 27/-29 novembre 2014 pag. 32 n. 28.

**Laboratory for Mother and Child Health**

Bonati M. Perché non tutti i bambini del mondo ricevono le terapie necessarie. In: Tavola rotonda: “Salute, malattie e cure nel mondo globalizzato” MNIAA (Associazione Alunni del Mario Negri) il 3 novembre 2014 a Milano Eventi, presso Società’ Svizzera Milano Sala Meili III° piano (Via Palestro, 2) 2014.

"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 as 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 citizens

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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.

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 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

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 in the EPIFARM (Epidemiologia del farmaco) project funded by the Lombardy Region. During 2014 the activities regarded, in particular, the following topics:

a. Comparison of recurrent prescriptions in children receiving generic or brand name antibiotics;
b. Health care resources consumption in immigrant and native Italian paediatric population;

a. Comparison of recurrent prescriptions in children receiving generic or brand name antibiotics
- The aim of the study was to evaluate effectiveness and safety of paediatric generic antibiotic formations compared to the brand name ones. The rate of antibiotic recurrent prescription (therapeutic failure issue) and hospital admission (complication or severe adverse reaction issues) were compared in children and adolescents who had been prescribed a generic of a brand name formulation of the same antibiotic.
- Children receiving an antibiotic prescription (index prescription) between February and April 2010 who did not receive any prescription in the previous 28 days were identified through the administrative database of reimbursed prescriptions of the Lombardy region. A recurrent prescription was defined as a prescription occurring within 28 days from the initial one which was defined index prescription. It was evaluated if the child required a new antibiotic course (recurrent prescription) or hospital admission in the 28 days period following the index prescription. The rates of recurrent prescription and hospital admission were calculated for amoxicillin, amoxicillin clavulanate, clarithromycin, and cefaclor, and stratified by age class. For these four active substances a paediatric generic formulation was available from at least 2 years.
- The percentage of children and adolescents that received at least one recurrent prescription was 17.7%. The rate of recurrent prescription was slightly lower in children receiving at least a generic formulation at the index prescription (OR 0.96; 95% IC 0.93-0.98) compared to those receiving a brand name one. The percentage of hospital admission was 1.01% (95% IC 0.98-1.08) in children receiving a brand name formulation, and 1.03% (95% IC 0.96-1.06) without significant differences (p=0.43).
Children treated with a generic antibiotic did not show different outcome measures compared to those treated with a brand name one. The results provide more evidence about the effectiveness and safety of generic antibiotics.

b. Health care resources consumption in immigrant and native Italian paediatric population:

The study compared the health care resources consumption (drug prescriptions, diagnostic exams, specialist visits) in 12,827 children and adolescents less than 18 years old born in low-middle income country outside the EU-27 and 24,574 Italian children, matched for age and physician.

In all, 4,673 immigrants (38%) and 11,723 natives (48%) received at least a drug prescription. The mean number of prescriptions was respectively 3.2 (SD 5.4) in immigrants and 3.3 (SD 4.3) in natives (t=0.42; p=0.67).

Italian children and adolescents received more frequently anti-asthmatic and systemic steroid prescriptions. On the contrary, the percentage of anthelmintic and antianemic drugs was two-fold higher in immigrant than in Italian children.

The rate of hospital admission was comparable in the two groups (3.7 and 3.5%), however immigrant children received less frequently diagnostic exam prescriptions (40 vs 53%) and specialist visits (49 vs 58%).

Differences in the prevalence of prescription among different geographical areas of origin have been observed, with the lowest value observed in immigrants from East Asia (immigrants/Italians ratio 0.5)

c. Psychotropic drug prescription in Italian children: a multiregional study:

The 2006-2011 prescription trend of psychotropic drugs in the paediatric population was evaluated in seven Italian region: Friuli-Venezia-Giulia, Veneto, Lombardy, Emilia Romagna, Abruzzo, Lazio e Puglia.

The source of data were the regional administrative prescription databases. Prevalence and incidence rates by age and sex were calculated for any psychotropic drug, antidepressants, antipsychotics, and ADHD medications.

In 2011 the prevalence was 1.76‰ (95% IC 1.72-1.80), ranging between 1.56 and 2.17‰ among regions. The incidence of new psychotropic drug users was 1.03‰ (1.00-1.06). Antidepressants were the most prevalent class (1.02‰), followed by antipsychotics (0.70‰) and ADHD medications (0.19‰). Prevalence rate increased with age until 4.2‰ in the 12-17 years old age class. Males were more exposed than females (ratio = 1.23). Antipsychotics were mostly prescribed in males, and antidepressants in females. In the 2006-2011 period the prevalence rate was stable (p=0.97).

The prevalence rate of psychotropic drugs in Italy in 2006-2011 did not change and was lower than what has been observed at the international level.

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 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 NICUs, located in 28 different countries, were selected and contacted and about 200 NICU completed the entire questionnaire. The results, published in the Scientific Reports journal, 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 survey also allows the identification of the NICUs where the trial will be conducted. The project is ongoing and is expected to end in 2015. However, to allow the clinical trials to be carried out, a request for a project extension will be made to the EMA.

www.tinn2-project.org/

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 2014, 2,061 patients have been included in the register, 1,210 of whom had a confirmed ADHD diagnosis, 631 were not diagnosed for ADHD, and 220 were still under diagnostic evaluation. In the most cases patients were referred to the Centers by the school (34%) or the parents (22%). In all, 80% of the 1,210 patients with a confirmed ADHD diagnosis received a non pharmacological prescription, 3% only a pharmacological one, 13% both the prescriptions, and the remaining patients are still awaiting for any therapy. The most frequent comorbidities were learning disabilities (35%), sleep disorders (14%) 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
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

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. On June 17, 2014 was released on the 7th up Reports on the monitoring of the Convention on the Rights of the Child in Italy, in the presence of the Minister for Labour and Social Policy Giuliano Poletti and the Guarantor for the 'childhood and adolescence, Vincenzo Spadafora.

The 87 associations Group CRC are turning to the representatives of the new government and parliamentarians, as well as to local authorities hope that each institution may, within its field of action, grasp the importance and urgency of the critical issues raised and take action to resolve them, taking charge of the recommendations contained in the Report.

With the publication of the 7th Report of the Group updated CRC continue the monitoring of the implementation, in our country, the UN Convention on the Rights of the Child (CRC) and its Optional Protocols, undertaken starting from processing 1st Supplementary Report in 2001.

In over ten years of the CRC Group has published seven reports annual update and two Supplementary Reports that were sent to the UN Committee to help with the Government Report to the analysis of the implementation of the Convention in Italy. The annual publication testifies to the perseverance and the commitment of the associations in ensuring a constant and regular update on the implementation of the rights of children and adolescents in our country, even when the different institutional levels are not always able to maintain the rights of ‘childhood on the political agenda.

The report can be downloaded from the website: http://www.gruppoerc.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.

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

Head
Vittorio BERTELE’, M.D.

Senior scientist
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Senior scientist
Pasquale Lorenzo MOJA, Dr. Med.

Chir, PhD

Ricercatore junior
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Visiting scientist
Roberta JOPPI, Chem Pharm. D

Visiting scientist
Stefanos BONOVAS, Dr. Med. Chir.

Visiting scientist
Koren Kwag, Dr. Biol
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 publications 2005-2014

9. Garattini S, Bertele’ V. The scientific community should lobby to be able to apply for drug licences. BMJ 2012; 344 : e3553.
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. Coordination of the evaluation process conducted by the Board and external peer-reviewers.

Follow up of the clinical trials selected by the ECRIN-IA (European Clinical Research Infrastructure Network-Integrating Activities) Working Package 7 competitive call aimed to facilitate the conduction of multinational studies by providing free services for their multinational implementation.

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 Working Package 8 of the ECRIN, European Clinical Research Infrastructure Network.

Participation to the other activities of the ECRIN-IA project.

Support to the preparation of grant proposals for the European Programme Horizon 2020.

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

Critical appraisal of eleven multinational clinical trial protocols to be conducted with the methodological and operating support of ECRIN.

Follow up of the clinical trials selected by the ECRIN–IA WP7 competitive call to support multinational clinical trials in Europe.
Support to the development of the National ECRIN network (Ita-CRIN).

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 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.

Systematic reviews with meta-analysis of biotech drugs for the treatment of senile macular degeneration (Cochrane methodology).

Systematic review with meta-analysis of rehabilitation interventions.

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
ISDB - International Society of Drug Bulletins
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 (2014)


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 learnings, 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.
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. 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 STARPAN
  - 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

- Development 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
- Development Internal Timesheet Management System
- Planning and Development new web site of Institute “Mario Negri”
THE CATULLO AND DANIELA BORGOMAINERIO CENTER

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

Due to a contribution from the Regione Lomabardia, from every computer in the Institute it is now possible to have access, in addition to the Library personal collection, 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


PUBLICATIONS

Balduzzi S, Mantarro S, Guarneri V, Pistotti V, Moja L, D'Amico R
Trastuzumab-containing regimens for metastatic breast cancer
Cochrane Database Syst Rev 2014 Issue 6 : CD006242
IF: 5.939
Negri Bergamo Laboratories

ANNUAL REPORT 2014

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., Ph.D.
CURRICULA 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 Nephrology and Dialysis, Ospedali Riuniti di Bergamo, Italy; in 1982 Post Doctoral Fellow, Centre Regional de Transfusion Sanguigne 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. 2013-2014: 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. Since 2013 member of the Visiting Committee d'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:


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.

**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**
- S. Tomasoni, L. Longaretti, C. Rota, M. Morigi, S. Conti, E. Gotti, C. Capelli, M. Introna, G. Remuzzi, A. Benigni. Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells. Stem Cells Dev 2012

**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.

Member of European Complement Network Board from 2013.
Selected publications


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.

**Educational training:** in 1989-1991 Graduate student, University of Milan; in 1991-1994 PhD student, University of Milan; in 1994 Research Fellow, Renal Division, Brigham & Women’s Hospital, Harvard Medical School, Boston, USA; 1995-1998: Post Doctoral Fellow, IRFMN, Bergamo, Italy.

**Areas of interest:** construction of adenoviral vectors for gene therapy; in vitro and in vivo gene transfer techniques; use of adenoviral and adenov-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 cell from adult somatic cells; differentiation of iPS renal progenitor cells and endothelial cells; use of stem cells for tissue regeneration in acute and chronic renal failure; study of the renal regeneration mechanisms.

**Employment:** in 1998-2000 Scientist, IRFMN, Bergamo, Italy; in 2000-2010 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. From January 2010 to December 2014 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:
Daniele Corna obtained her degree in Industrial Chemistry in 1985, and the degree in Biochemical Research Technicians in 1988-1989 at IRFMN, Bergamo, Italy.

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.

**Educational Training:** 1989-1994 research fellow, IRFMN, Bergamo.

**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 Molecular Biology of Transplantation Tolerance, Transplant Research Center “Chiara Cucchi de Alessandri e Gilberto Crespi”, Ranica.

**Selected Publications:**


**Daniele Corna** obtained her degree in Industrial Chemistry in 1985, and the degree in Biochemical Research Technicians in 1988-1989 at IRFMN, Bergamo, Italy.

**Educational Training:** 1986-1989 researcher fellow, IRFMN, Bergamo.

**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 fibronectin 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.

**Educational training:** in 1981-1983 Post Doctoral Fellow, Istituto di Patologia Speciale Medica dell'Università degli Studi di Milano, Italy; in 1985 - 1989 Post Doctoral Fellow, IRFMN, Bergamo, Italy; in 1989-1991 Post Doctoral Fellow at Scripps Clinic and Research Foundation, Laboratory of Thrombosis and Hemostasis, La Jolla, CA, USA; in 1991-1995 Post Doctoral Fellow, IRFMN, Bergamo, Italy.

**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) identification of mechanisms of tissue regeneration induced by renoprotective therapies. Role of renal progenitors;
3) generation of new functional nephrons starting from renal embryonic cells;
4) derivation of induced pluripotent stem cells from patients affected by rare diseases and set up of differentiation protocols to generate renal progenitors.
5) understanding the mechanisms underlying endothelial cell dysfunction in thrombotic microangiopathies and hyperacute rejection of xenograft;
6) 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;
7) 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) therapies with stem cells of different origin to cure acute and chronic kidney diseases (bone marrow, umbilical cord, amniotic fluid);
3) glomerular and tubular cell lines;
4) induced pluripotent stem cells (iPS);
5) generation of renal organoids by using tissue engeneering approaches;
6) in vitro models to assess the interaction of vascular endothelial cells with leukocytes and platelets under controlled flow conditions;
7) 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 accomodation;
8) gene transfer of viral constructs carrying genes encoding immunomodulatory molecules to overcome acute rejection of allotransplantation avoiding immunosuppression;
9) identification of candidate genes with linkage analysis and search for mutations as well as assessment of gene polymorphisms.

**FINDINGS/MAIN RESULTS**

Identified a method to reprogram human bone marrow stromal cells into functional renal proximal cells using cell-free extracts.

Identified a new role of SIRT3 in protecting mitochondrial dynamics in acute kindey injury by preventing fission, membrane depolarization and mitophagy.

Identification of a new method for in vitro generating chimeric renal organoids by using suspensions of mouse embryonic kidney cells, in which human cells can be incorporated.

Identified in the kidney of diabetic rats miRNAs that contribute to understanding molecular mechanisms involved in the pathogenesis of diabetic nephropathy.

An unanticipated role for survivinG in protecting renal tissue from ischemia/reperfusion injury in kidney transplantation.

Identified a novel hybrid CFHR1/CFH gene in patients with atypical hemolytic uremic syndrome.

Functional characterization of identified nucleotide changes in FB is mandatory to confirm atypical hemolytic uremic syndrome association.

A new test assays endothelial complement activation in patients with atypical hemolytic uremic syndrome and monitors Eculizumab therapy.
Genetic analysis of complement genes in the donors is mandatory to prevent and/or treat posttransplant aHUS recurrences.

**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
CORT, Padova
Clinica di Pediatría Oncoematologica, Università di Padova
Fondazione I.R.C.C.S. Policlinico San Matteo, Pavia
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
Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Pozzuoli (Napoli)
International Centre for Genetic Engineering and Biotechnology, Molecular Medicine Group, Trieste
Istituto di Medicina Interna e Geriatria e Centro di Ricerca Emostasi, Università Cattolica, Roma
Istituto Nazionale dei Tumori Regina Elena, Roma
Laboratorio di Biologia dello Sviluppo, Dipartimento di Biologia Animale, Università degli Studi di 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
U.O. di Ostetricia e Ginecologia, Azienda Ospedaliera Spedali Civili di Brescia
School of Medicine, University of Milano-Bicocca
Stem Cell Processing Laboratory, Clinic of Paediatric Oncohematology, University of Padova
Stem Cells and Regenerative Medicine Lab, Foundation Institute of Paediatric research, Fondazione Città della Speranza, Padova
Unità di Nefrologia e dialisi, Ospedale Pediatrico “Bambino Gesù”, Roma

**INTERNATIONAL COLLABORATIONS**

Assistance Publique-Hôpitaux de Paris, Hôpital European Georges-Pompidou, Service d’Immunologie Biologique, Paris, Francia
Academisch Ziekenhuis Maastricht, Interne Geneeskunde, Maastricht, Olanda
Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA
Biogazelle NV, ZwiJnarde, Belgio
Centro de Investigaciones Biológicas and Centro de Investigacion Biomedica en Enfermedades Raras, Madrid, Spagna
Charité Universitätsmedizin Berlin, Germania
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
Department of Medicine, Renal Research Institute, New York Medical College, Valhalla, NY, USA
Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Institute of DNA Medicine, Tokyo, Japan
Division of Pathology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden
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
Institut of Experimental Immunology, University of Bonn, Bonn, Germany
Icahn School of Medicine at Mount Sinai, New York, New York, United States
Klinikum der Ludwig Maximilians 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
Molecular Medicine Research Center, Laboratory of Molecular and Medical Genetics, University of Cyprus
Molecular Otolaryngology and Renal Research Laboratories, Division of Nephrology, Department of Internal Medicine, University of Iowa, Iowa City, USA
Mosaiques Diagnostics GmbH, Hannover, Germany
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, Olanda
University of Pittsburgh School of Medicine, Pittsburgh, USA
Wake Forest Institute of Regenerative Medicine, Wake Forest University School of Medicine, Winston- Salem, NC, USA
Weizmann Institute of Science, Rehovot, Israel

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PEER REVIEW ACTIVITIES

American Journal of Kidney Disease
American Journal of Pathology
American Journal of Pathology-Renal Physiology
American Journal of Transplantation
Annals of Hematology
Apoptosis
Blood
Biodrugs
British Journal of Pharmacology
Cell Transplantation
Clinical Journal of the American Society of Nephrology
Clinical and Experimental Immunology
Cytotherapy
Diabetology and Metabolic Syndrome
Disease Models & mechanisms
EMBO Molecular Medicine
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Experimental Biology and Medicine
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Journal of the American Society of Nephrology
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Physiological Reports
Plos One
Stem Cells International
Stem Cell Research & Therapy
Stem Cells Translational Medicine
Thrombosis Research
Transplantation

PARTICIPATION IN EVENTS IN WHICH THE DEPARTMENT WAS INVOLVED

Fourth Annual Meeting SysKid, FP7, Berlin, Germany, January 26-28, 2014

Ninth International Workshop on Structure and function of the vascular system, Parigi, Francia, February 6-8, 2014
Up to date 2014: Diagnositca e Terapie delle Malattie Rare, Bari February 12, 2014

Meeting STELLAR Project III, Ranica (BG), March 19, 2014

The IX International Conference on Hypertension and the kidney” Madrid, Spagna, March 19-21, 2014

5th Expert Meeting MISOT, Ranica (BG), March 20-21, 2014

La primavera nefrologica, Bertinoro (Forlì), March 24-29, 2014

ISN Nexus Symposium, Bergamo, April 3-6, 2014

Le cellule staminali cureranno davvero le malattie dell’uomo?, Conferenza ARMR, Cremona, April 15, 2014

Cypriot Congress of Medicine, Nicosia, Cyprus, May 10-11, 2014

51st ERA-EDTA congress, Amsterdam, Netherlands, 31 May-1 June, 2014

“Focus su E.coli produttori di tossina Shiga-like (STEC)” , Lecco, June 5, 2014

32 Vicenza Course HD-PD, Vicenza, June 11, 2014

2014 aHUS Genetic Testing Round Table Roma, June 13-14, 2014

Simposio: La conoscenza delle malattie rare, Napoli, June 24, 2014


Academy of Ideas, Biogem, Ariano Irpino, Italy, September 1-3, 2014

XXVI European Congress of Pathology , Londra UK, September 2, 2014

XXXVIII Congresso Nazionale della Società Italiana Trapianti D’Organo, Siena, Spetember 24-26, 2014

The 7th International Conference: Living Donor Abdominal Organ Transplantation, Padova, September 26-27, 2014

8th Bari International Conference, Bari, Italy, October 3-5, 2014

55° Congresso Nazionale Società italiana di Nefrologia, October 8-11, 2014

Lecture ISN Program, University Hospital Moscow, October 14, 2014

Eurenomics Steering Committee Meeting, Parigi, October 20, 2014

Lecture University of Turin, Italy, October 27, 2014


5th World Congress on Targeting Mitochondria, Berlin, Germany, October 29-31, 2014
Il Cuore della Scienza: Le storie degli scienziati che scelgono l’Italia, Roma, November 10, 2014

Giornate Ematologiche Vicentine VI Edizione. Vicenza, November 19-21, 2014

ASN Kidney Week, Philadelphia, PA, November 13-16, 2014

Meeting STELLAR Project IV, London, UK, November 24-25, 2014

Nephrology Educational Winter Symposium III Barcellona, Spagna, November 28, 2014

VII Meeting della Rete di Ricerca Italiana per la lotta alla glomerulosclerosi focale, Milano, November 28, 2014

Fifth Annual Meeting SysKid, FP7, Vienna, Austria, December 1-3, 2014

Kidney-connect: a platform for European podocyte research, Milano, December 5, 2014

Donazione e trapianto in Regione Lombardia: Stato dell’arte, innovazione, prospettive, Milano, December 17-18, 2014

GRANTS AND CONTRACTS

Comitato Telethon Fondazione ONLUS
Commissione Europea
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.
ADIENNE Srl
Bayer Pharma AG
Celldex Therapeutics, Inc
Chemocentryx, Inc
Novartis Farma SpA
Omeros Corporation
Sigma-Tau SpA

SELECTION OF SCIENTIFIC PUBLICATIONS (2014)


RESEARCH ACTIVITIES

Laboratory of Cell Biology and Regenerative Medicine

Reprogramming of human bone marrow stromal cells into renal cells using cell-free extracts

In collaboration with the laboratory of Gene Therapy and Cellular Reprogramming and with the laboratory of Rare Diseases Documentation and Research

Acute and chronic kidney diseases are life-threatening conditions that affect hundreds of million people in the world, and the number of patients is rapidly increasing worldwide. To date, dialysis and renal replacement therapy remain the only treatments available. However, the shortage of transplantable organs creates an imperative need for finding innovative interventions for the cure of renal diseases. Recent studies demonstrated that cell therapy based on the use of stem cells seems to be one of the promising approaches of regenerative medicine. In the kidney field, the search for a renal specific stem cell led to the identification of progenitor cells able to induce regeneration of the damaged tissue. In this context, our laboratory identified a method to reprogram human bone marrow stromal cells (BMSCs) into renal proximal tubular-like epithelial cells using cell-free extracts of proximal tubular cells (HK2). Results showed that BMSCs exposed to HK2-cell extracts underwent morphological changes like formation of “domes” and tubule-like structures and acquired specific epithelial markers as E-cadherin, Aquaporin1 and ZO-1. Transmission electron microscopy of cell-extract treated BMSCs revealed the presence of brush border microvilli and tight intercellular contacts, suggesting a phenotypic switch of the reprogrammed cells towards a proximal tubular-like epithelial phenotype. To chronologically trace the reprogramming process, we used an eGFP plasmid containing the promoter region of E-cadherin. From day 5, we observed the presence of reprogrammed BMSCs positive for eGFP, suggesting that BMSC reprogramming was achieved using this treatment. To study whether the epithelial-like phenotype of reprogrammed BMSCs was maintained during passages, 50 clones were generated. Five out of 50 clones showed the most epithelial-like morphology, and cells from clone 17 (CL17) were the most similar to the HK2 cells and maintained stable phenotype and morphology throughout passages. Global transcriptome analysis revealed a higher degree of similarity between CL17 and HK2 cells, than between CL17 and BMSCs. Moreover, cells of CL17 acquired epithelial functional properties as transepithelial resistance and albumin binding and uptake. Finally, in in vivo studies we showed that infusion of reprogrammed BMSCs in immunodeficient mice with cisplatin-induced...
acute kidney injury engrafted into proximal tubuli, reduced renal injury and improved function. In conclusion, our results provide evidence that human BMSCs can be directly reprogrammed into cells that closely resemble renal proximal tubular epithelial cells using cell extracts. This approach could potentially fulfill the quest to utilize somatic cell types in a precursor state for cell therapy.

**Sirtuin 3 and mitochondrial dynamics: new perspectives in acute kidney injury**

*In collaboration with the Laboratory of Gene Therapy and Cellular Reprogramming*

Acute kidney injury (AKI) affects more than 13 million people worldwide and it is associated with a high mortality rate, the development of long-term chronic kidney disease, and other types of organ dysfunction in a substantial percentage of patients. Despite the extensive research in the last twenty years, effective therapeutic tools that could improve survival after an AKI episode have not been identified so far. For this reason the identification of a plausible key mediator capable of hastening renal repair after an AKI episode remains one of the critical issues in designing innovative therapies. A previous study by us showed that elevated renal expression of Sirtuin 3 (SIRT3) reduces ROS and ameliorates mitochondria dynamics that translate into the longevity phenotype in mice. SIRT3 is the major mitochondrial deacetylase that maintains basal ATP levels, as well as ROS homeostasis through the regulation of detoxifying enzymes. Building on this evidence we thought that looking at SIRT3 could be of interest to identify a plausible therapeutic target in AKI. We found that in a murine model of cisplatin-induced AKI, oxidative stress and mitochondrial fragmentation are associated with reduced renal mitochondrial SIRT3. SIRT3-deficient mice are more severely diseased and die suggesting that SIRT3 is not dispensable in preventing mitochondrial damage allowed for its repair. Pharmacological manipulations that increase SIRT3 including the AMPK-agonist, AICAR, or the antioxidant agent acetyl-L-carnitine (ALCAR) improve renal function and decrease tubular injury in WT but not SIRT3-deficient mice. In vitro, studies in cultured human tubular cells overexpressing or silenced for SIRT3 allow demonstrating the functional role of SIRT3 in regulating mitochondrial dynamics in AKI. The mechanism of SIRT3 protection depends on its capacity to preserve mitochondria integrity, thereby limiting organelle fission and membrane depolarization, which represent the prerequisites for mitophagic processes. Our present findings point to SIRT3 as a new therapeutic target for the development of compounds that are able to activate its function. Should one of those molecules become available in future for human use, many chronic conditions still lacking effective treatment, including metabolic syndrome, premature aging, cancer and AKI, could benefit.

**Generation of 3D renal structures starting from suspensions of mouse embryonic kidney cells and human cells**

*In collaboration with the Laboratory of Gene Therapy and Cellular Reprogramming*

Engineering kidneys *de novo* to provide transplantable tissues represents an intriguing option for facing the problem of the shortage of transplantable organs. Studies have suggested the possibility of employing xenogeneic embryonic kidneys, the metanephroi, as a source of immature renal cells able to recreate *in vitro* an organotypic renal structure, the renal organoid. This is made possible by the capacity of single-cell suspensions dissociated from isolated metanephros to reaggregate to form the organotypic renal structure. However, *in vitro*, the formation of glomeruli is not possible. Thus, a modified culture system that resembles *in vivo* organogenesis more closely is needed. By using an optimized reaggregation system we recently constructed *in vitro* organoids using suspensions of fully dissociated mouse kidney cells. These renal organoids integrated into a living recipient and grew to form vascularized glomeruli that exhibited well-formed capillary structures and filtration slits. They displayed most of the features of fetal kidney anatomy and were also competent at exerting kidney-specific functions in terms of blood filtration, tubular reabsorption of macromolecules and erythropoietin
production. Based on this evidence, we may expect that mouse self-organizing organoids can be used as “bio-scaffolds” to integrate human cells, in order to create 3D chimeric renal tissues. To this end, we used human cells displaying a renal tubular epithelial phenotype, called CL17. These cells were obtained by human bone marrow stromal cells (BMSCs) reprogrammed into renal proximal tubular-like epithelial cells by using tubular cell extracts (human kidney-2, HK2 cells). Thus, human CL17, HK2 cells or BMSCs were mixed with kidney cells derived from E11.5 mice, and grown in vitro for 5 days. At day 1, CL17 integrated into the condensing metanephric mesenchyme (MM) around the ureteric bud (UB) tips, identified by co-expression of neural cell adhesion molecule (NCAM; marker of condensated MM) and paired box 2 (Pax2; marker of both MM and UB). At day 5, chimeric aggregates of human and mouse cells grew into elongating tubular structures that were surrounded by laminin positive basement membranes. Remarkably, tubular structures containing CL17 or HK2 cells were in closed vicinity to glomerular-like structures expressing the early podocyte marker WT-1. BMSCs neither formed nor contributed to renal structures indicating a non-nephrogenic potential. These data indicate that CL17 acquired a renal phenotype, supporting the efficacy of cellular reprogramming, and contributed to tubular structures in renal organoids in vitro. Conclusively, we successfully demonstrate that our method provides the possibility of generating in vitro 3D chimeric renal structures, in which human cells can be incorporated. Finally, chimeric organoids represent a tool useful for testing the nephrogenic potential of human cells of various origins.

Laboratory of Immunology and Genetic of Rare Diseases and Organ Transplantation

An unanticipated role for survivin in organ transplant damage

Ischemia/reperfusion (I/R) injury is a major determinant of graft survival in kidney transplantation. Survivin, an inhibitor of apoptosis that participates in the control of mitosis and cell cycle progression, has been implicated in renal protection and repair after I/R injury; however, no study has been performed in the transplant setting. We investigated the role of survivin in modulating posttransplant I/R injury in syngeneic and allogeneic kidney grafts, and studied whether protection from I/R injury impacted on the recipient immune system, on chronic allograft nephropathy and rejection. We used genetically engineered mice with survivin haploinsufficiency and WT mice in which survivin over-expression was induced by genedelivery. Survivin haploinsufficiency in syngeneic grafts was associated with exuberant I/R tissue injury, which triggered inflammation eventually resulting in graft loss. Conversely, survivin over-expression in the grafts minimized I/R injury and dysfunction in syngeneic grafts and in a clinically relevant fully MHC mismatched allogeneic combination. In the latter, survivin over-expression translated into limited antidonor adaptive immune response and less long-term allograft injury with protection from renal parenchymal damage. Our data support survivin over-expression in the graft as a novel target for protocols aimed at limiting tissue damage at the time of transplant ultimately modulating the recipient immune system.

A Novel Atypical Hemolytic Uremic Syndrome–Associated Hybrid CFHR1/CFH Gene Encoding a Fusion Protein That Antagonizes Factor H–Dependent Complement Regulation

Genomic aberrations affecting the genes encoding factor H (FH) and the five FH-related proteins (FHRs) have been described in patients with atypical hemolytic uremic syndrome (aHUS), a rare condition characterized by microangiopathic hemolytic anemia, thrombocytopenia, and ARF. These genomic rearrangements occur through nonallelic homologous recombinations caused by the presence of repeated homologous sequences in CFH and CFHR1-R5 genes. In this study, we found heterozygous genomic rearrangements among CFH and CFHR genes in 4.5% of patients with aHUS. CFH/CFHR rearrangements were associated with poor clinical prognosis and high risk of post-transplant recurrence. Five patients carried known CFH/CFHR1 genes, but we found a duplication leading to a novel CFHR1/CFH
hybrid gene in a family with two affected subjects. The resulting fusion protein contains the first four short consensus repeats of FHR1 and the terminal short consensus repeat 20 of FH. In an FH-dependent hemolysis assay, we showed that the hybrid protein causes sheep erythrocyte lysis. Functional analysis of the FHR1 fraction purified from serum of heterozygous carriers of the CFHR1/CFH hybrid gene indicated that the FHR1/FH hybrid protein acts as a competitive antagonist of FH. Furthermore, sera from carriers of the hybrid CFHR1/CFH gene induced more C5b-9 deposition on endothelial cells than control serum. These results suggest that this novel genomic hybrid mediates disease pathogenesis through dysregulation of complement at the endothelial cell surface. We recommend that genetic screening of aHUS includes analysis of CFH and CFHR rearrangements, particularly before a kidney transplant.

Complement factor B mutations in atypical hemolytic uremic syndrome—disease-relevant or benign?

Atypical hemolytic uremic syndrome (aHUS) is a genetic ultrarare renal disease associated with overactivation of the alternative pathway of complement. Four gain-of-function mutations that form a hyperactive or deregulated C3 convertase have been identified in Factor B (FB) ligand binding sites. Here, we studied the functional consequences of 10 FB genetic changes recently identified from different aHUS cohorts. Using several tests for alternative C3 and C5 convertase formation and regulation, we identified two gain-of-function and potentially disease-relevant mutations that formed either an overactive convertase (M433I) or a convertase resistant to decay by FH (K298Q). One mutation (R178Q) produced a partially cleaved protein with no ligand binding or functional activity. Seven genetic changes led to near-normal or only slightly reduced ligand binding and functional activity compared with the most common polymorphism at position 7, R7. Notably, none of the algorithms used to predict the disease relevance of FB mutations agreed completely with the experimental data, suggesting that in silico approaches should be undertaken with caution. These data, combined with previously published results, suggest that 9 of 15 FB genetic changes identified in patients with aHUS are unrelated to disease pathogenesis. This study highlights that functional assessment of identified nucleotide changes in FB is mandatory to confirm disease association.

Dynamics of complement activation in aHUS and how to monitor eculizumab therapy

In collaboration with Unità Interazione Piastrine-Endotelio Vascolare

Atypical hemolytic-uremic syndrome (aHUS) is associated with genetic complement abnormalities/anti-complement factor H antibodies, which paved the way to treatment with eculizumab. We studied 44 aHUS patients and their relatives to (1) test new assays of complement activation, (2) verify whether such abnormality occurs also in unaffected mutation carriers, and (3) search for a tool for eculizumab titration. An abnormal circulating complement profile (low C3, high C5a, or SC5b-9) was found in 47% to 64% of patients, irrespective of disease phase. Acute aHUS serum, but not serum from remission, caused wider C3 and C5b-9 deposits than control serum on unstimulated human microvascular endothelial cells (HMEC-1). In adenosine 5'-diphosphate-activated HMEC-1, also sera from 84% and 100% of patients in remission, and from all unaffected mutation carriers, induced excessive C3 and C5b-9 deposits. At variance, in most patients with C3 glomerulopathies/immune complex-associated membranoproliferative glomerulonephritis, serum-induced endothelial C5b-9 deposits were normal. In 8 eculizumab-treated aHUS patients, C3/SC5b-9 circulating levels did not change posteculizumab, whereas serum-induced endothelial C5b-9 deposits normalized after treatment, paralleled or even preceded remission, and guided drug dosing and timing. These results point to efficient complement inhibition on endothelium for aHUS treatment. C5b-9 endothelial deposits might help monitor eculizumab effectiveness, avoid drug overexposure, and save money considering the extremely high cost of the drug.

Kidney Transplantation From a Donor With Acute Kidney Injury: An Unexpected Outcome
A 30-year-old male received a kidney graft from a 19-year-old lady dead from a brain trauma complicated by massive gastrointestinal bleeding and nonoliguric acute kidney injury. Surgery was uneventful, but kidney function did not recover. On day 6, a second transplant biopsy again showed fresh microthrombi in approximately 30% of glomeruli, without signs of rejection. Renal function progressively deteriorated and the patient resumed hemodialysis. The explanted graft disclosed chronic allograft nephropathy. A subsequent deceased-donor transplant was uneventful.

Several years later, sequencing DNA from the first donor for genes coding circulating (C3 and Factors H, B and I) and cell-bound (membrane cofactor protein and thrombomodulin) complement regulators revealed a heterozygous missense mutation in the thrombomodulin gene causing the p.V81I change in the N-terminal lectin-like (D1) domain. Conceivably, the unexpected outcome of our patient was likely explained by the mutated donor thrombomodulin that sustained kidney thrombi formation even posttransplant. Thrombomodulin is a transmembrane glycoprotein expressed on endothelial surfaces that exhibits various anti-coagulant and anti-inflammatory properties. In particular, the D1 domain, housing the V81I change found in our patient, is directly involved in complement system inhibition through factor I–mediated C3b inactivation. D1 heterozygous mutations have been recently identified in 2% of atypical hemolyticuremic syndrome (aHUS) cases, mostly children with viral prodromes, and the 81I variant has a severely impaired protective effect on CHO-K1 cell surface from activated complement. The fact that the thrombomodulin defect was restricted to the graft may also explain why, despite severe and irreversible renal microangiopathy, systemic signs of HUS were subtle in the recipient. Thus, we suggest that trauma-induced complement activation might have precipitated aHUS in a donor predisposed to the disease because of a thrombomodulin defect and, posttransplant, defective thrombomodulin might have sustained the microangiopathic process in the graft.

Laboratory of Pathophysiology of Experimental Renal Disease and Interaction with other Organ systems

RAS-FGF23-Klotho, RAS-ET-1: relevant pathways for the treatment of diabetic nephropathy

Activation of the renin angiotensin system (RAS) has long been recognized as a key component of renal disease progression. The effectiveness of RAS blocking agents depends on time when treatment is started, and imperfect renoprotection may occur if beginning therapy at an advanced phase of disease, especially in diabetic nephropathy (DN). Looking for new targets and molecules to associate to conventional therapy, we studied in experimental type 2 diabetes potentially significant pathways, among which RAS-FGF23-Klotho axis. FGF23 is a phosphaturic hormone mainly produced by bone in response to high levels of plasmatic phosphate, that acts in the kidney by binding to FGF receptors, requiring Klotho as a co-receptor. We investigated whether the kidney could be a site of FGF23 synthesis during the development of renal disease in Zucker diabetic fatty (ZDF) rats, a model resembling human type 2 DN, characterized by obesity, dyslipidemia, insulin-resistance, progressive renal scarring and cardiovascular abnormalities. FGF23 expression in the kidneys of ZDF rats increased with time as diabetic disease progressed, in parallel with decrease of Klotho expression. ACE inhibitor treatment, besides limiting proteinuria, attenuated renal expression of FGF23 and ameliorated Klotho expression in ZDF rats. Thus we demonstrated, for the first time, that the kidney is a source of FGF23 and that there is a link between RAS activation and FGF23-Klotho axis. The local production of FGF23 might represent an adaptive response to early kidney injury as a mechanism by which the ZDF rat kidney could maintain phosphate homeostatis. The recovery of renal Klotho expression after ACE inhibitor allowed the re-engagement of serum, and residual renal FGF23 to exert phosphaturic activity, resulting in normalization of serum phosphate levels in ZDF rats. Thus in experimental diabetes there is a complex interaction between angiotensin II-FGF23-Klotho-phosphorus, and disturbances of this axis may
contribute to disease progression. Interfering with this delicate balance implies potential clinical implications.

The endothelin system has been the subject of large number of studies in our department for many years. Endothelin-1 (ET-1) is a powerful vasoconstrictor peptide with proinflammatory, mitogenic and profibrotic properties. ET-1 exerts these biological actions principally through binding to ETA receptor. In the kidney of rats with streptozotocin-induced type 1 diabetes, we observed an increased expression of ET-1 which correlated with progressive glomerular extracellular matrix deposition and tubular interstitial damage. We demonstrated that adding of ETA receptor antagonist on top of ACE inhibitor led to the normalization of proteinuria, provided protection against tubule interstitial damage and promoted regression of glomerular lesions. ETA receptor antagonist acts through promoting peritubular vascularization and perfusion, while the ACE inhibitor limits proteinuria, by preserving the glomerular permselective properties. In the ZDF rat model of type 2 diabetes, the combined treatment of ETA receptor antagonist and ACE inhibitor led to substantial albeit not complete renoprotection, which was mainly due to ACE inhibitor. The combined therapy also showed cardioprotective properties, with a reversal of cardiomyocyte hypertrophy, amelioration of myocardial structure and capillary network and increased expression of VEGF/VEGF receptor, which were mainly dependent on the contribution of ETA receptor antagonist. Actually, the main objective to achieve in the clinical use of ETA receptor antagonists is to overcome problem of fluid retention and edema, which in recent trials however, could be mitigated through proper patient selection, drug dosing and diuretic administration.

Renal expression of miRNAs in a model of diabetic nephropathy

In collaboration with the Laboratory of gene therapy and cellular reprogramming

MicroRNAs are rapidly emerging as important regulators of gene expression in physiological and pathophysiological processes. Recent evidence indicated that miRNA dysregulation plays critical roles in processes involved in the development of renal diseases. In this context, specific goal of our activity was to identify altered miRNAs in the kidney of rats with type 2 diabetic nephropathy (DN) during the progression of the disease, and to study their functional role. Large-scale miRNA analysis was performed in the kidney of Zucker diabetic fatty (ZDF) rats, a model of human type 2 DN, characterized by obesity, dyslipidemia, insulin resistance, proteinuria, progressive renal injury and cardiac abnormalities. Differentially expressed miRNAs were obtained between ZDF rats and control lean rats at early (2 months of age) and late (8 months of age) phase of the disease. Expression profiling revealed that 11 miRNAs were significantly altered in the kidney of ZDF rats versus lean rats at 2 months of age: 6 were upregulated and 5 were downregulated. Moreover, 15 miRNA were significantly altered between ZDF and lean rats at 8 months of age: 10 were upregulated and 5 were downregulated. The expression levels of two of the most upregulated miRNAs in the kidney of ZDF rats at 8 months were then validated by quantitative real-time PCR. Using bioinformatic algorithms, potential gene targets were identified which are involved in fibrosis and inflammation and now are under investigation. In situ hybridization experiments are being performed to establish the renal cell population expressing the upregulated miRNAs. Next, experiments in in vitro settings will allow to define which mediators of kidney damage may act as stimulus for the specific miRNA. This study could provide insights on molecular mechanisms underlying renal disease progression in DN.

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
We recently demonstrated that bone marrow-mesenchymal stem cells (BM-MSC) ameliorate renal dysfunction and repair tubular damage of acute kidney injury (AKI) by locally releasing growth factors but also through the release of microparticles and exosomes enriched in mRNAs. The release of exosomes is a phenomenon common to different cell types including renal cells and particularly glomerular cells. Currently, we are evaluating the role played by exosomes released by different renal cells with the specific aim to evaluate whether they can be considered as a therapeutic active component of cells.

**Induction of pluripotent stem cells from somatic cells**

*In collaboration with the Laboratory of Cell Biology and Xenotransplantation*

We generated human induced pluripotent stem cells (iPS) by reprogramming human neonatal fibroblasts by using a lentiviral vector containing a unique reprogramming cassette including the four transcription factors OCT4, KLF4, SOX2 and cMyc. Four clones of iPS were obtained and characterized for their pluripotency. In collaboration with the Laboratory of Cell Biology and Xenotransplantation, we developed a protocol to differentiate iPS into renal progenitor cells.

To avoid insertions of viral sequences into the cellular genome, we are now generating iPS through the Sendai virus system and we obtained iPS clones from both normal human adult fibroblasts and PBMC from a patient with a genetic disease. We are going to characterize and differentiate the new clones into glomerular visceral epithelial cells and into endothelial cells, which will enable us to better understand the molecular mechanism underlying the renal pathology.

**Gene therapy to prevent chronic rejection of a solid organ**

*In collaboration with the Laboratory of Immunology and Genetic of Rare Diseases and Organ Transplantation*

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 clinics, 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 autotransplantation 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. To this aim, in collaboration with the Laboratory of Immunology and Genetic of Rare Diseases and Organ Transplantation, we are studying the possibility to perform gene delivery into the mouse kidney, which will enable us to better
understand how the local expression of LEA29Y is able to create a pro-tolerogenic environment. We modified our previously established gene delivery protocol for rat kidney by reducing the ischemia time necessary to still have a functional mouse graft. By RT-PCR experiments, we have demonstrated the transgene expression into a syngeneic mouse kidney 30 day after surgery. We are now going to evaluate whether this expression exerts any effect on the allograft function and survival.

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. Investigating the molecular mechanism activated by Ang II in diabetic nephropathy is crucial.

We documented that in isolated rat kidney the perfusion of Ang II induced a reduction of nephrin expression with concomitant loss of the functionality of the glomerular filter. 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 rats and patients with diabetic nephropathy. In both experimental and clinical diabetes, 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. The present study demonstrated how Ang II is crucial in perpetuating the glomerular damage in both experimental and human diabetic nephropathy via a persistent activation of Notch1 and Snail signal in podocytes and the consequent reduction of nephrin expression.

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.

To shed light on the pathogenesis of extracapillary glomerulonephritis, we are now investigating other mechanisms underlying the development of crescentic lesions such as Bowman's capsule and glomerular basement membrane rupture and the role of the inflammatory cells infiltrating the glomeruli. Furthermore, in order to strengthen the results obtained in the previous study, we are increasing the number of patients analyzed after treatment with ACE inhibitor.
DEPARTMENT OF BIOMEDICAL ENGINEERING

STAFF

Head Andrea REMUZZI, Eng. D.

Laboratory of Renal Biophysics
Head Daniela MACCONI, Biol.Sci.D.

Laboratory of Biomedical Technologies
Head Bogdan ENE-IORDACHE, Eng.D.

Unit of Medical Imaging
Head Anna CAROLI, Ph.D.

Unit of Informatics for Clinical Research
Head Sergio CARMINATI, IT.

Laboratory of Tissue Engineering for Regenerative Medicine
Head Marina FIGLIUZZI, Biol.Sci.D.
Andrea Remuzzi

**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**


Daniela Macconi

**Areas of interest**: role of angiotensin II in progressive kidney disease; podocyte and glomerular barrier dysfunction in proteinuric nephropathies; glomerular remodeling induced by renin angiotensin system blockade; intracellular molecular mechanisms underlying kidney repair/regeneration; epigenetics in glomerular and tubulointerstitial damage; role of reactive oxygen species in chronic kidney disease; relationship among albuminuria, immune response and renal disease progression.

**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, IRFMN, Bergamo, Italy; 1989-94 Scientist, IRFMN, Bergamo, Italy; 1985-89 post-doctoral fellow, IRFMN, Bergamo, Italy; 1982-83 fellow Laboratory of the Division of Nephrology e Dialysis, Ospedali Riuniti di Bergamo, Bergamo, Italy.

**Selected publications**


Bogdan Ene-Iordache got an MSc in Mechanical Engineering in 1990 at the Petroleum & Gas University in Ploiesti (Romania).

Training activities: 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.

Areas of interests: Renal research (hemodynamics and remodeling 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 NegriBERGAMO Laboratories, Bergamo (BG). Since January 2000 he is the Head of the Laboratory of Biomedical Technologies, part of the Department of Biomedical Engineering, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Ranica (BG).

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, immunosolilation devices for pancreatic islets, differentiation of progenitor pancreatic cells in insulin containing cells, immunohistochemistry.

Chronology of appointment: From 2014 Head Laboratory of Tissue Engineering, Department of Biomedical Engineering; 2000-2014 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 fields.

Roles: since 2004, researcher at the Laboratory of Epidemiology and Neuroimaging, IRCCS Fatebenefratelli, Brescia, Italy. Since 2008 to 2012, researcher at the Medical Imaging Unit, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri” in Ranica (BG). In July 2012 she became head of the Medical Imaging Unit, part of the Laboratory of Biomedical Technologies inside the Bioengineering Department, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Ranica (BG).

Most relevant publications:

Sergio Carminati got an Accountancy Diploma in 2000 at Istituto Tecnico Commerciale Camanghè, Zogno (BG).

Training activities: He completed his training at the Clinical Research Center “Aldo e Cele Daccò” in Ranica (BG). In 2001 he obtained a master in Computer Programming at ENAIP Lombardia, Bergamo (BG).

Areas of interest: Data management for clinical trials, web-based applications for epidemiology and outcome research. Support activities for clinical web sites, local area network (LAN) system administration, videoconferences and meetings.

Roles: since 2001 to 2002 he did the civil service in the Laboratory of Biomedical Technologies. Since 2002 to 2011 he was a researcher at Laboratory of Biomedical Technologies. Since February 2010 he became the Head of the Unit of Clinical Research Informatics, Department of Bioengineering, IRCCS - Istituto di Ricerche Farmacologiche “Mario Negri”, Ranica (BG).

Selected publications

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, Università degli studi di Bergamo, Dalmine
Facoltà di Medicina e Chirurgia, Università degli Studi di Milano, Milano
Brembo S.p.A. Stezzano, Bergamo
Italcementi Group, CTG S.p.A. c/o i.lab, Bergamo
Ospedale Generale Regionale Miulli, Acquaviva delle Fonti, Bari
Azienda Ospedaliera Cannizzaro, Catania
Ospedale Annunziata, Cosenza
INTERNATIONAL COLLABORATIONS

Massachusetts Institute of Technology, Cambridge MA, USA
Academisch Medisch Centrum, Amsterdam, the Netherlands
Ghent University, Ghent, Belgium
Technical University, Eindhoven, The Netherlands
University Hospital, Maastricht, The Netherlands
The University of Sheffield, Sheffield, United Kingdom
ESAOTE, Maastricht, The Netherlands
Beta O2, Tel Aviv, Israel
Fresenius Medical Care, Frankfurt, Germany
University Hospital Zurich, Switzerland
Technische Universität München (TUM), Munich, Germany

EDITORIAL BOARD MEMBERSHIP

International Journal of Artificial Organs (Andrea Remuzzi, Editor in Chief)

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
Applied Numerical Mathematics
ASME Journal of Biomechanical Engineering
Artificial Organs
Biomaterials
Cell Transplantation
Cells Tissues Organs
Cells & Materials Journal
CNS Neuroscience and Therapeutics
Computers in Biology and Medicine
Contemporary Clinical Trials
Current Alzheimer Research
Current Genomics
Experimental Biology and Medicine
Endocrine
Frontiers in Endocrinology
Frontiers in Experimental Pharmacology and Drug Discovery
Hemodialysis International
IEEE Transactions on Biomedical Engineering
IEEE Transactions on Image Processing
International Journal of Alzheimer's Disease
International Journal of Artificial Organs
EVENT ORGANIZATION


May 16th 2014, Club: "INTERCheck: uno strumento informatico per la valutazione dell'adeguatezza prescrittiva" Flavio Suardi, Istituto Mario Negri, Centro Anna Maria Astori – Bergamo.


April 15th 2014, Club: "Sviluppo e validazione biologica di sistemi sperimentali per l'ingegneria del tessuto renale" Michele Rosati, Politecnico di Milano.

PARTICIPATION IN EVENTS IN WHICH THE DEPARTMENT WAS INVOLVED

MASTER UNIVERSITARIO DI II LIVELLO 1° Corso Residenziale: “Propedeutica clinica e fisiopatologia dell'accesso vascolare”, 29-30 Gennaio 2014, Pisa, Italy.

Corso Teorico-Pratico Avanzato Di Eco Color Doppler Carotideo E Vertebrale (Con sessioni applicative pratiche), 27-28 febbraio / 1 marzo 2014, Milano, Italy.

IV Congresso Gruppo Nazionale Bioingegneria GNB - 25-27 giugno 2014, Pavia, Italy.

18th European Vascular Course - VAS, 12-14 Maggio 2014, Maastricht, the Netherlands.

7th World Congress of Biomechanics, WCB 2014, 6-11 Luglio 2014, Boston, USA.

XLI Annual ESAO Congress, European Society for Artificial Organs, 17-20 Settembre 2014, Roma, Italy.

FASEB Meeting: “Polycystic Kidney Disease: From Molecular Mechanism to Therapy”, 3-8 Agosto 2014, Lucca, Italy.

La Mano e il Piede Ischemico Nel Dializzato: Verso Nuovi Approcci, 16 e 17 ottobre 2014, Villa Romanazzi Bari, Italy.


Seminario Avanzato CKD-MBD 2014, 8 e 9 maggio 2014, Milano, Italy.

“Tissue Engineering & Regenerative Medicine International Society” European Chapter Meeting, 10-13 Giugno 2014, Genova, Italy.

3rd International Conference on Tissue Science & Regenerative Medicine, 24-26 Settembre 2014, Valencia, Spain.

55° Congresso Nazionale della Società Italiana di Nefrologia, 8-11 Ottobre 2014, Catania, Italy.

Young investigators meeting on career planning, 28 March, 2014, Aachen, Germany.

CampING - Advanced Imaging and Visualization Workshop, 19-22 May 2014, Innsbruck, Austria.

Advanced Statistics and Data Mining Summer School, 23 June - 4 July, 2014, Univ. Politécnica de Madrid, Spain.

Medical Imaging Summer School, 27 July - 2 August, 2014, Favignana, Italy.

GRANTS AND CONTRACTS

Research grants AIFA - studi clinici controllati (ARCADIA, ANSWER, CRESO2).

Research grant Baxter – ASAP trial “Acute Start Access Programme”.

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Research grant ABBOT – PROCEED trial

Project - FP7 UE - RESET "Dreaming of no more renal dialysis: how self-derived tissue and cells can replace renal function". FP7- 268632 - Project Coordination.


Project COBRA in collaboration with Brembo S.p.A.: “Valutazione del rischio potenziale legato alla produzione di pastiglie per freni e al loro utilizzo”.

Project with Fresenius S.p.A. “Studio pilota per valutare l’impatto del modello matematico predittivo (AVF.SIM) nella pratica della clinica convenzionale”.

Project - FP7 UE TranCYST (Marie Curie Initial Training Network on Polycystic Kidney Disease - FP7-PEOPLE-MCA-ITN-317246).

Research grant ERA – EDTA - EuroCYST (Building a network of ADPKD reference centers across Europe).

**SELECTION OF SCIENTIFIC PUBLICATIONS (2014)**


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 MWF rats with advanced nephropathy has documented a progressive rarefaction of the renal vasculature affecting intermediate and small size vessels. Treatment of animals at advanced stage of the disease with renin angiotensin system inhibitors halted vascular rarefaction and even increased the volume density of kidney vasculature as compared to pre-treatment suggesting a regenerative process. 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 assessing 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.

We have recently applied the microCT to the 3D analysis of cardiovascular structures and cystic kidney in experimental models of end stage kidney disease.

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. This prompted us to assess 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. Our results show that Ang II promotes insulin resistance through mitochondrial ROS that lead to Sirt3 dysfunction impairing mitochondrial antioxidant defense and the activity of AMPK-activated protein kinase, a key regulator of energy metabolism and glucose transport in skeletal muscles. Acetyl-L-carnitine improves insulin sensitivity of Ang II-treated cells by preventing Sirt3 dysfunction.

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 (Remision 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). 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). 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.

**Web EMR – a web-based tool for electronic management of clinical activity in Daccò Center**

Clinical activities carried out since the ‘90s, and especially the recent transformation of the Daccò Clinical Centre in IRCCS requires complete computerized management of clinical data of the patients followed in the Centre. The Unit of Informatics for Clinical Research has been involved in the re-engineering of the electronic medical record (EMR) concerning the clinical activities of the Centre. The project was aimed at integrating under a unique, centralized system, the management of clinical data generated in the Day Hospital, with the data collection systems of other laboratories involved in the controlled clinical trials (like Chemical Analysis, Rare Diseases and Immunology and Genetic Transplantation).

In September this year we replaced the "old" medical records with a new web-based version (https://clinicalweb.marionegri.it/emr/), a system reliable and complete for the management of clinical data. We have given special priority to the recovery of historical clinical data collected in more than 20 years of clinical activity. For this data recovery plan, we have set complex import procedures on the old clinical database. Available via Internet through the https protocol, the medical web-EMR can be accessed from any device (desktop or mobile) with built-in browser, and meets all the technical and safety requirements for medical software and implements Good Clinical Practice (GCP) for clinical trials. The web-EMR uses the LAMP (Linux Apache MySQL PHP) server technology and is hosted in the server-farm facilities in Milan.

**INTERCheck® WEB- a tool for evaluating appropriateness of prescriptive therapy**

(*In collaboration with the Department of Neuroscience*)

Our Unit of Informatics for Clinical Research has developed a support system for evaluating the appropriateness of drug therapies and possible drug interactions. In a first step, we developed a system that allows on-line management and easy maintenance of the database interactions between drugs. The maintenance of the database is performed by specialized researchers from the Department of Neuroscience. This database is the relational engine of the new online version of the application INTERCheck® WEB (https://www.intercheckweb.it/). The tool was aimed at improving the appropriateness of prescriptive therapy through pharmacology-based standards: inappropriate medication according to several known criteria (Beers 2012, START/STOPP, AIFA indications), estimation of Anticholinergic Cognitive Burden scale, and dosage in patients with impaired renal function (GerontoNet ADR Risk Score) for the identification of subjects at risk of drug side-effects.

INTERCheck® WEB has several interesting features, such as the possibility of saving patient data, or the integration with other centralized systems through dedicated web services.

**Haemodynamics and vascular pathology**

During the last twenty years, the existence of a tight relationship between haemodynamics 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-colour 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 haemodialysis 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 nature of the disturbed flow has been recently characterised in details by fluid dynamic simulations in patient-specific models of vascular access for haemodialysis treatment, with particular attention to the laminar-turbulent transition of the flow.

Computer-aided planning of vascular surgery for the creation of vascular access for haemodialysis

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, a preliminary validation showed that these tools can reliably predict vascular access function from pre-operative evaluations and could help the surgeon in best planning the creation of vascular access for each individual patient. A multicentric pilot clinical study aimed at proving AVF.SIM validity and usability in support of vascular surgical planning in clinical practise is still ongoing.

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. By developing 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 S.p.A., 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.
Laboratory of Tissue Engineering for Regenerative Medicine

Islet transplantation as a cure for type 1 diabetes

Type 1 diabetes is an autoimmune disease that results from destruction of insulin-producing beta cells of the pancreas. Death of pancreatic beta cells is associated with hyperglycaemia which is the main determinant of long-term complications in diabetic patients. Type 1 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. At the current time the only way to restore normal blood glucose level in diabetic patients is pancreas or pancreatic islets transplantation. Transplantation of pancreatic islets is currently limited because of the need of immunosuppressive therapy. Development of immunoisolation technique by separation of implanted cells from the host immune system is a strategy to prevent immunorecognition and rejection. The aim of this project is to develop new immunosolation devices for pancreatic islets transplantation and to test the efficacy and in vivo resistance of the devices. We have developed a device 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. 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. Then, we have also evaluated whether transplantation of pancreatic islets can induce regression of diabetic complications in a model of allotransplantation in rats with chemically induced diabetes. We observed that neurological parameters observed in diabetic rats significantly ameliorate in transplanted rats as well as a consistent reduction in proteinuria. When the blood glucose concentration was normalized by islet transplantation we also observed an important regeneration of beta cells.

Development of methodologies for kidney regeneration

(In collaboration with the Department of Molecular Medicine)

Chronic kidney disease is a pathological condition that affects millions of people worldwide, and the number of patients is increasing. The poor quality of life of patients on dialysis and the shortage of transplantable organs require efforts to develop therapeutic alternatives. The overall focus of this project is to recreate a new organ in the laboratory starting from a native kidney completely decellularized and subsequently recellularized with mouse embryonic stem (mES) cells. To this aim we have developed a perfusion system for decellularization and recellularization of intact rat kidneys. We have previously shown that perfusion of rat kidney with the anionic detergent sodium dodecyl sulfate (SDS) allowed to obtain optimal cellular removal and maintenance of intact 3D architecture of renal extracellular matrix. Complete decellularization and preservation of ultrastructure and vasculature were assessed by optical and electron microscopy and microCT. The recellularization technique has been modified to allow the cells to reach tubular compartment. mES cells were infused through renal artery and subsequently through ureter. A negative pressure was applied outside of the scaffold during ureter infusion in order to increase tubular membrane permeability and promote cell distribution along the collecting system without damaging extracellular matrix. Recellularized scaffolds were then 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 mES cells. Histological analysis demonstrated that mES cells infused through the renal artery were uniformly distributed in the vasculature and in glomerular capillaries while cells infused through the collecting system reached tubular compartments. Our findings indicate that kidney scaffolds can be suitable for adhesion, survival and differentiation of mES cells.
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. Particularly, our interest focuses on the failure of native arteriovenous fistulas, used for hemodialysis treatment, mainly due to neointimal hyperplasia. The aim of our study is to investigate the roles of wall shear stress, calculated in different fistula areas, in the failure of arteriovenous access. Thanks to innovative technologies developed in medical imaging and mathematical modelling, the researchers of the Laboratory of Biomedical Technologies are able to reproduce accurately patient-specific hemodynamic force distribution in vascular access for hemodialysis. The values estimated in a side-to-end fistula were imposed on endothelial cells with a programmable system based on a cone-plate geometry developed in our laboratory. The device was able to generate highly unsteady, unidirectional or oscillating shear stress waveforms. Our results indicate that shear stress measured in the high risk stenosis area elicits up-regulation of pro-inflammatory signals (i.e. monocyte chemo-attractant protein 1 and interleukin 8) and down-regulation of protective genes such as the Krüppel-like factor 2, if compared to levels elicit by shear stress waveforms derived from areas not prone to disease development.

Development of methodologies for cytotoxicological assessment of particulate matter

*(In collaboration with the Laboratory of Environmental Chemistry and Toxicology)*

Particulate matter (PM) is a complex mixture of organic and inorganic components originate from natural sources, industrial processes and traffic. Particularly, brake wear is estimate to contribute 50% of non-exaust traffic related PM emissions. Coarse (diameter >10 μm), fine (diameter <2.5 μm) and ultratine (diameter <0.1 μm) particulate is deemed one of the most dangerous factors to human health. Our Department is involved in a research project in collaboration with Brembo S.p.a., CTG S.p.a. and PNO Consultants Limited. The aim of the project is to demonstrate a novel brake-pad technology where a cementitious material replace state of the art phenolic resins. The environmental benefits will be energy saving, and thus CO₂ reduction, water saving and avoidance of the emission of PM 0.1. Our object is to evaluate the toxicity associated to the pollutants emitted from the old and new brakes. The toxicity of pollutants and dusts generated from braking tests on human target organs is investigated using different human cell lines cultured in vitro, as model of respiratory tract, whole organ (3D culture of liver cells) and blood as potential target of organs and systems. Toxicity is investigated at different levels, including effects on cell proliferation cytotoxicity, genotoxicity, production of oxygen free radicals and inflammation.
Aldo and Cele Daccò Center

Ranica (Bg)

ANNUAL
REPORT 2014
departments and laboratories
DEPARTMENT OF RENAL MEDICINE

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CURRICULA VITÆ

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.


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:

**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 2007 researcher at the Mario Negri Institute, till 1995 at Molecular Medicine Department and then at Renal Medicine Department.  
**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:**

Giulia Gherardi got her Scientific High School Diploma in 1989 at the Liceo Scientifico Marie Curie in Zogno (Bergamo), the Nurse Diploma in 1995 at the Scuola per Infermieri Professionali, Ospedali Riuniti, Bergamo and the 1st Level Master in Clinical Research in 2008 at the Medicine and Surgery Faculty of the University in Milan.

**Educational training:** Clinical Research Nurse Diploma on 1997 at IRFMN – Daccò Center.

**Areas of interest:** statistical methodology of long-term randomised clinical trials in nephrology, and diabetology; the coordination, conduction and monitoring of controlled clinical trials.


**Selected publications:**
Norberto Perico got his Medicine degree in 1983 at the University of Verona, 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 2004 he’s Member, ISN Research & Prevention 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 nephrology and diabetology, 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 - IRCCS IRFMN, 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 (hyperinsulineemic euglycemic 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 and 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 (since 1990), of the Union of Scientists in Bulgaria (since 1991), and member of the Union of Medical Doctors and Dentists, Bergamo, Italy (since 05.03.2009).

Selected publications:


Nadia Rubis got her degree in licensed practical nurse in 1995 at the Nursing School of Azienda Ospedaliera Ospedali Riuniti di Bergamo. **Education training:** she completed her research training at IRFMN - Centro di Ricerche Cliniche per le Malattie Rare Aldo e Cele Daccò – Bergamo, Clinical Research Nurse Course (1998).

**Areas of interest:** coordination of clinical studies, monitoring activities and data management.


**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 microvascular 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 Polycystic 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, Romano di Lombardia (BG)
- AO Treviglio, Poliambulatorio extra-ospedaliero, Brembate (BG)
- ASL Bergamo, Bergamo
International Collaborations

- University Medical Center, Ljubljana (Slovenia)
- Service de Pharmacologie Clinique, Faculté de Médecine, Lyon (France)
- Hospital Universitario de Canarias, La Laguna, Tenerife (Spain)
- Drug Prescribing Unit Navarre Regional Health Service, Pamplona (Spain)
- Department of Primary Health Care, University of Oxford, Oxford (UK)
- The Ottawa Hospital, Centre for Practice-Changing Research, Ottawa (Canada)
- Department of Clinical Sciences/Diabetes & Endocrinology Lund University, Skåne University Hospital, Malmö (Sweden)
- University Medical Center Groningen, Groningen, (The Netherlands)
- Department of Clinical Pharmacology, Groningen (The Netherlands)
- Leiden University Medical Center, Leiden (The Netherlands)
- University Medical Center, Groningen (The Netherlands)
- Department of Gastroenterology & Hepatology, Radboud University Nijmegen Medical Center, Nijmegen (The Netherlands)
- Department of Nephrology, Radboud University Nijmegen Medical Center, Nijmegen (The Netherlands)
- Mariinskaya Hospital, Saint-Petersburg (Russia)
- Moscow State University of Medicine and Dentistry, Mosca (Russia)
- Chişinău Hospital, Chişinău (Moldova)
- Damanhour Medical National Institute, Damanhour, Beheira (Egypt)
- Health Sciences University, Ulaanbaatar (Mongolia)
- BP Kerala Institute of Health, Dharan (Nepal)
- Division of Cardiology, Brigham and Women's Hospital, Boston, MA (USA)
- National Kidney Foundation, New York, NY (USA)
- New England Medical Center, Boston, MA (USA)
Complejo Hosp Metropolitano de la Caja de Seguro Social, Panama City (Panama)
Hospital Juan XXIII, La Paz (Bolivia)
Hospital Maciel, Montevideo (Uruguay)
Cochrane Collaboration, Cochrane Renal Group, Centre for Kidney Research, NHMRC Centre for Clinical Research Excellence in Renal Medicine, The Children's Hospital at Westmead, Westmead, (Australia).

EDITORIAL BOARD MEMBERSHIP

Current Diabetes Reviews (Piero Ruggenenti)
Journal of Nephrology (Piero Ruggenenti)
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
PloS Medicine
PloS One
The International Journal of Artificial Organs
The Lancet
Translational research
PARTICIPATION IN EVENTS
IN WHICH THE DEPARTMENT WAS INVOLVED


*HUS updated for the transplant nephrologist.* Hot topics in renal transplantation: endothelial injury. Genova (Italy), April 12th 2014.

*Therapies for ADPKD: somatostatin therapy.* 51° ERA-EDTA Congress. Amsterdam (Holland), June 2nd 2014.

*MSCs in transplantation: immunoregulatory or pro-inflammatory.* Clinical Scientist Summer Symposium on Translational Medicine. Berlin (Germany), June 19th 2014.

*Long-acting somatostatin analogue and renal function in ADPKD patients: the ALADIN trial.* Science Research Conferences of the Federation of American Societies for Experimental Biology. Lucca (Italy), August 5th 2014.

*La malattia renale policistica autosomica dominante.* Il rene nelle malattie rare (e dintorni…). Piacenza (Italy), September 26th 2014.

*L’eculizumab contrasta la microangiopatia trombotica complemento-mediata e migliora la prognosi in pazienti adulti con sindrome emolitico-uremica atipica (SEUa).* 55° Congresso Nazionale della Società Italiana di Nefrologia. Catania (Italy), October 8th-11th 2014.


*Pathogenesis of and novel treatments for Autosomal Dominant Polycystic Kidney Disease (ADPKD).* ISN-SRC Program. Moscow (Russian Federation), October 14th 2014.


*Mesenchymal stromal cells: can they make the difference?* ESOT-AST Joint Meeting. Madrid (Spain), October 19th 2014.

*Glomerular hyperfiltration in renal disease, 30 years of debate.* Continuing Medical Education Course “DIABESITY: Diabetes and Obesity in Renal Disease”. Tenerife (Spain), November 1st-2nd 2014.
Calorie restriction in renal disease. Continuing Medical Education Course “DIABESITY: Diabetes and Obesity in Renal Disease”. Tenerife (Spain), November 1st-2nd 2014.

Estimation of GFR in diabetes and obesity: is it reliable?. Continuing Medical Education Course “DIABESITY: Diabetes and Obesity in Renal Disease”. Tenerife (Spain), November 1st-2nd 2014.

Future directions in Diabesity and renal disease. Continuing Medical Education Course “DIABESITY: Diabetes and Obesity in Renal Disease”. Tenerife (Spain), November 1st-2nd 2014.

Hypertension associated with CKD. American Society of Nephrology Kidney Week. Philadelphia (Pennsylvania, USA), November 11th-16th 2014.


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
Bayer AG
Baxter SpA
Bio3 Research Srl
Bracco Imaging SpA
Genzyme Europe BV
Sanofi-Aventis SpA

SELECTION OF SCIENTIFIC PUBLICATIONS (2014)


**RESEARCH ACTIVITIES**

**Laboratory of Biostatistics**

**A Long-term effects of the somatostatin analogue octreotide LAR in ADPKD patients with polycystic liver disease: a pre-specified substudy of the ALADIN trial**

Short-term studies suggest that somatostatin-analogues may have a beneficial effect in patients with polycystic liver disease (PLD) associated with Autosomal Dominant Polycystic Kidney Disease (ADPKD) or considered as a single disease entity. Whether and to what extent these medications are effective and tolerated in the long-term is unknown. In this substudy of 27 ADPKD patients with PLD, randomly allocated to 40 mg Octreotide-LAR (n=14) or placebo (n=13) monthly administered in the context of the “A Long-Acting somatostatin analogue on Disease progression In Nephropathy due to autosomal dominant polycystic kidney disease (ALADIN)" prospective, randomized, single-blind, parallel-group trial, we primarily evaluated the absolute and percent changes in total liver volume (TLV), as assessed by repeated Magnetic Resonance Imaging (MRI), at three years of treatment and two years after treatment withdrawal (Recovery) versus baseline. Analyses were by modified intention to treat. This study was registered with ClinicalTrials.gov, NCT00309283. Recruitment was between April 27, 2006, and May 12, 2008. In 2014 we performed final statistical analyses of the ALADIN-liver substudy.

**Glomerular hyperfiltration is a common risk factor for accelerated GFR decline in young adults with autosomal polycystic kidney disease (ADPKD)**

Glomerular hyperfiltration is an independent risk factor for accelerated GFR decline in ADPKD children. Here we aimed to assess whether and to what extent this finding can be extended to young <35 years ADPKD adults. Among 900 ADPKD outpatients of the "Papa Giovanni XXIII" Hospital (Bergamo, Italy), we identified all consecutive young adults with a baseline creatinine clearance (CrCl) >140 mL/min/1.73m², followed for at least 1 year with a minimum of 3 CrCl measurements. We identified 91 patients (45 males, 49.5%) aged<35 years with a
baseline CrCl of 110 (IQR: 86-129) mL/min/1.73 m². Eleven patients (12.1%) were hyperfiltering. In 2014 we performed statistical analyses of the above study.

Anti PLA2R antibody titer predicts post-rituximab outcome of membranous nephropathy

Rituximab induces nephrotic syndrome (NS) remission in two thirds of patients with primary membranous nephropathy (MN), even after other treatments have failed. To assess the relationships among treatment effect, circulating nephritogenic anti phospholipase A2 receptor (PLA2R) autoantibodies and genetic polymorphisms predisposing to antibody production we serially monitored 24-hour proteinuria and antibody titer in patients with primary MN and long-lasting NS consenting to rituximab (365-mg/m2) therapy and genetic analyses. Over a median (range) followup of 30.84 (6.00-145.36) months, 84 of 132 rituximab-treated patients achieved complete or partial NS remission (primary endpoint) and 25 relapsed after remission. In 2014 we performed statistical analyses of the above 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): first interim analysis

VALID is a multicenter, Prospective, Randomized, Open label, Blinded End point (PROBE) trial of 3-year treatment with halved doses of benazepril (10 mg/day) and valsartan (160 mg/day) given in combination, or full doses of both benazepril (20 mg/day), or valsartan (320 mg/day) given alone in 120 consenting patients >40 year old, with type 2 diabetes (WHO criteria), serum creatinine >1.8 mg/dl and < 3.5 mg/dl, spot morning urine albumin to creatinine ratio >2000mg/g and no specific contraindications to the study drugs. Primary efficacy variable is ESRD and primary comparison will be between the benazepril plus valsartan and valsartan alone groups. During 2014 the first interim analysis has been completed.

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): first interim analysis

The ATHENA study primarily compared the incidence of biopsy-proven chronic allograft nephropathy (CAN) three years post-transplant in kidney recipients randomly allocated to MMF or AZA, after induction therapy with basiliximab and low-dose RATG, and sequential steroid and CsA withdrawal. Secondly, the study compared acute rejections after CsA withdrawal, long-term patient and graft survival, and graft function and prevalence/severity of CAN at study end. This was an open, randomized, prospective, multicenter study to compare the cost/efficacy of low-dose MMF versus AZA as the sole immunosuppressive therapy in preventing CAN after induction therapy with basiliximab plus low-dose RATG combined with CsA during the first year after surgery in kidney transplant recipients. Two-hundred-twenty-four kidney transplant recipients from deceased donors given induction therapy with two 20 mg basiliximab injections 4 days apart and a seven-day course of RATG (0.5 mg/kg/day), were randomly allocated on a 1:1 basis to 3-year treatment with low-dose MMF or AZA, added-on CsA maintenance therapy. During 2014 the first interim analysis has been completed.

A prospective, randomized, prospective, open label, blinded end-point (PROBE) trial to evaluate whehter, at comparable blood pressure control, ACE inhibitor therapy more effectively than nonRAS inhibitor therapy reduces cardiovascular morbidity and mortality in chronic dialysis patients with left ventricular hypertrophy and/or arterial hypertension (ARCADIA study): report for Agenzia Italiana del Farmaco (AIFA)
Angiotensin-converting-enzyme (ACE) inhibitors have a specific cardioprotective effect and, compared to treatment not directly interfering with the renin-angiotensin-system (RAS), significantly reduce cardiovascular (CV) mortality and morbidity in subjects with normal renal function.

Patients with end stage renal disease (ESRD) have a 10-20 fold excess cardiovascular risk compared to subjects with normal renal function and excess risk is even higher in those with left ventricular hypertrophy (LVH). Despite CV events are the leading cause of death in these patients, no adequately powered trial so far evaluated the specific cardioprotective effect of ACE inhibitors in this population. This prospective, randomized, open label, blinded end point (PROBE) trial is primarily aimed at evaluating whether, at comparable blood pressure (BP) control, ACE inhibitor as compared to non-RAS inhibitor therapy significantly reduces the incidence of a composite end point of CV death (including sudden death) and non-fatal myocardial infarction or stroke in 624 patients with arterial hypertension (pre-dialysis systolic/diastolic BP >140/90 mmHg or postdialysis systolic/diastolic BP >130/80 mmHg or antihypertensive therapy) and/or echocardiography evidence of LVH (cardiac mass index >130 g/m² for men and 100 g/m² for women) who are on dialysis therapy since at least six months. Secondarily, the study will compare the incidence of single components of the primary outcome, new onset paroxysmal or persistent atrial fibrillation, thrombosis of the arterio-venous fistula, new onset, progression or regression of LVH, changes in components of the metabolic syndrome, the safety profile of the two treatment regimens and their cost/effectiveness. During 2014 the ‘Agenzia Italiana del Farmaco’ (AIFA) report has been completed.

Rituximab versus Cyclophosphamide therapy in Idiopathic Membranous Nephropathy: a propensity score guided comparison

Idiopathic membranous nephropathy (IMN) is the most common cause of the nephrotic syndrome in adults. The initial treatment of iMN patients who present with nephrotic syndrome consists of anti-proteinuric and blood pressure lowering drugs such as ACE inhibitors and/or ARBs. In addition, for patients at high risk of progressive kidney failure KDIGO guidelines recommend treatment with cyclophosphamide, which is proven to be effective in reducing the risk of end stage kidney disease in iMN patients. However, the use of cyclophosphamide is associated with severe side effects. Rituximab is a monoclonal antibody against CD20 that can be found on B-lymphocytes. We recently reported the successful use of rituximab in a cohort of 100 consecutive patients with iMN. Our data suggested that rituximab as effective as cyclophosphamide, but with a more favourable safety profile. However no head-to-head comparisons of the currently recommended cyclophosphamide based regimen and rituximab have been reported. Clinical trials are difficult to perform in iMN due to its relative rarity and the long time follow-up needed to evaluate clinical end points, such as end stage renal disease and mortality. As a consequence very few randomized trials have been performed in iMN. Therefore, many guidelines rely on observational data to aid clinical decision making. Unfortunately, traditional regression models used to adjust for confounding factors in observational studies are hampered by sparse data as well. As a consequence, either over-fitting of a model to the data or residual confounding occurs. Propensity score methods, however, use a two stage approach in order to achieve confounder balance between treated and untreated patients before comparing outcomes in selected patient groups. Thus, propensity score methods are very close to randomization conceptually, and may offer a solution next best to a clinical trial in comparing treatments for iMN. In the present study, we pooled data of cohorts of iMN patients, treated in two different centers, who have used treatment regimens using either rituximab or cyclophosphamide respectively. We used propensity scores to pseudo-randomize patients, and directly compared the safety profiles of rituximab and the restrictive cyclophosphamide regimen in the treatment of iMN. In 2014 we performed statistical analyses of the above study.

The Glu936Asp CFH gene variant and Renal and Cardiovascular Events in Type 2 Diabetics: BENEDICT Study Group
Despite optimal blood pressure and metabolic control and early treatment with angiotensin converting enzyme inhibitors (ACEi), approximately one third of type 2 diabetic patients develop microalbuminuria (i.e., a urinary albumin excretion [UAE] rate of 20-200 μg/min). These patients may progress to macroalbuminuria taken as an early marker of diabetic nephropathy, have progressive deterioration of renal function and are at excess risk of cardiovascular events. Indeed, forty to 50 percent of patients with type 2 diabetes who have microalbuminuria eventually die of cardiovascular disease; this is three times as high a rate of death from cardiac causes as among diabetic patients with no evidence of renal disease. On the other hand, ACEi therapy may induce regression to normoalbuminuria in 50% of microalbuminuric type 2 diabetic patients and regression is associated with 50% reduction in cardiovascular risk. Evidence is available that complement-mediated inflammation is implicated in the pathogenesis and progression of renal and extra-renal complications of type 2 diabetes. In a large and homogenous cohort of normoalbuminuric type 2 diabetic patients prospectively followed in the context of a randomized clinical trial, we evaluated the association between carriers of the Asp/Asp genotype of the Glu936Asp FH polymorphism and risk of progression to microalbuminuria than carriers of one or two wild-type Glu alleles. In 2014 we performed statistical analyses of the above study.

**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 collaborate 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**

**Effects of L-Cysteine administration compared to placebo on residual renal function in stable peritoneal dialysis patients**

Over the last decades, peritoneal dialysis has grown worldwide to become one of the most common modalities of renal replacement therapy, particularly in developing or newly industrialized countries. Peritoneal dialysis has been associated with an initial survival benefit compared to hemodialysis, although this advantage becomes less apparent over time, likely due to the progressive loss of residual renal function and the development of pathological alterations of peritoneum.

It has been recently shown that antioxidant therapy by N-acetyl-cysteine oral supplementation may improve residual renal function in peritoneal dialysis patients.

This finding may have major clinical relevance, since preserving residual renal function in peritoneal dialysis patients may be associated with improved survival.

Thus, we planned a randomized, double-blind, crossover study to confirm the preliminary evidence of the beneficial effects of antioxidant agents on residual renal function by using the L-enantiomeric form of cysteine in 10 prevalent peritoneal dialysis patients with residual diuresis higher than 100 mL. Iohexol was selected as a gold standard for renal function determination.
An intravenous bolus of 20 mL of iohexol solution was injected and 12 blood samples were drawn between 15 and 480 minutes after injection of the marker. Iohexol plasma clearance was determined according to a 2-compartment open model and a simultaneous urinary collection was performed to calculate renal clearance of the marker. Iohexol dialytic clearance was determined by measuring the concentration of the marker in the dialysate exchanged during the nocturnal dialytic session.

The effect of the antioxidant therapy by N-acetyl-cysteine oral supplementation was also assessed by evaluating the peritoneal equilibration test (PET). In addition to routine laboratory parameters evaluation, serum, plasma and dialytic liquid samples were collected for the determination of markers of inflammation as well oxidative stress.

The study is in progress, and 4 out of the 6 enrolled patients, completed the study.

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 diabetes 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. Our 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 unrealistically 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 severe renal insufficiency (ALADIN II study)**

There is an 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 treatment with octreotide-LAR, a synthetic long-acting analogue of somatostatin, 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, and also ameliorated glomerular hyperfiltration in patients with ADPKD and relatively preserved renal function, with acceptable safety profile (The Lancet 2013; 382 : 1485-1495). 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.

**In patients with autosomal dominant polycystic kidney disease and renal insufficiency sirolimus therapy fails to slow disease progression and is unsafe**

In patients with autosomal dominant polycystic kidney disease (ADPKD) and normal renal function or mild renal insufficiency sirolimus, an inhibitor of the mammalian target of rapamycin (mTOR) enzyme, has been suggested as suitable therapeutic option to slow the progressive renal cyst growth. To assess the long-term outcomes of sirolimus treatment on kidney disease progression, an academic, prospective, randomized trial was performed in 41 patients with ADPKD and severe renal insufficiency, randomized to either sirolimus on top of
conventional treatment (n= 21) or to conventional treatment alone (n= 20). The primary outcome was change over baseline of glomerular filtration rate (GFR) measured by iohexol plasma clearance at 1 year and 3 year of follow-up. At one year of follow-up, a planned interim analysis showed that sirolimus, as compared with conventional treatment, did not improve the slope of GFR decline and was associated with an increase in albuminuria and 24-hour proteinuria. Mean total kidney volume similarly increased in the sirolimus and the conventional treatment group. These observations, coupled with evidence of an excess risk of treatment-related side effects, including peripheral edema, aphthous stomatitis, upper respiratory tract infections, and acne, prompted the independent Data and Safety Monitoring Board to recommend early termination of the trial. These findings indicate a lack of efficacy of sirolimus in slowing renal function deterioration in ADPKD patients with advanced renal insufficiency and point toward an unfavorable risk-benefit profile associated with sirolimus treatment.

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).

Paricalcitol for secondary hyperparathyroidism in renal transplantation (APPLE study)

Secondary hyperparathyroidism contributes to post-transplant chronic kidney disease mineral and bone disorder. Paricalcitol, a selective vitamin D receptor activator, decreased serum parathyroid hormone levels and proteinuria in patients with secondary hyperparathyroidism. This single-center, prospective, randomized, crossover, open-label study compared the effect of 6-month treatment with paricalcitol (1 µg/d for 3 months and then uptitrated to 2 µg/d if tolerated) or nonparicalcitol therapy on serum parathyroid hormone levels (primary outcome), mineral metabolism, and proteinuria in 43 consenting recipients of renal transplants with secondary hyperparathyroidism. Participants were randomized 1:1 according to a computer-generated sequence. Compared with baseline, median (interquartile range) serum parathyroid hormone levels significantly declined on paricalcitol from 115.6 (94.8-152.0) pg/mL to 63.3 (52.0-79.7) pg/mL (P<0.001) but not on nonparicalcitol therapy. At 6 months, levels significantly differed between treatments (P<0.001 by analysis of covariance). Serum bone-specific alkaline phosphatase and osteocalcin decreased on paricalcitol therapy only and significantly differed between treatments at 6 months (P<0.001 for all comparisons). At 6
months, urinary deoxypyridinoline-to-creatinine ratio and 24-hour proteinuria level decreased only on paricalcitol (P<0.05). L3 and L4 vertebral mineral bone density (assessed by dual-energy x-ray absorption) significantly improved with paricalcitol at 6 months (P<0.05 for both densities). Paricalcitol was well tolerated. Overall, 6-month paricalcitol supplementation significantly reduced parathyroid hormone levels and proteinuria, attenuated bone remodeling and mineral loss in renal transplant recipients with secondary hyperparathyroidism. Long-term studies are needed to monitor the effects of paricalcitol treatment on renal function, ensure that the bone remodeling and mineral effects are sustained, and determine if the reduction in proteinuria improves renal and cardiovascular outcomes. The results of this study have been published in J Am Soc Nephrol. 2014 Sep 5. pii: ASN.2013111185.

Functional and biochemical characterization of B cells and HLA antibodies in kidney transplantation

One of the most important advances in transplantation medicine has been the recognition that alloimmune response, mediated by donor-specific anti-human leukocyte antigen antibodies (DSA), plays a key role in the failure of kidney allografts. Although DSA are considered to be harmful, there is a wide spectrum of graft injury related to these antibodies, ranging from no recognizable damage to florid rejection. Preliminary observation in a cohort of 60 kidney transplant patients at our Center showed that 30% developed DSA later than 1 year post-transplantation, but graft function progressively deteriorated only in 60% of them during a 5-year follow-up. Such a varied effect underscores the need to define distinct patient graft phenotypes and outcomes according to the presence and characteristics of DSA after transplantation. From a prognostic perspective, predictive factors could be: i) peculiar phenotype of peripheral B cells emerging post-transplantation that could instruct maturation of aberrant DSA responses post transplantation; ii) differential glycosylation of DSA, as minor variation in the attached sugar moiety can switch an IgG antibody from an anti-inflammatory to a pro-inflammatory activity; iii) capacity to activate terminal complement cascade which may determine the cytotoxic potential of DSA. Taking advantage of serum, peripheral blood mononuclear cells and biopsy samples stored at our Center and sequentially collected at different time points after kidney transplantation, we will define the relative role of B cell phenotype, antibody glycosylation status and/or the capacity to bind and activate complement cascade fractions in predicting patients at risk of progressive long-term deterioration of graft function. It can be hypothesized that unique subsets of circulating B cells early post kidney transplantation may anticipate the development of DSA detrimental to the graft in the long-term due to their peculiar glycosylation pattern and/or their complement activating capacity. It can be expected that the identification of unique effector/regulatory B cells and DSA with abnormal glycosylation, and/or complement binding properties enhance risk stratification for long-term kidney allograft failure.

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\(^{+}\)CD25\(^{high}\)FoxP3\(^{-}\)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.

**Allogenic Mesenchymal Stromal Cells to induce tolerance in recipients of liver transplant from deceased donor**

For technical reasons, cell therapy with autologous mesenchymal stromal cells (MSC) can be used as an immunomodulatory tool only in the setting of living donor organ transplantation. Indeed, for deceased donor organ transplant programs, preparation of autologous MSC cannot be programmed, and theoretically all patients on waiting list should undergo bone marrow aspiration to isolate, expand and store MSC to be subsequently used when a deceased donor is available. Thus, an individually tailored autologous MSC preparation for every patient on the waiting list would be too time consuming and expensive. On the basis of these evidences, we started a phase I clinical study to test infusion of allogeneic, bone-marrow-derived MSC as a strategy to induce immune tolerance in liver transplant recipients with a deceased donor. Ten patients will be randomized to receive a single infusion of allogeneic MSC (1-2 x 10^6/Kg of body weight) few hours before liver transplant while 10 patients will be randomized to no cell infusion (control group). All patients will receive induction therapy with low-dose Thymoglobulin and maintenance immunosuppression with tacrolimus and mycophenolate mofetil. MSCs will be prepared according to an established protocol starting from the remnants of the filtration of the bone marrow collected from normal donors. To assess whether MSC infusion promotes a pro-tolerogenic immune environment biologic/mechanistic studies will be performed. In particular, the percentage and count of peripheral blood cells (CD4+ T cells, CD8+ T cells, naïve and memory T cells, B cells and NK cells) will be assessed before transplantation, 7 and 14 days, and 1, 6 and 12 months post-surgery. Moreover, circulating T cell subpopulations with immunoregulatory phenotype (CD4+CD25highCD127-FOXP3+) will be monitored before transplant, 1, 6 and 12 months post-transplant. Finally, mRNA expression of Transferrin receptor CD71 (TFRC) and Hepcidin antimicrobial peptide HAMP genes will be quantified in liver tissue biopsy samples collected at month 12 post-transplant since it has been reported that the expression of these genes selectively increased in operationally tolerant liver transplant recipients. The same evaluations will be performed in case of acute rejection episodes. 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 \( \alpha \)-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 \(^{[3]}\text{H}\)-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, \( \text{CD}^{8^+} \) 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 \( \text{CD}^{8^+} \) 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 \( \text{CD}^{8^+} \) T cells assessed by spots for granzyme-B. \( \text{CD}^{8^+} \) T cell activation was reduced following perfusion of human blood in kidneys of GAL-KO pigs. The lack of expression of galactose-\( \alpha \)-(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.

Management of chronic kidney disease and its risk factors in Eastern Nepal

Non-communicable diseases are the most common causes of morbidity and premature mortality worldwide. Among non-communicable disorders, chronic kidney disease ranks 18th among the global causes of death. One potential outcome of chronic kidney disease is end-stage renal disease (ESRD), requiring costly renal replacement therapy in the form of dialysis and transplantation. In low-income countries these life-saving therapies remain unaffordable for the majority of ESRD patients and cause severe financial hardship for those who have access to it. The high costs associated with kidney failure have motivated attention to earlier and milder forms of renal impairment as well as to their risk factors in order to prevent late disease consequences especially in developing countries. In the past two decades community screening programs have been progressively increasing in rural and urban areas of low- and middle-income nations in the attempt to identify individuals who will benefit the most from preventive measures. However, screened individuals are seldom maintained on active follow-up even when risk factors for chronic kidney disease and other non-communicable diseases have been identified. This particularly applies to patients living in rural areas. In this context, the major barrier to follow-up and management is the general lack of awareness and, more important, lack of suitable transport infrastructures and precarious family financial conditions that preclude young adults to stay away periodically from work for one or two days to reach hospitals in cities where the few nephrologists or specialized doctors operate. Nepal, one of the poorest countries in the world, has grossly limited treatment options for chronic kidney disease. The cost per month treatment per patient for dialysis makes the renal replacement therapy a rare choice for most of the ESRD patients. In 2003, the B.P. Koirala Institute, the second largest University Hospital in the country, located in the city of Dharan, Eastern Nepal, activated a program for early detection of kidney disease and risk factors. While the screening program was successful, it was soon realized that those identified as patients and asked to come periodically to the hospital at the outpatient clinic, were progressively lost to follow-up. To overcome this limitation, the Department of Medicine of the B.P. Koirala Institute, in collaboration with the
International Society of Nephrology and the Clinical Research Center for Rare Diseases of the Mario Negri Institute in Bergamo, strategically decided to move to the rural communities of Eastern Nepal. Under a centralized coordination in Dharan, an affordable program was set up involving community volunteers and local leaders to assure long-term sustainability and follow-up of screened participants in these rural areas. Among 20,811 individuals previously screened in communities, a three-year follow-up of 4471 participants found to have high blood pressure and/or fasting blood glucose, proteinuria or impaired renal function was set-up. The aim was to assess the impact of the local management of these patients in the community on the control of blood pressure, glycemia, proteinuria, and progression of renal dysfunction. Among 3419 participants on active monitoring after three-years (73%), systolic, diastolic blood pressure, or glycemia normalized in 53%, 63%, and 55% of individuals with high values at baseline, respectively. Participants with dipstick proteinuria ≥ 1+ at baseline progressively declined with value normalization in 63% of them over the three-year follow-up. In 45 to 48% of participants with estimated glomerular filtration rate <60 ml/min/1.73 m² at baseline, renal function improved during the same period. None of participants progressed to end-stage renal disease, requiring renal replacement therapy. The prevalence of participants with predicted 10-year cardiovascular risk ≥ 10% was 27.3% at baseline and declined to 16.9% after three-year follow-up. An affordable local organization delivers substantial population-wide benefits to manage chronic kidney disease and its risk factors in the resource-poor setting of Eastern Nepal. This program would translate in less need of renal replacement therapy and reduced cardiovascular mortality on the long-term. The results of this study have been published in Lancet Glob Health. 2014; 2:e506-7.


Up-to-date trends for age-sex-specific all-cause and cause-specific mortality is essential for the formation of global, regional, and national health policies. In the Global Burden of Disease Study 2013 (GBD 2013) we estimated yearly deaths for 188 countries between 1990, and 2013. We used the results to assess whether there is epidemiological convergence across countries. For all quantities reported, we computed 95% uncertainty intervals (UIs). Global life expectancy for male and female increased from 65·3 years (UI 65·0–65·6) in 1990, to 71·5 years (UI 71·0–71·9) in 2013, while the number of deaths increased from 47·5 million (UI 46·8–48·2) to 54·9 million (UI 53·6–56·3) over the same interval. Global progress masked variation by age and sex: for children, average absolute differences between countries decreased but relative differences increased. For women aged 25–39 years and older than 75 years and for men aged 20–49 years and 65 years and older, both absolute and relative differences increased. Decomposition of global and regional life expectancy showed the prominent role of reductions in age-standardised death rates for cardiovascular diseases and cancers in high-income regions, and reductions in child deaths from diarrhoea, lower respiratory infections, and neonatal causes in low-income regions. HIV/AIDS reduced life expectancy in southern sub-Saharan Africa. For most communicable causes of death both numbers of deaths and age-standardised death rates fell whereas for most non-communicable causes, demographic shifts have increased numbers of deaths but decreased age-standardised death rates. Global deaths from injury increased by 10·7%, from 4·3 million deaths in 1990 to 4·8 million in 2013; but age-standardised rates declined over the same period by 21%. For some causes of more than 100 000 deaths per year in 2013, age-standardised death rates increased between 1990 and 2013, including HIV/AIDS, pancreatic cancer, atrial fibrillation and flutter, drug use disorders, diabetes, chronic kidney disease, and sickle-cell anaemias. Diarrhoal diseases, lower respiratory infections, neonatal causes, and malaria are still in the top five causes of death in children younger than 5 years. The most important pathogens are rotavirus for diarrhoea and pneumococcus for lower respiratory infections. Country-specific probabilities of death over three phases of life were substantially varied between and within regions. For most countries, the general pattern of reductions in age-sex specific mortality has been associated with a progressive shift towards a larger share of the remaining deaths caused by non-communicable disease and injuries. Assessing epidemiological...
convergence across countries depends on whether an absolute or relative measure of inequality is used. Nevertheless, age-standardised death rates for seven substantial causes are increasing. Yet, important gaps exist in the empirical data for cause of death estimates for some countries. The Department of Renal Medicine of the Mario Negri Institute, on behalf of the International Society of Nephrology, have contributed to data collection related to renal diseases for this GBD 2013 Study. The results of this study have been published in Lancet. 2014 Dec 17. pii: S0140-6736(14)61682-2.

**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 primary membranous nephropathy (MN) through the secretion of autoantibodies directed against kidney constituents. Among them, autoantibodies against the phosfolipase A2 receptor (PLA2R) seems to play an important role.

On the basis of this finding, 10 years ago we started to treat patients with MN 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 primary MN 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 MN 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.

Thus to study the relationship between anti-PLA2R antibody levels, genetic polymorphisms, and treatment effects, we treated with Rituximab 132 patients with primary MN who were followed for a minimum of 6 months up to 12 years. Serial autoantibody measurements were done in collaboration with the clinical researchers of the Tenon Hôpital in Paris. Results from this study have been recently accepted for publication in the American Society of Nephrology Journal and showed that most patients with primary MN had detectable autoantibodies at onset, reflecting previous data from the literature; however our study show for the first time a series of data that can be summarized as follows:

- The presence or absence of detectable anti-PLA2R antibodies did not influence the prognosis;
- Among patients who had detectable anti-PLA2R antibodies at onset, a higher autoantibody titer independently predicted a worse prognosis while a lower titer predicted the remission of the nephrotic syndrome;
- Patients with detectable antibodies at onset who achieved complete depletion of anti-PLA2R within 6 months of follow-up had a higher probability of achieving the complete remission of the nephropathy; the antibody depletion preceded the complete remission by approximately 10 months;
- The reappearance of autoantibodies after initial depletion in patients who achieved at least the partial remission of the nephrotic syndrome preceded and predicted the occurrence of nephrotic syndrome relapse;
- These results were independent from the HLADQA1 and PLA2R1 gene polymorphisms distribution among individuals with primary MN thus suggesting that studying such genetic characteristics may not add further information to the prediction of clinical outcome in this patient population.

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 (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.

At completion of the two month run-in period, subjects who fulfill the selection criteria will be Throughout the study, patients will also be 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.

Eligible patients entered a two-month run-in treatment period with standardized and stable dose of renin angiotensin system (RAS) inhibitor therapy with Ramipril and Irbesartan. Treatment will be titrated to blood pressure and proteinuria. On December 17, 2014 patient enrollement was completed: 53 patients were included into the study; they were stratified in two subgroups depending on serum phosphate (≤ o > 4mg/dl) and then 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. During the whole study period patients will be followed by a nutritionist to verify adherence to a diet controlled for the amount of sodium and protein intake. To date 22 patients concluded the study and the remaining 31 are on active follow-up. We can conclude that sevelamer carbonate treatment was safe in all patients who concluded the treatment period.

Prospective, randomized, open-label, double blinded and point (PROBE) and cross over trial to in which the end-point is evaluated in blind, crossover to compare the effects of Telmisartan and Losartan on metabolic profile in patients with kidney transplantation (COSTANT study): final analysis

In renal transplant recipients, the most frequently and effective anti-rejection therapy is based on the combined use of steroids and/or calcineurin inhibitors (cyclosporine and tacrolimus). These drugs, may induce several side effects such as arterial hypertension, dyslipidemia and impaired
glucose tolerance do to reduce insulin activity. The combination of these side-effects has been described as the ‘metabolic syndrome’. The key point of this syndrome is represented by a reduced insulin sensitivity of the whole body, in large part due to immunosuppressive drugs. Improving insulin sensitivity represents therefore the ideal treatment to ameliorate the metabolic profile, reduce the cardiovascular risk and increase patient and graft survival in renal transplantation.

Recently, new antidiabetic drugs such as thiazolidinediones became available. These drugs bind the PPARγ (peroxisome proliferators-activated receptor gamma) that activates the transcription of a specific group of genes that, among other positive effects on the metabolic profile, are able to improve the insulin sensitivity and change the adipocyte differentiation. On the other hand, a relatively frequent side effect of drugs acting on the PPARγ is fluid retention and edema, presented in about 5% of treated patients and, in some cases, heart failure. The most severe side effect is hepatotoxicity.

A new drug, which has been used since 2004 for controlling blood pressure, Telmisartan, exerts the same positive activity on insulin sensitivity throughout the activation of PPARγ receptor and, tested in large multicenter clinical trials, did not show significant side effects related to fluid retention or to other adverse events. These angiotensin II type 1 receptor blockers (ARBs), in addition to the above mentioned positive effect on the metabolic syndrome, exerts nephroprotective effects through blockade of the renin angiotensin system and proteinuria reduction. These metabolic and renal effects of Telmisartan have been already reported in patients with diabetes nephropathy. The present study tests the hypothesis that Telmisartan improves insulin sensitivity also in kidney transplant patients when compared with another ARB agent, losartan, devoid of PPARγ receptor effect.

Therefore, the results of this study could provide precise information not only about the risk of developing diabetes linked to transplantation, but particularly regarding pre-diabetic states that are associated with insulin resistance, hyperglycemia and glucose metabolism imbalance related to anti-rejection therapy and could be demonstrated with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) tests. These data could provide a broader understanding of the mechanisms underlying the development of diabetes, metabolic syndrome and increased cardiovascular risk after renal transplantation.

Thus, this is an academic, prospective, randomized, open-label crossover study, in which the end-point is evaluated in blind to compare the short-term effects of Telmisartan and Losartan on insulin resistance in kidney transplants patients with stable renal function and concomitant treatment with steroids and/or calcineurin inhibitors. Secondarily, this trials will compare changes in systemic efficacy (systolic and diastolic blood pressure profile and 24 hours), metabolic (fasting glucose, glucose tolerance, glycated hemoglobin, fasting insulin, HOMA index, lipid profile ) and renal variables (UAE, GFR / RPF, Albumin, fractional clearance of IgG). Patients were randomized in a 1: 1 ratio according to a computer-generated randomization list. This study was registered in the website ClinicalTrials.gov number NCT01224860. In 2013 final statistical analysis were performed.

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 reno-protective 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 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 Papa Giovanni XXIII Hospital in 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 at the end of 2014, 2210 patients were included of which 1800 have completed the study. Urinalyses were completed by July 2014. Approximately 11.5% (253 of 2210) of screened patients presented alterations of the urinalysis/sediment or other urinary parameters considered (proteinuria/albuminuria/sodium excretion) and 79 of these were advised to perform a nephrology outpatient visit at the Nephrology Unit of the Papa Giovanni XXIII Hospital in Bergamo, as foreseen by the protocol. Since it was not expected to continue with a follow-up of the screened patients, the enrolment was stopped after data analysis was collected. Preliminary study results show that, as in other epidemiological studies, a clear association between increased albuminuria levels and high blood pressure in the general population.

Prospective, randomized, cross-over, double-blind, placebo controlled study to assess whether the effectiveness of paricalcitol in reducing proteinuria in patients with diabetic nephropathy and in stable therapy with an angiotensin receptor blocker (ARB) is modified by sodium diet (PROCEED study)

Proteinuria is an independent risk factor for cardiovascular morbidity and mortality and progression to renal failure. Higher levels of proteinuria predict higher risk for developing end stage renal disease (ESRD). Moreover, in chronic kidney disease patients, due to diabetes or not, interventions that reduce the urinary protein excretion slow the progression to ESRD. Therefore, the main objective of "nephro-protective" therapies is to minimize the proteinuria. Drugs that block the renin angiotensin system (RAS) have a specific renoprotective action that is largely explained by their antiproteinuric effect. These drugs are, among other antihypertensive, those that can more effectively prevent or slow the decline of kidney function. However, this effect varies from patient to patient and can be inhibited in patients with high sodium intake with diet. In addition to RAS inhibitors, other drugs such as Vitamin-D Receptor Activators (VDRA) are effective in reducing proteinuria in patients with and without diabetes. Among them, an analogue of vitamin D, paricalcitol, widely used since 1998 in patients with advanced renal failure and dialysis as a treatment for secondary hyperparathyroidism, has shown good safety profile and tolerability. The VITAL study (Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes), published in the Lancet, showed that this effect is clinically relevant only for patients that exceed 12 grams of daily sodium intake while it is very modest in those who take little sodium in the diet. This action seems to mirror that of the inhibitors of the RAS and suggests that paricalcitol could be particularly effective in those patients who respond poorly to these drugs because they take too much salt in the diet. The PROCEED study was designed to evaluate whether the antiproteinuric effect of paricalcitol in patients with diabetic nephropathy and already on stable therapy with Losartan is modified by concomitant salt intake. During the study there will be two treatment periods: one with placebo and the other one with paricalcitol at a dose of 2 micrograms per day. Patients will be started on a random sequence in which neither the patient nor the doctor knows that treatment sequence (study in cross-over, double-blind). It is expected to include 112 adult subjects with diabetic nephropathy (albuminuria> 200 g / min) before treatment with Losartan at a dose of 100 mg/day for at least a month, creatinine <2 mg / dl, PTH ≥ 20 pg/ml, Calcium <9.5 mg/dl, phosphorus <5 mg/dl, controlled blood pressure (systolic/diastolic blood pressure <140/90 mmHg) and without vitamin D or derivatives therapy. The primary outcome is to compare changes in albuminuria after one month of treatment with 2 µg/day of Paricalcitol in conditions of high versus low sodium intake. Secondary objectives are designed to evaluate the effect of treatment on blood pressure, pulse pressure, ambulatory pressure profile, pulse wave velocity (PWV) and other markers of vascular stiffness. Glomerular
filtration rate (GFR) measured by ioexol technique, fractional albumin clearance and IgG, A/C index, plasma renin activity, plasma renin levels, pro-renin, angiotensin II, aldosterone and CNP. Excretion of aldosterone, phosphate, calcium, magnesium, urea, sodium, and 25 OH Vit D, MCP-1, TGF beta, RANTES and albumin in the urine of 24 hours; serum levels of biomarkers of mineral metabolism (vitamin D, PTH), plasma levels of Ca, P, PTH, bone FA, total cholesterol, HDL, LDL, triglycerides, apolipoprotein A and B, C-reactive protein (CRP). Also, parameters of vascular stiffness will be measured by non-invasive tonometry. The potentially eligible patients will enter a one month run-in period. During this period patients will be started or continued (in case of previous Losartan treatment) on Losartan up to 100 mg daily and placebo. Successively, will be randomized to a sequence placebo - paricalcitol or paricalcitol - placebo and to a low or high sodium diet. Each patient will complete one period of treatment and the will be cross-overed to reverse the initial sequence placebo - paricalcitol or paricalcitol - placebo; however, diet prescription (high or low intake) will be the same for the patient from randomization up to study end. Throughout the study, patients will also be followed by a nutritionist who will verify diet adherence. Since start up in November 2011 at the end of the year 2014 193 patients were enrolled and 112 were randomized; thus, all who had been considered necessary by the initial estimated sample size. Currently, more than 100 patients have already completed the study and the other 12 are in active follow-up. To date, Paricalcitol has been well tolerated and proven safe in most patients treated. It is expected to conclude with all visits in the coming months.

**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 researchers is a part of the job of the senior personnel. For this purpose, in 2014, was set up a course for Clinical Monitor which involves the participation of students to 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 students 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 2014 were coordinated and monitored the follow studies:

Studio ALADIN 2 - 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.

Studio ANSWER – 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.

Studio ARCADIA - 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.

Studio ATHENA - 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.

Studio CE-US – 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.

Studio CINDY – A prospective, randomized, double-blind, cross-over study to evaluate the renal and biohumoral effects of L-cystatine compared to placebo in stable peritoneal dialysis patients with residual diuresis.

Studio CRESO2 – Long-term effects of caloric restriction on metabolic, renal and retinal healthin subject effected by obesity and type 2 diabetes.

Studio EAGLE – Evaluating the morphofunctional effects of Eculizumab therapy in primary membranoproliferative glomerulonephritis: a pilot, single arm study in ten patients with persistent heavy proteinuria.

Studio LIMONE - 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.

Studio MSC-CIS - 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.

Studio MSC-KTX - Mesenchymal stem cells under basiliximab/low dose RATG to induce renal transplant tolerance.
Studio MSC Liver – Third-party bone marrow-derived mesenchymal stromal cells to induce tolerance in liver transplant recipients.

Studio PREDICTION – A prospective study to compare early graft function or recipients of single or dual kidney organs stored in ice cold solution or pulsed perfusion.

PRIORITY - Proteomic prediction and renin angiotensin aldosterone system Inhibition prevention of early diabetic nephropathy In type 2 diabetic patients with normoalbuminuria

Studio PROCEED - 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.

Studio REMISSION CLINIC: 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.

Studio VALID – 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.

Studio VARIETY – 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, 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. Particular attention is paid to the preparation of the patient information form and obtaining of informed consent, especially in reference to the protocols involving genetic analysis and minors (children and adolescents). 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 accreditiation 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 2014, 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 has been prepared the final report of the following 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 (COSTANT study).

Starting from February 2014, the staff of the Laboratory trained, in their areas of expertise, the students of the Course for Clinical Research Monitor ongoing in Ranica.
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). Educational training: Clinical Research Nurse Diploma on 1996 at IRFMN – Daccò Center.
First Level Master on Clinical Research at Milan University on 2008.

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 participate 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

- Krulichova I, Gamba S, Ricci E, Garattini L on behalf of the Italian Takayasu Arteritis Study Group. Direct medical costs of monitoring and treating patients with Takayasu arteritis in Italy. Eur J Health Econ Vol. 49, August 2004

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

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 whit 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 11357 patients affected by 945 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 2244 patients with rare conditions and their families have been collected. Disease registries include 1561 cases.

The Centre has established contacts with more of 300 Italian Associations for rare diseases. It was even possible that patients with 92 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
UNIAMO - Rare Diseases Italian Federation
Papa Giovanni XXIII Hospital, Bergamo
Assessorato alla Sanità, Lombardy Region
University of Turin, Department of Experimental Medicine and Oncology, Master in Rare Diseases
AO Niguarda Cà Granda, Milan, Nursing course degree
University of Florence, School of Nephrology
University Consortium for Applied Economic Research in Health (CREA Health), Rome
Scientific Committee of the project: "Rare diseases: recognition of care needs and the definition of measures to support" (Decree no. 7771 of 11.09.2012, DGR, Lombardy Region)

INTERNATIONAL COLLABORATIONS

EURenOmics: European Consortium for High-Throughput Research in Rare Kidney Diseases – 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

Working group with functions of operational coordination and sharing of common strategies for prevention, surveillance, diagnosis, treatment of rare diseases, according to the M.D. 18/5/2001, N°279, established by 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.
Technical Working Group for the scientific analysis and processing of data from the National Register of Rare Diseases.
Working group “Rare diseases and orhan drugs” (DG Sanità – Lombardy Region).
Technical interregional permanent table for rare diseases, established in 2006 by Health Committee - coordination of regional councilors and PPAA.
Steering Group for Project CAROUSEL (Law 383/2000, letter f, year 2013), co-financed by the Ministry of Labour and Social Policy, in collaboration with the Italian Federation of Rare Diseases UNIAMO-FIMR Onlus.

EVENT ORGANIZATION

Open Day: information, research and treatments
A guided tour to learn about the research on Rare Diseases
Ranica, (Bergamo) February 26, 2014
CONFERENCE AND WORKSHOP CONTRIBUTIONS

Genetica delle Microangiopatie Trombotiche a patogenesi complemento-mediata
Impiego dell’eculizumab nella MPGN
17^° Convegno Patologia Immune e Malattie Orfane 2014
Torino, January 30-31, 2014

La Rete per le malattie rare ed il Registro regionale
La diagnosi, le normative e la gestione delle malattie rare
Milano, March 29, 2014

I Percorsi diagnostici terapeutici assistenziali: traguardi e criticità
Malattie rare: i pazienti ed il Presidio ospedaliero di riferimento
Milano, April 10, 2014

The Lombardy Regional Registry for Rare Diseases: an Example of Record Linkage
Across Different Data Sources
PHD Student Meeting
Milano, June 24-25, 2014

La rete per le malattie rare
Corso organizzato da AO Niguarda Cà Granda
Milano, June 25, 2014

Helpline service for Rare Diseases
Toward Europe 2020: Counseling and telepsychology for health
Comparing professional experiences between counseling centres and helplines
Firenze, June 30 – July 1 2014

Whole mRNA sequencing on Ion PGM is a reliable method for differential expression
analysis
ION TORRENT USER GROUP MEETING
Bologna, September 30 – October 01, 2014

Rituximab as pre-emptive therapy in patients with thrombotic thrombocytopenic purpura
and anti-ADAMTS13 antibodies: effectiveness of a single infusion
Bari International Conference
Bari, October 3-5, 2014

Sindrome emolitico-uremica atipica e glomerulonefriti da disfunzioni della via alterna del
complemento
55^° Congresso Nazionale Società Italiana di Nefrologia
Catania, October 08-11, 2014

Oggi possiamo curare le malattie auto inflammatorie?
Autoinflammation 2day
Sesto S. Giovanni (MI), October 11, 2014

C3 glomerulopathy and Related Disorders
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 counselling is also provided to patients and relatives, when appropriate.

Through this Registry, data of about 1100 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 EURenOmics, 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.

There are different forms of SRNS: Diffuse Mesangial Sclerosis (DMS) is mostly diagnosed in children, whereas Focal Segmental Glomerulosclerosis (FSGS) is mostly diagnosed in young adults. 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, WTI, 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.

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 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.

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 the main aim of this project. Another aim is to finding targeted and effective therapies, through the evaluation of specific complement inhibitors for the disease cure and the prevention of disease recurrence on transplantation in a mouse model and in a pilot study of patients with primary MPGN at risk of progression.

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.

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.

Data and biological samples of 230 patients, referred from 35 Italian and 15 international Nephrology Units, have been collected. A genetic defect has been found in 18% of patients.

EAGLE: Evaluating the morphofunctional effects of eculizumab therapy in primary membranoproliferative glomerulonephritis

Membranoproliferative glomerulonephritis (MPGN) is a rare kidney disease characterized by nephrotic syndrome primarily of children and young adults, and is the third or fourth leading cause of end stage renal disease among the primary glomerulonephritis. Hyperactivation of complement system plays a main role in the pathogenesis of MPGN.

The lack of randomized, controlled trials and the current understanding that multiple pathogenic processes lead to MPGN make it impossible to give strong treatment recommendations in this patient population, in particular for those with primary forms and more severe disease.

The recent introduction in clinical practice of Eculizumab, a humanized monoclonal antibody that inhibits C5 activation, may offer the unique opportunity to explore the role of complement inhibition in the treatment of more severe cases of primary MPGN.

The main goal of the study is to assess whether Eculizumab therapy may achieve changes in clinical and morphofunctional abnormalities of the disease, including biomarkers of activation.
of the complement system and circulating levels of its final effector complement system membrane attack complex (MAC) or sC5b-9, that might translate into improved disease outcome in the long term.

Ten patients, identified through the MPGN Registry coordinated by our Laboratory, will be included in the study. In 2014, eight patients have been enrolled in the study and started Eculizumab therapy. The enrollment phase will end in February 2015. The ten patients will follow a treatment period of one year.

**EURenOmics - European Consortium for High-Throughput Research in Rare Kidney Diseases**

The Laboratory take part of EURenOmics, a Consortium that will integrate several established consortia devoted to rare kidney diseases with eminent need and potential for diagnostic and therapeutic progress. The Consortium has access to the largest clinical cohorts assembled to date (collectively >10,000 patients) with detailed phenotypic information and comprehensive biorepositories. The project aims to (1) identify the genetic and epigenetic causes and modifiers of rare kidney diseases and their molecular pathways; (2) redefine their classification; (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.

**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.

**The Register of Rare Diseases of Lombardy**

Since December 2006 the implementation of the Register of Rare Diseases of Lombardy (italian acronym: ReLMar) is web-based using a specific software called “Sistema Malattie Rare”, operating within the information system Regional Services Card - Health Care Information System (italian acronym: CRS-SISS). The data management of ReLMaR is a task of the
Coordination Center that provides for their validation and analysis, production of reports and sending the shared dataset (subset of data required by the National Register of Rare Diseases) to Istituto Superiore di Sanità (Rome).

The reports on the activities of ReLMaR describe the data filed by the medical specialists of Regional Centres of Reference and validated by the operators of the Coordination Centre. The reports can be viewed and downloaded from the website of the Coordination Center (http://malattierare.marionegri.it).

During 2014, the Coordination Centre has worked on the development of an Information System for the management of information on rare diseases and in particular on the development of a procedure for record linkage which will allow to verify the epidemiology of rare diseases at regional level with particular reference to their prevalence, to the characteristics of the patients and to the activities of the different Centres of Reference of the Regional Network.
INTERNATIONAL RELATIONS OFFICE
OF RARE DISEASES

STAFF

Head
Arrigo SCHIEPPATI, M.D.
CURRICULUM VITAE

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, Bergamo

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). Working group with functions of operational coordination and sharing of common strategies for prevention, surveillance, diagnosis, treatment of rare diseases, according to the M.D. 18/5/2001, N°279, established by Lombardy Region (Delibera Regione Lombardia N°7328, 11/12/2001).
Scientific Committee A.O. Bolognini di Seriate
Ethical Committee, Papa Giovanni XXIII Hospital, Bergamo

EVENT ORGANIZATION

Open Day: information, research and treatments
A guided tour to learn about the research on Rare Diseases
Ranica, (Bergamo) February 26, 2014

International Workshop on Orphan Drugs
Ranica, (Bergamo) March 25, 2014

PARTICIPATION IN EVENTS IN WHICH THE LABORATORY WAS INVOLVED

La sindrome emolitico-uremica
Il rene nelle malattie rare (e dintorni…)
Piacenza, September 26, 2014

GRANTS AND CONTRACTS

European Commission (DG SANCO)

SELECTION OF SCIENTIFIC PUBLICATIONS (2014)

The social burden and quality of life of patients with haemophilia in Italy
Blood Transfus 2014 ; 12 Suppl 3 : s567-s575

Immunosuppressive treatment for idiopathic membranous nephropathy in adults with nephrotic syndrome
Cochrane Database Syst Rev 2014 Issue 10 : CD004293

Dramatic effects of eculizumab in a child with diffuse proliferative lupus nephritis resistant to conventional therapy
Pediatr Nephrol 2014 30 : 167-172

Eliminating treatable deaths due to acute kidney injury in resource-poor settings
Schieppati A, Perico N, Remuzzi G
Semin Dial 2014 E-pub :
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 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.

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.
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 7000. 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 Researchers (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 2013 the Mario Negri Institute awarded 7,827 grants**, 814 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”
STAFF
Executive Offices
Services and Offices
Prof. Silvio Garattini

Prof. 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 THREE locations -- Milan, Bergamo and Ranica (Bg) -- 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 LegalItaliana per la LottaContro i Tumori (LILT); President of V section 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" Foundation, permanent member of the CSS, member of the National Bioethics Committee, and of the Sicilian Regional Bioethics Committee.

Professor Garattini has received numerous awards for his work, including the French Legion d'Honneur for scientific merit, and the Medaglia d'Oro al Merito della Sanità Pubblica of the Italian Ministry of Health, the Medal of the Società Italiana di Chimica “Giulio Natta”, the Grand Ufficiale della Repubblica Italiana, honorary degrees from the Universities of Bialystok in Poland and Barcelona in Spain and in Milan.

Other awards include the HippocratesPrize, Mens Sana in Corpore Sano, Nuova Spoleto, Angelo dell’Anno, Alkmeneon 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 14,000 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 Bergamo hospital, 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 (World Congress of Nephrology: WCN 2011, Vancouver). In November 2011, he received the Third Edition of the International Award "Luis Hernando" assigned by the Iñigo Alvarez de Toledo Renal Foundation (FRIAT) in Madrid, Spain. Since June 2013, he is President of the International Society of Nephrology (ISN) for the biennium 2013-2015 and creator of the ISN initiative called "0 by 25": Nobody should die of preventable and treatable Acute Kidney Injury (AKI) by 2025. The hope is that the ISN will contribute during the next decade to ensure that we can reduce the mortality rate of acute renal failure at the global level.

Prof. Remuzzi has authored and co-authored more than 1269 scientific articles, reviews and monographs and regularly collaborate through the preparation of scientific articles with the Italian national newspaper “Corriere della Sera”.
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