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International Meeting

Needs and Challenges in Translational Medicine: filling the gap between basic research and clinical applications

Istituto Superiore di Sanità
Rome, Italy
October 1-3, 2008

ABSTRACT BOOK

Edited by
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This multidisciplinary international meeting is organized by the Istituto Superiore di Sanità, in collaboration with Alleanza Contro il Cancro (Alliance Against Cancer, the Network of the Italian Comprehensive Cancer Centres) and EATRIS (European Advanced Translational Research Infrastructure in Medicine). The primary goal of the meeting is to provide a scientific forum to discuss the recent progress in translational research. Moreover, a particular focus will be devoted to the identification of needs, obstacles and new opportunities to promote translational research in biomedicine. The scientific programme will cover a broad range of fields including: cancer; neurosciences; rare diseases; cardiovascular diseases and infectious and autoimmune diseases. Furthermore, special attention will be given to the discussion of how comprehensive initiatives for addressing critical regulatory issues for "First-In-Man" - Phase I clinical studies can potentially improve the efficiency and quality of biomedical and translational research at an international level.
Key words: Translational medicine, Biomedical research, Advanced therapies

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Questo convegno internazionale multidisciplinare è organizzato dall'Istituto Superiore di Sanità, in collaborazione con Alleanza Contro il Cancro (la rete italiana degli IRCCS oncologici) ed EATRIS (European Advanced Translational Research Infrastructure in Medicine). L'obiettivo principale del convegno è di rappresentare un forum scientifico per lo scambio di informazioni e di opinioni sui nuovi progressi nel campo della ricerca traslazionale. Un interesse particolare sarà rivolto inoltre all'identificazione dei bisogni, degli ostacoli e delle nuove opportunità per promuovere la ricerca traslazionale in biomedicina. Il programma scientifico coprirà una vasta area di campi di ricerca, tra cui cancro, neuroscienze, malattie rare, malattie cardiovascolari, malattie infettive ed autoimmuni. Particolare attenzione sarà rivolta poi al dibattito sul modo in cui iniziative di vasta portata, che riguardano gli aspetti regolatori critici per gli studi clinici di Fase I - "*First-in-Man*", possono migliorare potenzialmente l'efficienza e la qualità della ricerca traslazionale e biomedica a livello internazionale.
Parole chiave: Medicina traslazionale, Ricerca biomedica, Terapie avanzate

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PREFACE

The recent research progress has led to an accumulation of knowledge on the molecular mechanisms responsible for human diseases and to novel perspectives of medical intervention. However, the impressive new information stemmed from the results of basic research has not yet met the expectations of patients in terms of clinical applications and benefit for public health. Thus, several initiatives have recently been launched to promote biomedical translational research. While competition remains a driving force for the discovery process, strong initiatives based on an integrated and managed approach as well as on national and international collaboration are essential for a relevant advance in translational medicine. Such initiatives include the organization of international meetings for discussing the main challenges and critical features of translational medicine, including the design of infrastructures where all the relevant actors are engaged for breaking barriers and favouring bidirectional communication between basic research and clinic applications. In this context, the international meeting "Needs and Challenges in Translational Medicine: filling the gap between basic research and clinical applications" (Rome, October 1-3, 2008) represents a timely and important initiative for addressing some current critical issues in translational medicine. The event is organized under the auspices of the Italian Ministry of Health and sponsored by the Istituto Superiore di Sanità, Alliance against Cancer (Network of the Italian Comprehensive Cancer Centres) and EATRIS (European Advanced Translational Research Infrastructure in Medicine). The primary goal of the meeting is to provide a scientific forum where scientists and experts involved in both laboratory and clinical research in different fields of biomedicine can discuss the recent progress in translational research and the latest scientific discoveries from the laboratory and their translation into clinical applications. Moreover, a particular focus will be devoted to the identification of needs, obstacles and new opportunities to promote translational research in biomedicine. The scientific programme will cover a broad range of fields including: cancer; neurosciences; Rare Diseases; cardiovascular diseases and infectious and autoimmune diseases. Furthermore, special attention will be given to the discussion of how comprehensive initiatives for addressing critical regulatory issues for "First-In-Man"- Phase I clinical studies can potentially improve the efficiency and quality of biomedical and translational research at an international level.

On behalf of the Scientific Committee, I thank all the speakers, chairpersons and participants who will address during this meeting the emerging needs and challenges of translational medicine, thus rendering this event an important step for further international initiatives.

Enrico Garaci
President of the Istituto Superiore di Sanità
Honorary President of the Scientific Committee

Opening Lecture

NEEDS AND CHALLENGES IN TRANSLATIONAL MEDICINE: FILLING THE GAP BETWEEN BASIC RESEARCH AND CLINICAL APPLICATIONS

M. Hallen
European Commission, DG Research, Brussels, E.U.

The European Commission's Health Research Directorate has been funding collaborative research in Biomedicine for over 20 years. Major successes have been achieved in building world-class collaborations that have produced important advances ranging from basic biology to medical applications, from the human genome to the role of BSE "mad-cow" disease in human variant CJD.

In recent years, an increasing emphasis has been placed on "translational research", where the goal is to transfer knowledge and skills from basic research to applications. In order to achieve this change of emphasis from separated into connected research worlds, a number of specific actions need to be taken. Challenges include the traditional publication and promotions systems in basic research, which often favour a focussed rather than a multi-disciplinary approach.

Fortunately, the collaborative research mechanisms of the EC Framework Programme (FP) provide an ideal vehicle for overcoming these obstacles by providing scientific, managerial and financial arrangements that allow the assembly of multidisciplinary teams which unify a variety of skills that span basic research to application. This structure is supplemented by targeted research and infrastructure development that provide the basis for translational research projects. Key areas include bioinformatics, proteomics and model organism research and infrastructure, and systems biology research approaches to integrating knowledge.

The outcome of these years of capacity building is becoming evident in the current FP7, where projects such as APO-SYS embody the concepts of translational medicine. APO-SYS aims at understanding cell biology of apoptosis by applying systems biology approaches to cancer and AIDS. With 23 groups from 12 countries, it combines computational biology, lab work on model organisms and tissue samples from patients with cancer and AIDS undergoing clinical trials, to develop appropriate drugs and understand their applications.

Session 1

Cancer - Part 1

Chairpersons

F. Cognetti, P.G. Pelicci

CANCER PROTEOMICS IN THE DISCOVERY OF NEW BIOMARKERS FOR DIAGNOSIS AND TREATMENT

L.A. Liotta (1), E.F. Petricoin (1), C. Belluco (2), R. De Maria (3)
(1) Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA, USA
(2) Department of Surgical Oncology, CRO, IRCCS, National Cancer Institute, Aviano,Pordenone, Italy
(3) Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy

The field of proteomics is providing new technology for early diagnosis of cancer, prior to metastasis, and novel approaches for individualization of therapy. Each patient's cancer has a unique complement of pathogenic molecular lesions. There is a strong justification to develop a strategy that could select from a menu of treatment choices, or treatment combinations, those that best match the molecular profile of a tumor. Suppression of aberrant signaling is the goal of molecular targeted therapies of the future. A goal of functional proteomics is to develop "circuit maps" of protein pathways regulating proliferation, apoptosis, and differentiation in normal cells and diseased cells. Nanotechnology offers new approaches for amplification, stabilization, discovery and measurement of cancer biomarkers in blood and tissue. Protein microarrays provide a means to profile cellular signaling pathways in a manner not achievable by gene arrays. It is now possible to procure a biopsy, microdissect the cancer cells, and map the functional state of key signal pathways. Protein microarrays provide a read-out of the post-translational modifications associated with protein-protein interactions in the context of communication networks driving cancer pathogenesis. The information flow through a specific node in the proteomic network requires the phosphorylation of signal pathway proteins at a specific epitopes. By measuring the proportion of those protein molecules that are phosphorylated, we can infer the level of activity of that signal node. If we compare this measurement over time, or at stages of disease progression, or before and after treatment, a correlation can be made between the activity of the node and the biologic or disease state. Thus for the first time it is possible to identify molecular lesions within cooperating dysregulated pathways. These molecular lesions are the drug targets of the future. Protein array technology, in combination with gene arrays and serum biomarkers, will serve as a revolutionary foundation for the development of personalized cancer therapies. It is theoretically feasible to administer combination therapy targeting multiple interdependent points along a pathogenic pathway or targeting multiple distinct yet cooperating dysregulated pathways. The ultimate goal is tailored combination therapy which provides higher individual efficacy with lower toxicity.

MICRO-RNAs AND PERSPECTIVES FOR CANCER THERAPY

C.M. Croce
Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA

MicroRNAs (miRNAs) as short non-coding RNA molecules that modulate the expression of multiple target genes at the post-transcriptional level and are implicated in a wide array of cellular and developmental processes. In hematopoietic cells, miRNA levels are dynamically regulated during lineage differentiation and also during the course of the immune response. Mouse models have provided good evidence for miRNAs being key players in the establishment of hematopoietic lineages. Furthermore, miRNA-dependent alterations in gene expression in hematopoietic cells are critical for mounting an appropriate immune response to a wide range of pathogens, spontaneously emerging tumors, and autoimmune cells. Deregulation of hematopoietic-specific miRNA expression results in defects in both central and peripheral tolerance, hematopoietic malignancies, and sometimes both. Abnormal expression of miRNAs - has also been found in patients with rheumatoid arthritis. These findings identify miRNAs as critical targets for immunomodulatory drug development.

RATIONAL VACCINE DESIGN: IMMUNE APPROACHES TO CANCER AND INFECTIOUS DISEASES

G.J. Nabel
Vaccine Research Center, NIAID, National Institutes of Health, Bethesda, MD, USA

Advances in our basic understanding of the immune system have given us the tools to make a new generation of vaccines-rationally designed vaccines developed with a scientific understanding of the mechanisms by which microbes cause disease and an eye towards optimizing the immune response. To take one example, our laboratory was able to rationally design DNA vaccines and adenovirus-based vector vaccines against different strains of Ebola virus, an emerging microbial threat. Using a DNA prime followed by an adenoviral vector boost vaccine, or an accelerated immunization schedule using adenovirus alone, we have been able to generate protective immunity against viral challenge in non-primates models relevant to human disease, and we have begun phase I human studies. Gene-based vaccination with DNA liposome complexes has been utilized for cancer vaccines against melanoma with encouraging preliminary results. The efficacy of such vaccines can be further improved by structural biology: structural studies of the HIV envelope led to the design of vectors expressing envelope proteins containing alterations that improve immunogenicity to better elicit cellular immunity and broadly neutralizing antibodies. One of these candidates has been tested in a DNA prime, adenoviral boost combination in clinical studies, in combination with Gag, Pol, and Nef immunogens, and shown to elicit cellular and humoral immune responses. While these results are promising, translation from basic research into a successful vaccine release requires many steps. This talk will address the application of rational vaccine design to prevent and control diverse infectious disease agents such as HIV, Ebola and influenza as well as cancer.

CHALLENGES FOR TRANSLATIONAL RESEARCH ON CANCER AND THE ROLE OF ALLEANZA CONTRO IL CANCRO IN ITALY

M.A. Pierotti
Scientific Secretary, Alleanza Contro il Cancro, Rome, Italy

The European pharmaceutical industry is hampered by its limited ability to exploit research outcomes and to transform them into products and treatment methods. The growth of clinical trials is not matched by that of new drugs, most of which are modifications of already existing molecules. This is particularly evident in the cancer sector, where the gap in the development of translational research is caused by concurring factors which slow down competitiveness. In most of the developed countries, there is a trend towards improved survival. This is not generally due to the use of new molecules for innovative treatments, but rather to better early diagnosis, the rapid diffusion of highly specialised surgical techniques, the introduction of radioguided or robotic surgery, often associated with intraoperative radiotherapy, or the development of new accelerators which allow to treat rapidly, and sometimes radically, primitive tumours or secondary lesions. Since the incidence of tumours is also increasing, to cope with the problem we need to expand our basic knowledge on cancer, accelerating its transfer to the patients' bed, and to invest more in the so called targeted therapy, directly derived from the view of cancer as a disease of genes. However, notwithstanding a few successful examples, even this new therapeutic approach has shown some limitations from a biological, managerial and financial points of view. Better cancer treatment also depends on the ability of the healthcare systems to implement comprehensive cancer control programmes able to balance support to basic and epidemiological research with an agreed and coordinated translational approach which – in accordance with the general concept underlying modern oncology - aims at providing patients with the minimum effective treatment needed for better survival and an enhanced quality of life. A long-term approach to cancer control has both to exploit the huge possibilities offered by the new, molecular knowledge on cancer and to make good use of modern bio-immune therapies, while humbly cooperating with other scientific disciplines to identify possible applications of nanomedicine to the prevention and treatment of the disease. Coordination is needed in order to gather national and European funds and professionals, to give new impetus to the industry and concrete answers to patients' expectations.

Within this framework the Italian Ministry of Health has strongly supported the establishment of Alleanza Contro il Cancro (ACC), a network of CCCs and other research bodies involved in the oncological sector. ACC is chaired by the President of the National Institute of Health with the purpose of linking research and its applications, and of generating synergies with other sectors involved in health issues. ACC aims to promote translational research and to assure equal opportunity of care to cancer patients across Italy, thus reducing health migrations inside and outside the country. The coordination among groups working nationally in the same field is one of the main undertakings of ACC, as

well as the collaboration with other supranational bodies and programmes, such as the Organisation of European Cancer Institutes, with an eye to possible relationships outside Europe, for example with the American Association of Cancer Institutes. The international conference on translational research and the satellite meeting aiming to set up a group of member states interested in coordinating some aspects of translational cancer research represent an important achievement which, in the long term, will hopefully provide the right answer to the expectations of both industry and patients.

Session 1

Cancer - Part 2

Chairpersons

R. De Maria, L.G. Spagnoli

PREDICTING THE IMMUNOLOGIC CONSTANT OF REJECTION

A. Worschech (1,2), N. Chen (1), Y.A. Yu (1), Q. Zhang (1), M. Sabatino (3), A. Monaco (3), Z. Pos (3), H. Liu (1), R.M. Buller (4), E. Wang (3), A.A. Szalay (1), F. Marincola (3)
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(2) Virchow Center for Experimental Biomedicine, University of Würzburg, Am Hubland, Würzburg, Germany
(3) Department of Transfusion Medicine, National Institutes of Health, Bethesda, MD, USA
(4) Department of Molecular Microbiology and Immunology, Saint Louis University School of Medicine, St Louis, MO, USA

Based on hypothesis-generating clinical studies, we postulated that immune-mediated cancer rejection is part of a broader phenomenon shared by autoimmunity, allograft rejection and clearance of pathogens that we called "Immunologic constant of rejection" (ICR). ICR includes the combined expression of Interferon-stimulated genes (ISGs) and Immune effector functions (IEFs).

Here, we tested the predictive value of genes associated with the ICR using GLV-1h68, an attenuated recombinant Vaccinia Virus (VACV) that selectively colonizes established human xenografts inducing their complete regression. We explored human cancer cell/VACV interactions *in vitro* and xenograft/VACV/host interactions *in vivo* adopting organism-specific expression arrays. Indeed, tumor rejection was associated *in vivo* with activation of ISGs and IEFs as predicted in the ICR theory. As expected, the expression of CXCL9-11, CXCL12, CCL5 chemokines, Irf1, granzyme A and B, perforin and FAS which compose the ICR was highly predictive of tumor rejection in this xenograft model.

This study provides the first prospective validation of a universal mechanism associated with tissue-specific destruction observable across species that may represent a tissue-specific target of immune enhancement for the therapy of cancer and chronic viral infections or immune suppression in the context of allograft rejection or autoimmunity.

TUMOR-SPECIFIC IMMUNITY IN CERVICAL CANCER PATIENTS BY A HUMAN PAPILLOMAVIRUS TYPE 16 E6 AND E7 PEPTIDES VACCINE: PERSPECTIVES FOR THE DEVELOPMENT OF THERAPEUTIC VACCINES

C.J.M. Melief (1), M.J.P. Welters (1), M.J.G. Löwik (2), A.P.G. Vloon (1), J.W. Drijfhout (1), A.R.P.M. Valentijn (3), A.R. Wafelman (3), G.J. Fleuren (4), R. Offringa (1), S.H. van der Burg (5), G.G. Kenter (2)

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A therapeutic vaccine was designed based on long overlapping peptides covering the complete amino acid sequence of the HPV16 E6 and E7 oncogenic proteins, thereby harboring all potential T helper and CTL epitopes. Previously, we demonstrated that HPV16 specific T-cell immunity induced by this vaccine, delivered in Montanide ISA 51 adjuvant was able to terminate persistent infections and eradicate established $HPV16^+$ tumors in rabbits. Currently, 20 patients with histologically proven $HPV16^+$ vulvar intraepithelial neoplasia (VIN) grade III were vaccinated 4 times with a 3-week interval by s.c. injection of the peptides emulsified in Montanide ISA 51. Immunological monitoring was performed at the systemic level by the analysis of blood samples, drawn before each vaccination and after the last vaccination, and at the local level by the analysis of HPV16-specific T-cells in tissue biopsies of the VIN lesion (before and after vaccination) as well as a biopsy from the last vaccination site. In all 20 patients, already after 2 vaccinations strong and broad vaccine-induced systemic proliferative responses, accompanied with the production of IFNγ and IL-5 were detected. This type of response is similar to the memory T-cell responses observed in healthy individuals with HPV16-specific immunity. Importantly, circulating HPV16 E6 and E7 specific T-cells produced IFNγ upon stimulation with naturally processed and presented antigen. Notably, vaccination resulted in the induction of both $CD4^+$ and $CD8^+$ HPV16-specific T-cells. Multiple epitopes were recognized in each patient. A complete clinical response was seen in 5 out of 20 patients. 8 Of the remaining 15 patients experienced a partial remission of lesions and substantial improvement of disease-associated complaints. In conclusion, our peptide-based vaccine elicits a strong and broad HPV16-specific T-cell response that displays the capacity to migrate into the persistently HPV16-infected lesion of patients with high grade VIN and causes complete regressions in a substantial proportion of patients.

NOVEL IMAGING STRATEGIES FOR THE DETECTION AND MONITORING OF CANCER

C. Messa (1,2,3,4), M. Picchio (4), M.C. Gilardi (1,3,4), R.M. Moresco (1,3,4), C. Gelfi (3), N. Di Muzio (4), F. Fazio (1)
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(2) Department of Nuclear Medicine, San Gerardo Hospital, Monza, Italy
(3) Institute for Bioimaging and Molecular Physiology, National Research Council (IBFM-CNR), Milan, Italy
(4) Scientific Institute San Raffaele, Milan, Italy

Novel imaging strategies may be represented, at present, by "Molecular Imaging", defined as a "Spatially localised and/or temporally resolved sensing of molecular and cellular processes *in vivo*". The common background of "molecular imaging" methods is the selection and use of an "agent" that directly or indirectly interacts with a "target" molecule, being detectable *in vivo* and in a non invasive manner. Agents can be radiotracers, specific media used for MR imaging, substrates converted in a detectable form by the target enzyme activity (the "smart probes").

This technology is already changing the management of many diseases, including cancer, where it can define different aspects of disease (morphology, function, biochemistry) in a high accurate manner. Other than its clinical use in humans, a major goal of "Molecular Imaging" is to work as a "bridge" between basic and clinical research, having several characteristics that allow a mutual information between the experimental and the applicative phase of research. Such characteristics include: a) an adequate spatial and temporal resolution and contrast; b) the use of many different agents that reproduce *in vivo* important molecular functions, such as enzyme activity; c) the possibility to use the same technology for preclinical and clinical studies; d) the possibility of its direct application in humans in large population of patients. These devices include Magnetic Resonance Imaging (MRI, including high field systems), Magnetic Resonance Spectroscopy (MRS), Single Photon Emission Tomography (SPECT), Positron Emission Tomography (PET) and integrated PET/CT. Although at present employed for animal studies only, also Optical Imaging is a very promising tool to evaluate functions at molecular level. The accuracy and efficacy of "Molecular Imaging" methods will be discussed in view of the most relevant clinical oncological questions, including early detection of disease, prediction of treatment response and prognosis, early effects of new therapeutic approaches, targeted drug development.

THE GLOBAL PROBLEM OF CANCER: THE PRIORITY TO DEVELOP TRANSLATIONAL RESEARCH

P. Boyle
International Agency for Reseach on Cancer, Lyon, France

The majority of the global cancer burden now occurs in medium- and low-income countries. This is in marked contrast to the situation a few decades ago when cancer was considered to be a disease of westernised, developed countries. Given the continuing growth and ageing of the world's population, and the widespread introduction of cancer risk factors from developed countries, the global cancer burden will grow rapidly making cancer control an increasing priority worldwide.

In 2008, it is estimated that there will be 12.4 million new cases of cancer diagnosed worldwide, 7.6 million deaths from cancer, and over 28 million persons alive with cancer within five years of initial diagnosis. Taking account of the growth and ageing of the world's population, and factoring in an annual increase in cancer incidence and mortality of 1%, by 2030 it could be expected that there will be 26.4 million incident cases of cancer, 17.0 million cancer deaths annually, and 80 million persons alive within five years of initial diagnosis. Regions with the largest proportions of countries of low- or medium-income will be hardest hit and the impact in such countries, still faced with the burden of infectious disease and a low budget for health, will be considerable in terms of the treatment needs and the costs of treatment.

The world's growing cancer burden must be addressed. Priorities need to be realistic and achievable and include a focus on the identification, delivery, and assessment of effective cancer control measures. Depending on resources and competing health priorities, all steps must be taken to prevent those cancers which are preventable; to treat those cancers which are treatable; to cure those cancers which are curable; and to provide palliation and supportive care to those patients who need palliative care. The necessity for cancer control and capacity building in countries of limited resources is evident and urgent as is the need for Translational Research.

Translational research covers a wide spectrum of activities, including applications of findings from basic science into new treatments to sociological interventions on established risk factors in populations, that could lead to a reduction in cancer incidence and death. Translational Research covers the translation of research findings in any domain to cancer control ranging from "bench-to-bedside" to changing the lifestyle habits of populations. More than ever, successful innovative approaches are needed to reduce the world's cancer burden and its impact on society.

Session 2

Neurosciences

Chairpersons

E. Alleva, M. Pocchiari

RATIONAL THERAPEUTIC STRATEGIES FOR MODIFYING ALZHEIMER'S DISEASE: TRANSLATING BASIC RESEARCH ON A-BETA OLIGOMERS

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Compelling evidence shows that the Aβ-amyloid peptide is the central biochemical marker of Alzheimer's Disease, and is the most likely cause of the neurodegeneration manifest in synaptic dysfunction and eventual neuronal loss. Pathways up-stream of Aβ production provide therapeutic targets amenable to protease inhibition/modulation. Strategies which affect APP trafficking may also prove of value. Downstream, pathways promoting the degradation of Aβ or modulating clearance from the brain also offer windows for therapeutic opportunity.

Central interest lies in the mechanism through which Aβ undergoes toxic gain-of-function, inducing neuronal damage. This provides the most direct route for therapeutic intervention, with least risk of therapeutic side-effects, since Aβ toxicity is unlikely to mimic any normal function. Two principal hypotheses have emerged to explain Aβ toxicity: redox chemistry associated with the Cu/Zn metal binding sites on Aβ, and lipid interactions associated with the α/β conformation of the hydrophobic C-terminus. Drugs targeting these mechanisms are now in clinical development with encouraging preliminary results.

The normal function of APP remains elusive despite two decades of research, but we speculate that APP evolved as a metallo-protein, functioning through proteolytic release from the membrane and signalling through its cytoplasmic domain, in excitatory glutamatergic synapses. Perturbation of normal function or processing may lead to excessive production of Aβ peptide, accumulation of which leads to toxic gain-of-function. Either as a soluble oligomer or insoluble amyloid fibril, the accumulation of the Aβ fragment provides a pivotal biomarker, currently being developed as a neuroimaging target and a blood/CSF biomarker for efficacy of therapeutic intervention, and for gene-linkage discovery.

INHERITED DEMYELINATING NEUROPATHIES: FROM BASIC TO CLINICAL RESEARCH

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Neuron-glia interactions are fundamental to nervous system development. They include remarkable cellular specializations, such as axons that are physically enwrapped by a multilayered myelin sheath. The function of myelin for rapid saltatory impulse propagation is well known, but we recently discovered a myelin-independent requirement of ensheathing glial cells for the long-term function and survival of axons. Indeed, neurological diseases affecting glia in the CNS (multiple sclerosis, leukodystrophies) and PNS (neuropathies) are both associated with axon loss as a major cause of clinical disability. We have modeled inherited myelin diseases in transgenic and mutant mice, which allows us to study disease mechanisms and explore experimental therapies. The most frequent demyelinating neuropathy, Charcot-Marie-Tooth Disease type 1A (CMT1A), is caused by a small duplication of chromosome 17. Our transgenic rat model provides formal proof that increased Pmp22 gene dosage and 1.5-fold overexpression is the underlying cause of CMT1A. PMP22, a myelin membrane protein in Schwann cells, is expressed in part under control of the nuclear progesterone receptor. Using the CMT1A rat model, we have investigated the potential of an anti-progesterone drug (Onapristone), originally developed against breast cancer, as a therapeutic strategy to counteract Pmp22 overexpression and thus disease expression. A preclinical study has demonstrated that onapristine is able to improve the clinical phenotype of this neurological disease in the rat. Surprisingly, this effect is not mediated by preventing demyelination, but rather by rescuing the neuroprotective function of myelinating Schwann cells.

MULTIPLE SCLEROSIS: PHARMACOGENOMICS AND PERSONALIZED DRUG TREATMENT

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Multiple sclerosis is the most relevant cause of chronic and progressive neurological disability. "Disease-modifying therapies" have now been available for almost 20 years, new agents have been registered in the last couple of years and additional treatments will be available shortly. Nonetheless the problem is far from being solved. As for other multifactorial diseases, it is extremely difficult to design treatments with high therapeutic index in the absence of firm knowledge on the etiology of the disease. Current therapies can only target pathogenetic mechanisms which are essentially immunological in nature and, as such, indispensable for health and intrinsically redundant. As a consequence, any increase in efficacy is at risk of increased toxicity

While awaiting for definitive information on the etiology of the disease, which may allow the design of ground breaking therapies, we can try to increase the efficacy of current treatments by devising new therapeutic schedules, by combining them and/or by identifying those patients are more likely to respond to one treatment or another.

This can be done by studying the individual variations in the DNA sequence (pharmacogenetics), or the phenotypic changes as a result of genetic variations (pharmacogenomics) that may impact pharmacological responses. In multiple sclerosis, this kind of studies is still in an early phase. Nonetheless, some results are already being transferred to therapy. Examples of how this is being done will be presented.

LONG STORY SHORT: SEROTONIN TRANSPORTER IN EMOTION, SOCIAL COGNITION, AND DEPRESSION

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Depression is an etiologically heterogeneous group of brain disorders with complex genetics. Definition of clinical phenotypes are not rooted in their neurobiology and animal models of behavioral dispair have considerable limitations. Although research on the neurobiology of depression is still in its infancy, several milestones have already been reached: Variation in gene expression were confirmed to play a predominant role in individual differences in complex traits including personality and behavior; gene x environment interaction were established in humans and the nonhuman primate model; gene-phenotype correlations were substantiated by functional neuroimaging; as well as the notion that both genes and environmental factor impact on brain development and thus set the stage for the susceptibility to depression is increasingly appreciated. Investigation of subtle alterations in the expression of genes of the serotonergic pathway, such as the serotonin transporter (5HTT), of correlations between 5HTT genotype, brain activity and cognition, as well as of environmental variables interacting with 5HTT variants currently strengthen research on the genetics of depression. Given the etiological and psychobiological complexity of depression, it is not surprising that the identification of vulnerability genes and elucidation of their interaction with environmental stressors is extremely difficult and continues to be among the most daunting challenges for translational research comprising genomics, epigenetics, behavioral neurosciences, and neuropsychiatry.

CREUTZFELDT-JAKOB DISEASE AND TRANSLATIONAL MEDICINE

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Funding for research in prion diseases, including Creutzfeldt-Jakob Disease (CJD), increased significantly following the advent of Bovine Spongiform Encephalopathy (BSE) in the late 1980s and an important question is whether this has resulted in advances in patient care and treatment.

The prompt and accurate clinical diagnosis of CJD is of great importance to issue to patient and their families. Recognition of CJD can be difficult because of the varied clinical phenotypes in sporadic CJD and genetic forms of human prion disease and because of the occurrence of a zoonotic form, variant CJD with novel clinical characteristics. In the past decade there have been advances in diagnostic modalities with the development of the 14-3-3 CSF assay, the increasing use of gene sequencing to identify disease-associates mutations and the validation of MRI brain scan for diagnosis of variant and sporadic CJD. Disease classification has been enhanced by the development of prion protein biochemistry and the application of immunohistochemical techniques to brain histology, although the issue of strain variation in human disease remains unresolved.

Advances in basic science have led to increasing understanding of the pathogenesis of prion diseases, notably through the development of the protein misfolding cyclic amplification, and rational approaches to developing treatments for prion diseases are now feasible. However, despite some encouraging results in animal or cell models of disease, no proven therapy of CJD has yet been developed. It is hoped that collaborative studies in Europe will lead to advances in this area.

From a historical perspective the availability of data from basic scientific research, which at the time seemed of little relevance to human heath, was critical to the policy response the BSE epidemic. This underlines the difficulty in predicting which areas of basic science will eventually have practical implications and which will lead to advances in public health.

UNDERSTANDING THE MOLECULES THAT DETERMINE BEHAVIOR; A BASIS FOR DEVELOPMENT OF NEW DRUGS IN NEURO-PSYCHIATRIC DISEASE

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Neuro-psychiatric diseases are common and represent a major social-economic burden in virtually all European countries. Yet there has been relatively little progress with regard to therapy. Lithium has been used to treat mode disorders for more than one hundred years and is still the drug of choice. The principles for treatment of schizophrenia have not changed much during the last 40 years. The SSRI drugs, introduced for treatment of depression, are all relatively unspecific. Virtually all drugs used in neuro-psychiatric diseases are associated with several side effects. There are several reasons for the slow progress in this field. Patient and family organizations are weak and the molecular mechanisms behind neuro-psychiatric diseases are incompletely known. Yet the identification of novel therapeutic approaches and the development of more specific drugs with less side effects would have an enormous impact. In this presentation I will review some of the more specific potential therapeutic targets in neuro-psychiatric disease. The dopaminergic and glutamatergic systems interact to initiate and organize normal behavior, a communication that may be perturbed in many neuro-psychiatric diseases. Studies will be presented that indicate that the therapeutic approach in schizophrenia should involve both systems. The metabotropic glutamate receptor 5 (mGluR5) has also been implicated in behavior disorders related to schizophrenia. A newly discovered protein that plays a key role for mGluR5 signaling has been identified and may be a useful new target for therapy. The SSRI drugs all act to enhance the release and effect of serotonin, well known for its anti-depressive effects. Since however serotonin will activate several subtypes of receptors; many of which have different cellular read-outs, the effect of SSRI drugs will be rather unspecific and an effort should be made to identify the receptors that may play a key role for the anti-depressive effect of serotonin. One such receptor will be presented.

Session 3

Rare Diseases

Chairpersons

D. Taruscio, A. Macrì

CHALLENGES FOR TRANSLATION RESEARCH IN RARE DISEASES: THE E-RARE EXPERIENCE

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Rare Diseases are life-threatening or chronically debilitating diseases with a low prevalence (<5/10,000). The specificities of Rare Diseases - limited number of patients and scarcity of knowledge and expertise - make them a research area that can strongly profit from coordination on a transnational scale.

Nine partners from 8 European countries (Belgium, France, Germany, Israel, Italy, Netherlands, Spain and Turkey) have decided to join their efforts into the coordinated action "E-Rare". The general goal of E-Rare is to coordinate existing research programmes and to prepare joint and strategic activities to overcome some of the limitations imposed by scattered funding and fragmentation between national programmes.

Key activities:

- creation of a knowledge base for the development of joint and trans-national activities;
- definition of strategic research priorities;
- development of trans-national multidisciplinary approaches;
- implementation of trans-national calls for proposals.

A call for proposals for transational projects was launched in March 2007; 125 proposals were submitted, of which 13 were selected for funding. The participating researchers currently receive the required funds from their national agency. An analysis of the 47 pre-selected projects suggested that most of the projects:

- were intending to form networks and carry out research at the same time;
- contained both genetic and pathophysiology studies;
- envisioned therapeutic studies;
- had a multi-approach including the use of advanced scientific methods.

The transnational call for proposals revealed a large scientific potential however, translation of knowledge from basic science to patients must be improved.

Translational research needs interdisciplinarity and networking between basic researchers and clinicians. Networking among researchers is essential also for ensuring the most efficient use of limited resources. Future joint transnational calls and the opening of national programmes to international collaboration are envisioned by the E-Rare partners to strengthen the international collaboration necessary to achieve a better research.

In addition, the ERA-Net will continue its efforts to enable transnational research addressing issues like access to research infrastructures as well as rotational positions for clinical scientists. The ERA-Net, on the basis of the systematic exchange of information on different programmes, with a well established and successful coordination mechanism, a wide dissemination of the results and interactions with policy makers, will have an important effect in influencing research policy development within the partner countries and EU MS.

THE EUROPEAN NETWORK ON RETT SYNDROME

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Rett Syndrome (RS) is a severe neurological disorder primarily affecting girls, with an incidence of about 1/10,000 female births. 250 girls affected by RS are born each year in the European Union. Most Rett syndrome cases are caused by mutations in the methyl-CpG binding protein 2 (MeCP2). Atypical cases have mutations in the CDKL5 or the FOXG1 genes.

MeCP2 encodes two closely related proteins who act as transcriptional modulators. CDKL5 encodes a kinase which could mediate MeCP2 phosphorylation. However, the mechanisms leading to the severe, progressive and specific neuronal dysfunction when these genes are mutated are currently unknown. Several mouse models of RS have been generated. These models were used to show that the phenotype was due to Mecp2 dysfunction in post-mitotic neurons. Recent key experiments demonstrated that re-expressing Mecp2 in the knock-out mouse displaying overt symptoms was able to reverse disease progression. This possible reversibility fully justifies the development of therapeutic approaches for this disorder, especially pharmacological interventions. European teams are strongly involved in this research area.

EuroRett joins the forces of 17 research groups in Italy, France, Spain, Germany and Israel. Its objectives are:

- to improve phenotype-genotype correlations (building a large european cohort of patients);
- to study chromatin organization and identify MeCP2 and CDKL5 targets and interactors;
- to understand neuronal dysfunction in RS;
- to develop therapeutic approaches for RS.

Rett Syndrome is a model for autism-spectrum disorders. It is a severe phenotype for which there is currently no efficient treatment but that could be reversible. The strong commitment of parent associations to support research has generated a huge interest for the condition. The EuroRETT E-RARE network gives us the opportunity to organize research efforts at the european level to fight against this devastating neurological disease.

SYSTEMIC AMYLOIDOSIS: MOLECULAR MECHANISMS AND TARGETED THERAPY

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The elucidation of fundamental aspects of the pathogenesis of amyloidosis has a great potential to translate into better patients' care. In multifactorial diseases, such as systemic amyloidoses, the optimal therapeutic approach should exploit multiple strategies of attack, including suppression of amyloidogenic protein synthesis, stabilization of the amyloid precursor, inhibition of the toxicity exerted by the oligomers, interference with the facilitating effects of the common constituents, and promotion of amyloid reabsorption. It is now possible to reduce the amount of the amyloidogenic protein in several types of systemic amyloidoses using different approaches according to the source of the amyloid protein, for instance, stem cell transplantation and chemotherapy in light chain Amyloidosis (AL), and liver transplantation for certain hereditary amyloidoses. Innovative approaches targeting the mutated gene and its expression are under development in hereditary amyloidosis. Protein structural instability plays an important role in amyloidosis. Several small compounds, such as diflunisal and others, have been reported to bind to and stabilize transthyretin variants, preventing fibril formation. These drugs are being tested in patients. Furthermore, several short peptides able to interfere with the process of amyloid formation have been developed. Glycosaminoglycans (GAGs) mimetics were shown to efficiently inhibit the interaction between the amyloid protein precursor, serum amyloid A, and GAGs reducing amyloid fibril formation in an animal model. One of these compounds, eprodisate, produced clinical benefits in a controlled trial in patients with reactive amyloidosis. Reabsorption of amyloid deposits has been attempted using small molecules. We reported that an iodinated analog of anthracycline promotes fibril disaggregation and produced clinical responses in phase II trials in patients with AL amyloidosis. Similarly, tetracyclines showed disaggregating effects on transthyretin amyloid fibrils and they are being tested in patients. Another strategy aims at depleting serum amyloid P component from amyloid deposits favoring their resorption, using bivalent ligands. Several efforts have been directed at manipulating the immune system in order to prevent the formation of amyloid deposits and to favor their clearance. Future challenges include the understanding of the molecular mechanisms of tissue targeting and tissue dysfunction caused by amyloid proteins that will possibly open novel treatment avenues.

CLINICAL TRIALS FOR TREATMENTS FOR RARE DISEASES

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Rare Diseases are an important medical and social issue because the small numbers of patients causes a lack of interest in developing remedies. Besides the scarcity of financing for studying orphan drugs a number of methodological problems prevent proper evaluation of their efficacy and safety characteristics as is done for drugs for common diseases.

The lecture reports an analysis of 44 orphan drugs released on the market by EMEA between January 2000 and December 2007. Preclinical toxicological studies and characteristics of the clinical studies will be presented and discussed to demonstrate the heterogeneity of conditions leading to the approval of orphan drugs. In general these drugs are evaluated on the basis of surrogate end-points that do not always guarantee clinically significant advances for patients, also considering their high cost. In some cases, particularly in cancer, the application for the orphan drug pattern is an obvious excuse to enter a much larger market.

Session 4

Cardiovascular Diseases

Chairpersons

L. Frati, V. Macellari

NEW THERAPEUTIC APPROACHES FOR HEART FAILURE TREATMENT

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Heart Failure (HF) is a disease state affecting approximately 1% of the general population in Western Countries, representing a major socioeconomic burden. Its incidence is steadily increasing, also due to a significant improvement in the treatment of acute episodes of myocardial diseases. However, HF treatment is still unsatisfactory: drug weaponry did not change significantly during the last ten years, while the most significant progress is mainly due to electrical therapy. Molecular and cellular biology tools allowed however a very detailed identification of the mechanisms controlling cardiac function in the normal and failing heart.

Signal transduction molecules regulating cardiac contractility have been identified; progress has been made in particular in the knowledge of mechanisms of action of signal transduction from the beta-adrenergic pathway and in the Insulin/IGF1 signaling cascade and their role in controlling inotropism. Our group for instance determined that Akt, a serine/threonine kinase downstream of the Insulin/IGF1 receptor, regulates the ion flux of L-type Ca2+ channel (LTCC). The mechanism consists in the control of the maturation of the channel through phosphorylation of the b-2 subunit. New drugs interfering with this process by increasing the number of LTCC in cardiomyocytes during HF might be envisioned.

Further progress has been made in understanding the role of small RNAs in regulating critical cellular processes, not only in cardiovascular medicine. Among these small RNAs, microRNA have been subject of intensive studies during the last few years. These small molecules regulate gene function by binding to complementary sequences on the 3' untranslated regions of target genes, inhibiting translation. Our group focused on the role of few tissue specific microRNAs, among which miR 133.

We found that by regulating expression levels of this miR it is possible to increase or decrease cardiomyocyte cell size and function *in vitro* and *in vivo*. miR 133 seems to regulate protein levels of genes involved in different aspects of the hypertrophic response, such as gene expression, cytoskeletal organization and signal transduction. We are also studying other miRs which could be involved in the pathogenesis of the vascular degeneration during atherosclerosis and hypertension. The possibility to regulate cellular function with non-toxic, "natural" molecules is particularly fascinating and may lead to new therapeutic tools in the near future.

Another very promising field in HF research is regenerative medicine. Many investigators are determining the ideal cardiomyocyte progenitor cell. The initial reports on the possibility to generate new cardiomyocytes with bone marrow stem cells, opening the possibility to autologous transplantation, have not been confirmed by a number of other groups. On the contrary, cardiomyocytes from human embryonic stem cells were consistently generated in different research centers, although a number of problems, including ethical ones, make this therapeutic application unrealistic. A breakthrough in this

field might be represented by the newly discovered induced progenitor cells (iPS), generated *in vitro* by transfecting few embryonic genes in differentiated cells. These cells can differentiate cardiomyocytes from human iPS, rendering "personalized" cell therapy a serious possibility. Thus, a number of new tools are now available to generate new "biological" drugs for curing HF.

THE LONG PENTRAXIN PTX3: FROM INNATE IMMUNITY TO VASCULAR PATHOLOGY

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C reactive protein, the first innate immunity receptor identified, and serum amyloid P component are classic short pentraxins produced in the liver. Long pentraxins, the prototype of which is PTX3, are expressed in a variety of tissues. A subset of long pentraxins are expressed in the brain and some are involved in neuronal plasticity and degeneration. PTX3 is produced by a variety of cells and tissues, most notably dendritic cells and macrophages, in response to TLR engagement and inflammatory cytokines. PTX3 acts as a functional ancestor of antibodies, recognizing microbes, activating complement, facilitating pathogen recognition by phagocytes, hence playing a non-redundant role in resistance against selected pathogens. In addition PTX3 is essential in female fertility by acting as a nodal point for the assembly of the cumulus oophorus hyaluronan-rich extracellular matrix.

Thus, the prototypic long pentraxin PTX3 is a multifunctional soluble pattern recognition receptor at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. PTX3 will be used as a paradigm of the complexity and complementarity of the interplay between the cellular and humoral arm of innate immunity.

MOLECULAR MECHANISMS OF RETINAL VESSEL DYSFUNCTION AND CLINICAL IMPLICATIONS

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Information provided by the WHO has underlined that ocular pathologies are on the raise in both industrialized and not industrialized Countries, thus profoundly affecting the health management worldwide. Potentially blinding eye diseases, such as Age-related Macular Degeneration (ADM) and Diabetic Retinopathy (DR) result from complex systemic imbalances in which the interplay of genetic and environmental factors negatively impact organ homeostasis. In particular, ocular vascular dysfunction, as it occurs as consequence of diabetes, aging and prematurity, plays a pivotal role in the induction and progression of ischemic retinopathies. Experimental and clinical studies have emphasized that oxidative stress is critical to the development of retinal vessel dysfunction. Indeed, increased production of reactive oxygen species, at both extra- and intra-cellular level, profoundly affect redox-dependent biochemical pathways within the vascular cells population, leading to subtraction of nitric oxide and increased expression of pro-inflammatory mediators. In turn, the up-regulation of inflammatory cytokines and angiogenic factors, such as the Vascular Endothelial Growth Factors (VEGF), leads to macular edema, pathological neovascularization and, in most severe cases, to retinal detachment and blindness.

These studies, have therefore, suggested that treatment of affected patients with antioxidants (*e.g.* Vitamin A, C and E) should be of beneficial effect. However, the results of several clinical trials, particularly in the case of diabetic retinopathy, have been modest. This has been partially explained by inefficient drug delivery to the retina, and prompted ever more attention to bio-engineering solutions addressing this important limitation in the therapeutic management of retinal diseases. The results of these clinical studies have also revealed that oxidative stress at retinal vessel level is a complex a multifaceted process. Indeed, recent advances in the understanding of the molecular basis of oxidative stress have implicated the importance of endogenous detoxifying enzymes in the maintenance of the cell redox status and suggested new therapeutic approaches which are now under evaluation also for retinal diseases. In conclusion, the combination of clinical and experimental studies is critical to the resolution of important therapeutic and diagnostic problems which is essential to the management of ocular blinding diseases, as well as other important human pathologies.

GENOMICS, PROTEOMICS AND OTHER OMICS IN CARDIOVASCULAR MEDICINE

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The strategy of "omic" or systems biology and medicine is feasible due to a number of recent achievements in biomedicine, including the completion of the Human Genome Project, the emergence of cross-disciplinary biology and bioinformatics, with the latter serving as a link between different fields. Moreover, recent advances in high-throughput platforms for the first time permit simultaneous study of a large complement of genes, transcripts, proteins or small metabolites.

Last year has brought about a new dawn in the genomics of complex traits with the introduction of the Genomewide Association Studies (GWAS). For example, the Wellcome Trust Case Control Consortium (WTCCC) used 500,000 SNPs in 17,000 individuals to identify 24 genetic risk factors for 7 common diseases. Of the two cardiovascular diseases studied by the WTCCC, the coronary artery disease did much better than hypertension. Interestingly the reasons for these discrepancies are most likely related to the phenotyping of control subjects. Future strategies for cardiovascular GWAS include pharmacogenomic strategies as well as detailed analyses of target organ damage such as cardiac hypertrophy and microalbuminuria. Equally important place in biomarker discovery and cardiovascular prevention will to belong to high throughput proteomics and metabolomics, with emerging examples of biomaker patterns being useful in early diagnostics and monitoring of therapeutic interventions.

AUTOLOGOUS PROGENITOR CELL ADMINISTRATION FOR REGENERATIVE CARDIOVASCULAR THERAPIES

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Experimental studies suggest that the intravascular or intracardiac administration of either bone marrow or blood-derived autologous progenitor cells may improve contractile function after experimentally induced acute myocardial infarction. Initial clinical pilot trials as well as small randomised, but unblended studies in patients with acute myocardial infarction indicated that the intracoronary infusion of bone marrow-derived progenitor cells into the infarct artery may be associated with improved left ventricular function.

The recently completed double-blind, placebo-controlled multicenter Repair-AMI Trial investigating the effects of intracoronary infusion of bone marrow-derived progenitor cells versus placebo medium into the infarct artery inpatients with successfully reperfused acute myocardial infarction revealed a significantly greater improvement in global left ventricular ejection fraction combined with complete abrogation of left ventricular endsystolic volume expansion in the bone marrow-derived cell treated group compared to the placebo group. These findings were substantiated by MRI-analyses in a subset of patients at 1-year follow-up. Patients with the most severely depressed left ventricular function despite successful reperfusion therapy derived the most benefit from intracoronary cell therapy. Mechanistically, intracoronary Doppler flow velocity measurements revealed a complete normalisation of coronary flow reserve in the infarct artery treated with intracoronary infusion of bone marrow-derived cells. Finally, the group of patients receiving intracoronary infusion of bone marrow-derived cells had significantly fewer cardiovascular events up to 2 years follow-up compared to the patients receiving placebo medium infusion.

Taken together, evidence in emerging that the intracoronary administration of bone marrow-derived progenitor cells is associated with improved recovery of left ventricular contractile function in patients with acute myocardial infarction. Given the preferential effect in patients with large acute myocardial infarctions, studies are warranted to examine the potential effects of progenitor cell administration on morbidity and mortality in patients with extensive infarction and depressed left ventricular contractile function despite successful reperfusion therapy. Moreover, specific attention should be paid in the future to the process of cell isolation in order to maximise functionality of the transplanted progenitor cells, since functionality of infused cells was an independent predictor of functional outcome in Repair-AMI.

Session 5

Infectious and Autoimmune Diseases - Part 1

Chairpersons

A. Cassone, B. Ensoli

IMMUNE RESPONSE TO VIRAL INFECTION: CHALLENGES FOR THERAPEUTIC INTERVENTION

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The type of antiviral immune response mainly depends on the nature of the virus and the genetic background of the infected host. Virus properties such as the level of cytopathogenicity, tropism, virulence or persistence decide which arm of the immune system will be triggered and which effector mechanisms will act to control a virus infection efficiently. Our studies have been performed in mice with non-cytopathic Lymphocytic Choriomeningitis Virus (LCMV) and cytopathic Vesicular Stomatitis Virus (VSV) in the mouse.

Efficient control of a VSV infection requires early appearance of IgM and IgG virus neutralising antibodies at day 3 to 4 post infection. In contrast, early control of the non-cytopathic LCMV infection depends on efficient induction of cytotoxic T lymphocytes exhibiting perforin mediated cytotoxicity or secreting non-cytotoxic antiviral mediators. Neutralising antibodies appear rather late, around day 60 after LCMV infection. These neutralising antibodies, nevertheless, are important for long-term LCMV control.

Experiments with recombinant VSV expressing LCMV-Glycoprotein (LCMV-GP-1) instead of its own glycoprotein revealed a critical role for the glycoprotein but not for the viral backbone in determining the neutralising antibody kinetics. We also observed delayed but efficient control of LCMV by neutralising antibodies in the absence of CD8 positive T cells. However, virus variants emerged under these circumstances that escaped the established polyclonal neutralising antibody response against LCMV by changing their surface neutralising determinants. Similar phenotypes of viral infections could be observed with human pathogens such as hepatitis B and C and HIV viruses.

We found that wild-type mice developed LCMV-GP-1-specific antibodies already by day 8 after exposure to high but not low virus doses, demonstrating that naive GP-1-specific B cells were infrequent. Furthermore, the induced antibodies bound to the neutralizing GP-1 epitope but failed to neutralize the virus and therefore were of low affinity. In CTL-deficient mice, where massive viremia quickly levels initial differences in viral load, low and high doses induced low-affinity non-neutralizing GP-1-binding antibodies with kinetics similar to high-dose-infected wild-type mice. Only in CTL-deficient mice, however, the GP-1-specific antibodies developed into nAbs within 1 month. We conclude that LCMV uses a dual strategy to evade nAb responses in wild-type mice. First, LCMV exploits a "hole" in the murine B-cell repertoire, which provides only a small and narrow initial pool of low-affinity GP-1-specific B cells. Second, affinity maturation of the available low-affinity non-neutralizing antibodies is impaired.

Similar phenotypes of viral infections could be observed with human pathogens such as hepatitis B and C and HIV viruses.

THE QUEST FOR IMMUNE CORRELATES PROTECTION AGAINST AIDS: LESSONS FROM LONG TERM NON PROGRESSORS AND FROM THERAPEUTIC IMMUNISATION

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Developing a prophylactic or a therapeutic vaccine against HIV and AIDS aims at reducing viral replication in order to limit or delay both the incidence of AIDS and the transmission of HIV since it is now admitted that HIV cannot be eradicated by the immune system nor by the strongest antiretroviral drugs. It is therefore essential to define the effective immune responses that can ensure such a protection and be elicited by an HIV vaccine. We investigated this question in a cohort of HIV-infected Long Term Non Progressors and individualized a series of correlates of immune protection involving host genes, CD8 and CD4 T cell as well as antibody responses to HIV. We'll particularly focus on the various mechanisms involving the HLA restricting elements and the antigens targeted by which CD8 T cells can ensure protection against HIV disease.

IMPAIRED DENDRITIC CELL FUNCTION COMPROMISES IMMUNE FUNCTION IN TYPE 1 AND TYPE 2 DIABETES

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Diabetic subjects have increased incidence and persistence of infections. This suggests that the immune function may be impaired in diabetics. We postulate this to be associated with altered function of Dendritic Cells (DCs) as they are important initiators and regulators of T cell-mediated immune responses. We investigated functional responses of endogenous DCs in diabetes prone NOD mice and human diabetics. We observed no gross difference in number or maturation state of endogenous DC subsets in NOD as compared to non-diabetic mice. However, IL-12 production from NOD CD8α+ DCs was impaired. The IFN-α production by plasmacytoid DCs (pDCs) from diabetic NOD mice was much lower than in pre-diabetic NOD mice. There was more IL-10 secretion by diabetic NOD splenocytes as compared to controls. The IL-10 over-production may inhibit pro-inflammatory cytokines such as IL-12 from CD8α+ DCs. IL-10 regulation of DCs is a hallmark of tolerogenic DCs that activate regulatory T cells and are associated with protection from T1D. IL-10 is also associated with the inhibition of IFN-α production from human pDC. *In vitro* hyperglycemic conditions in our studies did not reduce IFN-α secretion from pre-diabetic NOD splenocytes. Therefore, abnormal DC function may relate to factors other than hyperglycemia.

As a translational study, we also characterized endogenous DC subsets in human diabetic subjects. We found DCs subsets to be at normal frequency and maturation state in these subjects. However, DCs are poor producers of the T helper 1 (Th1)-inducing cytokines IL-12p70 and IFN-α in T1D, while only IFN-α secretion was reduced in type 2 diabetic (T2D) subjects. In addition, DCs production of IL-10 is increased in both T1D and T2D subjects. Thus, we have identified similar functional differences in pDC of diabetic NOD mice and human diabetics. Our results indicate that poor glycemic control does not affect DC frequency or phenotype. DC regulation of T cell responses is found to be impaired. The inability of DCs to mount effective proinflammatory Th1 and/orTh17 responses is likely an important cause of poor control of infections in individuals with diabetes.

Session 5

Infectious and Autoimmune Diseases - Part 2

Chairpersons

G. Ippolito, S. Vella

THERAPEUTIC DENDRITIC CELL-BASED VACCINES AGAINST HPV-INDUCED TUMORS

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Radical hysterectomy with pelvic lymph node dissection and chemo-radiation therapy remain the cornerstone of treatment for invasive cervical cancer. Nevertheless, up to 35% of cervical cancer patients overall will experience persistent/recurrent, metastatic disease for which treatment results remain poor. Thus, novel therapeutic strategies effective in the treatment of recurrent/metastatic cervical cancer remain desperately needed. Because HPV infections mediated by high risk genotypes represent the most important risk factor for developing cervical cancer, and the human immune system is known play a major role in controlling HPV infections and HPV related lesions, therapeutic vaccines against HPV infected cervical tumors may represent promising and potentially effective therapeutic option in these patients. Fully mature Dendritic Cells (DC) represent the most potent professional antigen presenting cells present in the human body and autologous DC-loaded with HPV antigens have consistently been shown to induce effective activation of the human immune system against E6 and E7 oncoproteins *in vitro* and more recently *in vivo*. Our group has recently completed a Phase I study evaluating the safety and immunogenicity of HPV16 or HPV18 E7 antigen-pulsed mature DC vaccination in patients with stage IB or IIA cervical cancer. Escalating doses of autologous DCs (*i.e.*, 5, 10 and 15 x 106 for injection) were pulsed with recombinant HPV16/18 E7 oncoproteins and keyhole limpet hemocyanin, (KLH) and delivered through a total of 5 subcutaneous anterior thigh injections at 21 day intervals to 10 cervical cancer patients with no evidence of disease after radical surgery. The therapeutic vaccine was well-tolerated and no significant local or systemic side effects or toxicity were recorded. Ten out of ten (100%) of the patients developed humoral and cellular CD4+ T cell responses to the E7 vaccine as detected by ELISA and by ELISPOT. Swelling/induration (*i.e.*, a positive DTH response) to the intradermal injection of HPV E7 oncoprotein and KLH was detected in all patients after vaccination. On the basis of these promising results our research group is planning Phase II E7-pulsed DC-based vaccination trials in cervical cancer patients harboring limited tumor burden or at significant risk of tumor recurrence.

TLRs AND AUTOIMMUNE DISEASES

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Antiphospholipid Syndrome (APS) is an autoimmune disease characterized by antiphospholipid antibodies (aPL) and recurrent thrombotic events. In this study a human monoclonal aPL (HL5B) was used to investigate the stimulation and signaling of Toll-like Receptors (TLRs) in human peripheral blood mononcytes after activation by an aPL. TLRs are highly conserved receptors of the innate immune system specific for Pathogen Associated Molecular Patterns (PAMP's) and are crucial for early host defense. Activation of human peripheral blood monocytes by HL5B results in an upregulation of TLR8 mRNA expression level combined with an augmented secretion of tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL1-β) as well as their upregulation on mRNA level. This increase in cytokine secretion could be enhanced by adding ligands for TLR8, and was, in turn, subsequently neutralized by adding an inhibitory DNA oligonucleotide. Elevated amounts of TLR8 mRNA in APS patients suggests that a raised base level of procoagulant cytokines in patients with aPL lead to an increased risk of thrombosis in general and especially in case of infection.

INSIGHTS INTO THE MECHANISMS OF CROSS-PRESENTATION AND IMMUNE-REGULATION IN INFECTIOUS AND AUTOIMMUNE DISEASES

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Recently, we defined a new role of caspase-cleavage in the generation of fragmented antigens from apoptotic cells.

We demonstrated that this mechanism results instrumental to facilitate processing and cross-presentation of apoptotic antigens by Dendritic Cells (DCs). In addition, it is of interest the evidence indicating that cross-presentation of caspase-cleaved antigens contributes to establish the phenomenon of chronic immune activation, commonly observed during several viral and autoimmune diseases. In particular, we suggest that chronic immune activation is maintained by a continuous cross-presentation of apoptotic cells that are derived from chronically activated lymphocytes. The phagocytosis of apoptotic T cells by DCs leads to the generation of a huge number of apoptotic cell-derived epitopes that can be efficiently processed only from caspase-cleaved proteins. In a chronic inflammatory context, this activity also leads to the cross-priming of a large repertoire of apoptotic epitope-specific T cells, which in turn expand the inflammation and undergo apoptosis after they have performed their effector functions, and so on. Thus, the combination of cross-presentation of caspase-cleaved antigens and chronic immune activation can establish a milieu favouring the emergence and the maintenance of autoimmune responses, and ultimately contribute to the irreversible impairment of the immune system, such as in the case of HIV infection or in the final stages of several systemic autoimmune diseases.

Session 6

Critical Regulatory Issues for Promoting Biomedical Translational Research

Chairpersons

C. Pini, M. Bettoni

EMEA's ROLE AND ACTIVITIES FOR PROMOTING TRANSLATIONAL MEDICINE IN EUROPE

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Drug regulatory agencies have a pro-active role in facilitating access by patients to innovative drugs. On a general level this implies providing an enabling and predictable regulatory environment.

The European Medicines Agency (EMEA) has established a number of procedures and regulatory tools to support drug development in Europe, including translational medicine. This includes an increasing number of guidance documents, provision of scientific advice and protocol assistance for individual drug development programs, and a newly established scientific advice procedure for the qualification of biomarkers and other research tools or methodologies. On a more administrative level, EMEA has established a full-fledged support program for small and medium enterprises in the drug development field.

This presentation will provide a brief overview of these EMEA activities.

REGULATORY CHALLENGES IN FIRST IN MAN CLINICAL TRIAL WITH ADVANCED THERAPY

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Although the EU regulation created a centralized system for marketing authorization, one major barrier is the lack of a centralized system for clinical trials. Currently, industrial and academic sponsors have to approach each member state and adhere to their individual clinical trial requirements. This is particularly detrimental for Academic studies related to Advanced Therapy (AT) and represents a major bottleneck for translational research.

Huge efforts have been made by regulatory agencies by releasing guidance documents. Scientific guidelines are just one, albeit important, component of an increasingly complex legal/scientific environment in drug development. However, several obstacles remain. In the current European regulatory environment, a "global" guideline providing a reasoned framework for carefully assessing the potential risk of different innovative products, is still missing. Available EMEA guidelines stress the case-by-case approach that is reasonable for these products; however its inherent uncertainty leaves a degree of vagueness which, without further refinement may not provide the intended assistance to sponsors and national regulators in transitioning from non-clinical to early clinical development, particularly regarding their planning of how, when and where to conduct the First-in-Man (FIM) study.

For example, as review/approval of a Clinical Trial Application (CTA) is a national Competent Authority (CA) responsibility, it is not clear that the current EMEA guidelines provide sufficient detail to ensure a reasonable degree of harmony in the interpretation of individual cases. Will national guidelines be issued to clarify CA approaches? Would an European level survey or feedback mechanism of CA assessments be conceivable to review how the guideline is implemented in practice? The information for assessing an AT dossier requires iterative review and, with the implementation of 2001/20/CE and 2005/28/CE Directives, regulatory requirements provide formidable challenges for investigators. While biopharmaceutical companies employ contractors or staff to address and meet regulatory requirements, many academic institutions do not provide appropriate regulatory support. Academic research must learn to talk with regulatory body and the need of an Academic Regulatory Manager will be discussed in connection with advice on how to obtain more flexible access to scientific advice (national or EMEA), that would be a useful supplement to the guidelines.

In general, regulatory authorities are moving cautiously, seeking to ensure that they do not act prematurely in a fast-developing area of science. However, for Regulatory bodies assessing new product innovations some challenges are identifiable including: i) to establish advanced scientific basis assessment for making regulatory decisions concerning the clinical testing; ii) to develop a degree of oversight that retains appropriate safeguards and iii) to facilitate the necessary regulatory process by identifying critical issues early in product development in order to accelerat clinical trials and licensing of AT and medicinal products derived by translation research.

TOWARDS A EUROPEAN HARMONISATION OF THE REQUIREMENTS FOR FIRST CLINICAL USE OF ADVANCED THERAPY MEDICINAL PRODUCTS: PRECLINICAL REQUIREMENTS FOR CELL-BASED MEDICINAL PRODUCTS

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With the Regulation 1394/2007/EC, The European Community has recognized the necessity to set up a separated approach for the Advanced Therapy Medicinal Products (ATMPs). This category of medicinal products contains and define the products for Cell Therapy, Gene Therapy and Tissue Engineering. The Regulation has also introduced the legal concept of "Tissue engineered products" as medicinal products containing "engineered cells". The "engineered cells" legal definition is based on the extensive *in vitro* manipulation or the heterologous use. The introduction of the concept of heterologous use will help to recognize the use of cells as "pharmacological agents" but will also create a grey area with the transplant procedures. The transplant procedures authorization is delegated to the national competent authorities, making the development of a consensus not straightforward. At a minimum, a consensus by default will evolve through the exclusion from the transplant procedures of the "heterologous uses" recognized as Medicinal Products by EMEA.

According to the Regulation, the Commission received the task to revise the definition of advanced medicinal products contained in the Annex I of the Directive 2001/83 and 2003/63, which included cell therapy, gene therapy and xenogeneic medicinal products. The definition developed and under consultation, enlarge the use of the "engineered cell" definition also to the cell therapy product in an attempt to simplify the classification of the ATMPs.

One of the main reason to introduce the Regulation 1394/2007/EC was the profound differences between the ATMPs and small molecules or biotechnological medicinal products. The presence of cells or of gene therapy vectors in the ATMPs add a layer of complexity and of fuzziness to the pharmaceutical concepts of Identity and Potency making the definition of Identity, Potency and Dose quite challenging.

At the moment, there is no accepted paradigm on the preclinical characterization for the ATMPs. EMEA has just released the guideline EMEA/CHMP/410869/2006 coming into effect in September 2008 to address the Quality, Safety and Clinical issues linked to the development of Cell based Medicinal products.

This guideline also illustrate some of the possible approaches to define Identity and Potency in view of the Dose definition for the First in Man (FIM) application.

In particular, it has been introduced the possibility that in the absence of adequate biomarkers, the production process might be used to define at least partially the identity of the final product.

The use of Homologous Animal Models to circumvent the human-animal histocompatibility barrier has been proposed for the preclinical safety studies. For the

clinical studies, the EMEA guideline presents suggestion for novel definitions of Dose and the possibility that the definition of the administration procedures and concomitant treatments would be necessary for this purpose.

While this guideline is not legally binding for the national competent authorities involved in the authorization of the FIM studies with advanced therapy medicinal products, it represents a consensus between the national experts at EMEA and the industry and academic shareholders and a first step toward a harmonization of the preclinical studies in this field.

TOWARDS AN EUROPEAN HARMONISATION OF THE REQUIREMENTS FOR FIRST CLINICAL USE OF ADVANCED THERAPY MEDICINAL PRODUCTS: PRECLINICAL REQUIREMENTS FOR GENE THERAPY MEDICINAL PRODUCTS

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Efficient translation of research discoveries into industrial application is an essential element to maintain Europe's competitiveness in the biomedical and health industry. Advanced Therapy Medicinal Products (ATMP) represent a significant emerging field in which new treatment opportunities are offered to patients. However, bottlenecks possibly leading to delays of such innovative medicines development may arise from the current regulatory framework where advanced therapy medicinal products are registered and authorized for the market by the European Medicine Agency (EMEA) while as per Directive 2001/20/EC clinical trial approval is the responsibility of each Member State (MS).

Consequences are that in the case of multinational clinical trials, a separate authorisation procedure including a separate evaluation process will have to be carried out in each involved Member State. Particularly when aiming at treating an orphan disease, a multinational trial may be required to collect a sufficient number of patients.

Although not explicitly required by the Clinical Trial Directive, it seems highly desirable that a harmonised approach in the evaluation process be taken for European multinational trials and also for trials that are carried out in more than one MS. Since, at the end of clinical development, ATMP market authorisation application will be submitted through EMEA centralised procedure, a harmonised approach will facilitate clinical development processes in the EU and ensure uniform patients' access to treatment.

EMEA guideline on non clinical studies required before first clinical use of gene therapy medicinal products (CHMP/GTWP /125459/2006) has been developed with the aim at facilitating such a harmonised approach in the EU. It is addressed to scientists both at the production and at the regulatory side. It defines scientific principles and provides guidance to applicants developing gene therapy medicinal products, describing in detail the non-clinical studies required before first clinical use of a gene therapy medicinal product.

When applied to non-clinical data evaluation, it will allow a harmonised approach between Member States in the application of Directive 2001/20/EC. This would also set a positive example for future applications for marketing authorization, where a rigorous and aim-oriented approach would support the development and licensing of GTMPs.

THE SPONSORS' POINT OF VIEW ON THE CRITICAL REGULATORY ISSUES AT EUROPEAN LEVEL IMPACTING ON TRANSLATION FROM LABORATORY TO BEDSIDE

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The report from the EMEA/CHMP Think Tank Group on Innovative Drug Development dated March 2007 outlines some of the main technical and regulatory challenges faced by sponsors. It includes a section on "translational medicine" which stresses the importance of the identification and validation of predictive efficacy and safety biomarkers in future drug development. It also acknowledges that requirements may be irrelevant or redundant and that "modernisation of methods and procedures to develop and regulate medicinal products must be considered".

Several initiatives have been taken to try and address these needs *e.g.* organisation of workshops, pharmacogenomics briefing meetings, revision of Annex I to Directive 2001/83/EC as regards Advanced Therapy Medicinal Products; proposal in relation to an EMEA qualification process for biomarkers.

Principles contained in recently published general guidelines can also be usefully applied even though these documents (*e.g.* step 2 ICH M3(R2) or the July 2007 guideline concerning first-in-human clinical trials) were not specifically aimed at addressing translational medicines issues. However translation of basic research into real therapies will require that the legislation, the regulatory requirements and production practices accommodate an iterative research and development process and take into due consideration ethical principles and economic realities. The possibility of having ongoing personalised dialogue between sponsors and regulators will also be more than ever necessary.

HOW IMPLEMENTATION OF THE EUROPEAN CLINICAL TRIALS DIRECTIVE HAS IMPACTED ON THE APPROACH TO TRANSLATIONAL MEDICINE IN GERMANY

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The Paul-Ehrlich-Institut (www.pei.de) is responsible for vaccines and biologicals used in molecular medicine. Since August 1, 2004, when the European Clinical Trials Directive (ECTD; Directive 2001/20/EC) was transformed into German law, clinical trials of these medicinal products are authorised by the Paul-Ehrlich-Institut and receive a positive appraisal by the relevant ethics committee. In addition to the German Medicinal Product Act, a so-called GCP Ordinance regulates details of the authorisation procedure. A guidance document (3. Bekanntmachung zur klinischen Prüfung von Arzneimitteln) detailing requirements for a clinical trial application is available by internet (http://www.pei.de/cln_115/nn_160648/SharedDocs/bekanntmachungen/2006/banz-166-2-09-2006-6072-klin-pruef.html). English clinical trial applications are accepted. Where deliberate release needs to be authorised, *e.g.* when the medicianl product is or contains a Genetically Modified Organism, the deliberate release authorisation is included in the clinical trial authorisation by the Paul-Ehrlich-Institut.

Since 2004, the number of clinical trial applications received by the Paul-Ehrlich-Institut have steadily increased. In addition, applicants more frequently ask for scientific advice by the Paul-Ehrlich-Institut to improve their applications prior to the official start of the procedure. Academic groups in early product development consider it more difficult to fulfil the requirements necessary for first-in-man trials, but detailed proposals for system adaptations have not been made. Statistical data of clinical trial application review by the Paul-Ehrlich-Institut show that few applications failed and the legal timelines are met in almost all cases (http://www.pei.de/cln_115/nn_976132/DE/infos/pu/02-klinische-pruefung/ klin-pruef-statistik/klin-pruef-statistik-node.html?_nnn=true).

THE STATE OF THE ART OF REGULATORY ASPECTS OF THE USE OF ANIMALS IN BIOMEDICAL STUDIES: THE CASE OF NON-HUMAN PRIMATES

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Animals are kept in laboratories for scientific research and testing. Russell and Burch's three Rs (Replacement, Reduction and Refinement) are the basic principles underlying humane laboratory animal use and provide a systematic framework which underpins regulation and justification for animal use. Given that animals have the capacity to suffer, it is critical that the three Rs are applied such that the smallest number are used, their welfare is maximized, and the most reliable and valid scientific results are achieved from their use. The number of non-human primates used in biomedical research is less than a fraction of one percent of all animals, but non-human primates have special legislation regulating their use, given their close evolutionary history with humans. Although this similarity with humans makes them suitable models for certain types of research, the complexity of adequately providing for their social, behavioural and psychological needs in the laboratory, compounds their potential for suffering. In this presentation I shall argue for an improved evidence base to underpin changes to regulation of housing and husbandry standards, using the recently revised European guidelines on the accommodation and care of animals (Appendix A to ETS 123) to illustrate the issues. Further, I shall argue that improvements in the training of researchers and care staff, record keeping, review of research outcomes, and in information sharing will also promote good animal welfare and faciliate the most favourable advances in biomedical science.

Session 7

National and International Initiatives for Promoting Biomedical Translational and Clinical Research

Chairpersons

E. Garaci, G. Rasi

EATRIS: TOWARDS A EUROPEAN INFRASTRUCTURE FOR BIOMEDICAL TRANSLATIONAL RESEARCH

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Biomedical research needs infrastructure. The most important infrastructure consists of research driven hospitals in which preclinical and clinical research is fully integrated. Unfortunately many of the essential research infrastructure components necessary for such a "Under one roof" - concept are not available for European biomedical research. These include:

- state of the art animal facilities for preclinical proof of principle and proof of concept studies;
- small molecule screening facilities to identify and characterize new drug targets;
- high-resolution imaging facilities for preclinical and clinical validation;
- disease-specific patient and population cohorts to develop and validate new innovative diagnostic and therapeutic strategies;
- centralized GMP facilities for bioprocess development and manufacturing;
- facilities for Clinical Phase I studies.

In order to avoid unacceptable delays in the development of new innovative medicines a consortium of European research centers has been established with the mandate to develop a concept for European Advanced Translation Research Infra Structure (EATRIS). This will require the establishment of key preclinical and clinical components necessary to support the development of new diagnostic or therapeutic strategies at all stages of the biomedical R&D-process.

During the preparatory phase, EATRIS will work out a master plan describing in detail the establishment and mode of operation of the planned pan-European infrastructure during a later construction phase: This will include an agreement on the key legal, governance, strategic and financial issues as well as a concept to train and educate the next generation of biomedical translation researchers. Users of EATRIS will be biomedical researchers and clinical scientists located at universities, research institutions or SMEs that need to use this infrastructure in order to overcome specific bottlenecks and to move their research projects from a discovery to a preclinical and clinical stage.

ECRIN: TOWARDS A EUROPEAN INFRASTRUCTURE FOR CLINICAL RESEARCH

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Fragmentation of health and legislative systems in the European Union is an obstacle to multinational collaboration in clinical research. However efficient recruitment is a major challenge for clinical research, whereas quality of investigation, credibility of data, and compliance with regulation require professionalized support.

Networking of clinical research infrastructures leads to the development of common standards, of harmonised tools and practice, thus facilitating multicentre collaboration and fostering patient recruitment. Supported by the European Commission, the European Clinical Research Infrastructures Network (ECRIN) connects national networks to promote multinational collaboration within the European Union, taking advantage of the EU population.

ECRIN is designed to provide integrated, "one-stop shop" services to investigators and sponsors in multinational studies: patient recruitment and investigation, quality assurance, monitoring, ethics, regulatory affairs and adverse event reporting. Access to data centres and GMP manufacturing facilities for biotherapy products will also be provided. Users are investigators and sponsors in both the academic and industry sector. Services provided by ECRIN are particularly relevant for research on Rare Diseases and neglected diseases, for clinical trials in elderly and paediatric populations, for academic clinical research institutions, and for clinical trials steered by biotechnology SMEs.

BBMRI: THE PAN-EUROPEAN RESEARCH INFRASTRUCTURE FOR BIOBANKS AND BIOMOLECULAR RESOURCES

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Human biological samples, such as blood, tissues or DNA including associated medical data, as well as biomolecular research tools are a key resource in unravelling genetic and environmental factors causing diseases and influence their outcome. Furthermore these resources are required for biomarker discovery and development, identification of new targets for therapy, and may help to reduce attrition in drug discovery and development. The European roadmap for research infrastructures foresees a pan-European infrastructure to further develop these resources and to provide access to academia and industry. The planning of the construction of a pan-European Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) started in February 2008 and is funded with 5 mio € within the FP7 capacities program. BBMRI will build on existing sample collections, resources, technologies, and expertise, which will be specifically complemented with innovative components. In particular, BBMRI will comprise: i) all major population-based and disease-oriented biobank formats; ii) biomolecular resources, such as collections of antibodies and other affinity binders and a variety of molecular tools to decipher protein interactions and function; iii) bio-computing and sample storage infrastructure. All resources will be integrated into a pan-European distributed hub structure-like network, and will be properly embedded into European scientific, ethical, legal and societal frameworks. Specific tasks in the planning of BBMRI are the preparation of an inventory of existing resources, implementation of common standards and access rules, establishment of incentives for resource providers, and to develop solutions for international exchange of biological samples and data which properly consider the heterogeneity of pertinent national legislation and ethical principles. The planning consortium comprises 50 participants including several ministries and funding organizations from 21 European Member States and more than 180 associated organizations. A prototype of the infrastructure should be established by the end of 2010.

INFRAFRONTIER: THE EUROPEAN INFRASTRUCTURE FOR PHENOTYPING AND ARCHIVING OF MODEL MAMMALIAN GENOMES

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Mouse models are essential tools for the functional analysis of the mammalian genome and the molecular basis of human diseases. The European research community and international collaborative efforts will produce a large number of mouse disease models over the next years. The bottleneck for the exploitation of this valuable resource will be access to systematic functional and molecular characterisation. In addition, mouse models should be made available to the entire European mouse genetics, biomedical and translational research community which strongly depends on access to novel mouse disease models. The current resources to achieve this goal are limited. Existing facilities across Europe can only offer capacity for the systemic phenotype analysis, archiving and dissemination of a few hundred disease models per year.

To solve this problem, the Infrafrontier project, coordinated by Prof. Martin Hrabé de Angelis of the Helmholtz Zentrum München, will organise and establish an efficient distributed infrastructure for the systemic phenotyping, archiving and distribution of mouse models on a well-concerted, large-scale and pan-European level. Infrafrontier will organise two complementary and linked European infrastructure networks: Phenomefrontier for large scale and comprehensive phenotyping in a cross-laboratory effort (European mouse clinics); Archivefrontier for archiving and distribution of mouse mutant lines (organised by EMMA, the European Mouse Mutant Archive). Taken together, INFRAFRONTIER will bring the systemic phenotyping, archiving, and dissemination of mouse disease models to the next level and will contribute to maintaining Europe's leading role in the functional annotation of the mouse genome.

The Infrafrontier project unites 15 scientific partners and 12 ministries and funding agencies from 10 different European countries. It is included in the ESFRI (European Strategy Forum for Research Infrastructures) roadmap and receives Preparatory Phase funding from the European Framework Program 7 (Capacities).

THE INNOVATIVE MEDICINE INITIATIVE (IMI) AND CHALLENGES IN TRANSLATIONAL RESEARCH

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The Innovative Medicines Initiative is the most ambitious public private partnership ever undertaken in the life science sector. It brings together the European research-based pharmaceutical industry and the academic/public/SME sectors in a unique partnership designed to solve the major underlying problems besetting the discovery and development of new medicines. It focuses on stimulating the European life-science sector, both public and private, to collaborate in developing tools and technologies to improve the safety and efficacy of medicines through programmes of pre-competitive research. The huge advances in biology of the last decade or so have not (yet) led to an outpouring of revolutionary new treatments despite enormous investment. Nevertheless, the industry still believes that its future lies in science and technology as documented in the FDA's report of 2004 (Challenge and Opportunity on the Critical Path to New Medical Products) as well as the Strategic Research Agenda of the Innovative Medicines Initiative. The particular focus on translational medicine and the emphasis on predictive biomarkers of safety, efficacy, disease progression and treatment responsiveness are essential to reduce attrition and to produce better medicines more cost effectively. Success though will neither be trivial nor guaranteed. Better understanding of disease mechanisms will be needed to validate new tools and to move from treatment to prevention of disease. To this end, industry collaboration and productive external partnerships will be necessary as the pharmaceutical industry relies increasingly on the academic/biotech sector to provide it with the new ideas and early stage molecules it will develop into drugs. However, the promise of new scientific tools will not be realised if they are restricted to in-house drug discovery and if the development and approval path follows the conventional process that has been used for decades. It is essential that the new tools become part of the development process and that the regulatory agencies are open to greater acceptance of surrogates of efficacy, less formulaic clinical trial paradigms and better approaches to pharmacovigilance and benefit/risk analysis.

TRANSFORMING TRANSLATIONAL CANCER RESEARCH: THE ROLE OF THE NATIONAL CANCER INSTITUTE

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The National Cancer Institute (NCI) charged a Translational Research Working Group (TRWG) to evaluate the status of the NCI's investment in translational research and envision its future. The product of the TRWG's efforts includes a 150-page report detailing 15 specific recommendations for accelerating translational cancer research (www.cancer.gov/trwg). These recommendations, which include initiatives for co-ordinated management, tailored funding mechanisms, and enhanced operational effectiveness, are currently in the implementation phase.

Key to the vision of transforming translation is the TRWG's recognition that translational research requires a managed approach to product development, in contrast to the scientific discovery process that thrives on creativity and individualism. With this in mind, the TRWG generated a series of 6 Pathways to Clinical Goals that depict the steps involved in moving a basic science discovery to early phase clinical trials using an engineering flow diagram format. The 6 pathways focus on the generation of assessment tools, using either a biospecimen-based or image-based approach, and in the generation of four kinds of clinical interventions; agents (drugs and biologics), immune response modifiers, interventive devices, and lifestyle alterations. The TRWG pathways provide an operational definition of translational cancer research and a framework for the assembly of multidisciplinary teams to achieve the multiple steps required to move a credentialed discovery to the stage of clinical testing.

The TRWG recommended that a prioritization process be created to identify translational concepts that are ripe for development, and a new funding mechanism to provide the resources to carry prioritized concepts through a TRWG pathway. To initiate this process, the NCI is sponsoring the NCI Translational Science meeting to be held in the fall of 2008. The goal of the NCI Translational Science Meeting is to identify NCI-supported translational research projects that can be assembled to complete the steps of a TRWG pathway. This prioritized approach to translational research, in combination with the robust base of basic and clinical support and infrastructure provided by the NCI, is designed to accelerate the translational process for the benefit of cancer patients and the public.

COORDINATING CANCER RESEARCH IN EUROPE: THE ROLE OF MEMBER STATES

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Cancer is a major health challenge both worldwide and in Europe, where it is responsible for 25% of all deaths. Despite the excellence of biomedical research and the significant financial support provided to cancer research in Europe, more concerted efforts are required to translate research outcomes into clinical applications. From 2005 to 2007 the European Commission funded a Consortium of scientists, Cancer Research Centres and Funding Organizations (EUROCAN+PLUS), aimed to identify both the reasons for the European lack of competitiveness in translational research and the requirements for better coordination in cancer research within Europe, and to provide suggestions to meet these needs. There were two main recommendations from EUROCAN+PLUS: the establishment of a European platform for networking among funding bodies on translational cancer research and better cooperation between cancer research centres. The latter may involve basic research laboratories as well as clinical research centres. In the context of a EUROCAN+PLUS Work Package involving National Funding Organizations, consensus was reached on the need to launch an ERA-NET on Translational Cancer Research with the purpose of better coordinating within Europe those research activities, including health care research and prevention, aiming at the benefit of the patient and of the general population by taking findings from basic research to clinical research and also using feedback from clinical research to laboratory research to redefine research questions. Continuous education programmes on translational research could be part of this coordination. On the basis of this undertaking, representatives of Funding Organizations from France, Germany, Italy, Spain and the United Kingdom moved ahead by launching a debate with other Funding Bodies. It was decided to call for a meeting gathering a broad group of Funding Organizations from European Member States and Associated Countries to explore their possible engagement in future actions for the establishment of an ERA-NET on Translational Cancer Research as expected in a future FP7 call. The Health Directorate of the DG Research of the EC as well as the FP7 National Delegates to the Health Programme have been informed of this initiative. The meeting will be held at the Istituto Superiore di Sanità on October 1st, 2008 in conjunction with the international conference "Needs and Challenges in Translational Medicine: filling the gap between basic research and clinical applications". The main outcomes of the October 1st meeting will be presented.

COORDINATING TRANSLATIONAL CANCER RESEARCH IN EUROPE: THE ROLE OF THE OECI

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Modern biology offers new possibilities to improve cancer care and prevention. Clinical research is moving towards more complex multidisciplinary treatments.

Integration of research and cancer care is an increasing need. An important goal is personalized cancer medicine. By molecular pathology new subgroups of patients with unique sensitivities to treatments are identified. Availability of patients and biological materials together with more sophisticated research infrastructures make critical mass for a complete cancer research to an increasing problem. Single cancer centres will be unable to conduct an optimal research aiming at therapy development and personalized cancer medicine.

Analyses of European cancer research has pointed out fragmentation as the most important problem. The research process is fragmented due to insufficient communication between basic/preclinical and clinical research. Further, the lack of critical mass in single cancer centers is another important factor. OECI (Organisation of European Cancer Institutes), built on cancer centers/institutes as members, implements the concept of comprehensive cancer center in Europe. Comprehensiveness guarantees the integration of cancer care, prevention, research and education. If centers share organisation, create multidisciplinary cancer care and harmonize structures for translational cancer research, collaboration between centers will be facilitated and the problem of critical mass will find a solution.

A similar outcome was obtained from the project Eurocan, which critically analysed the European cancer research. One important proposal from the project is the formation of a European platform for translational cancer research linking the most research oriented comprehensive cancer centers and basic/preclinical cancer research centers. This will solve main problems behind fragmentation. A group of representatives of comprehensive cancer centers and basic/preclinical cancer research centers, mainly OECI members, has formed a working group, the Stockholm Group, to propose a worldclass infrastructure for translational cancer research. With such a structure the total cancer research process will be covered, critical mass for most types av cancer research will be reached and coordination of research as well as exchange of cancer reseachers within Europe will be facilitated. OECI:s accreditation/designation methodology will be used for quality assurance and harmonization of infrastructures of the participating centers.

THE ITALIAN INITIATIVE FOR BIOMEDICAL TRANSLATIONAL RESEARCH

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Recently, several initiatives have been launched to promote biomedical translational research. In fact, while competition is a driving force in the discovery process, an integrated and collaborative approach is essential for the success of translational research. In particular, well-designed infrastructures where all the relevant actors are engaged for breaking barriers and favouring bidirectional communication between basic research and clinical applications are urgently needed. The Italian National Institute of Health (ISS) has been appointed by the governmental partners as coordinator of the national initiative on translational research in the context of the EATRIS project. Italy is actively participating in the EATRIS preparatory phase playing a leading role in activities aimed at the preparation of documents/rules/initiatives regarding regulatory and ethical issues relevant for the construction of a pan-European research infrastructure for translational medicine. Moreover, Italy is contributing to the overall EATRIS activity by ensuring participation of experts belonging to different institutions, networks and consortia, including the Italian National Research Council, "Alleanza Contro il Cancro", IMINET (Italian Molecular Imaging Network) and the Mario Negri Institute for Pharmacological Research. Of note, in the context of the ECRIN project, the ISS is responsible for the joint ECRIN-EATRIS activities aimed at identifying the existing resources and needs of GMP facilities for biotherapy in Europe. In view of all this, the ISS has recently proposed the activation of the Italian Center of Translational Research in Biomedicine (Bio-CIRT), whose specific mission includes: i) ensuring a concerted Italian participation to all the European infrastructures relevant for Translational Medicine by coordination initiatives at both national and international levels; ii) providing services to the scientific community for the preclinical and clinical development, under the current GLP, GMP and GCP rules, of "tissue engineered products", which represent a new research frontier, where relevant regulatory issues still need to be defined; iii) providing information, education and training to scientists and clinicians by initiatives such "help-desk" and courses specifically focused on critical regulatory issues for translational medicine; iv) promoting research on selected preclinical and clinical aspects of novel biotherapies particularly relevant to face the emerging challenges of translational medicine in this field.

Poster Session

P 1 COMBINATION OF IMMUNOTHERAPY WITH CHEMOTHERAPY TO REDIRECT THE ANTITUMOR IMMUNE RESPONSE: FROM PRECLINICAL STUDIES TO PATIENTS

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Although immunotherapy has been considered an attractive antitumor strategy, its relationship with chemotherapy has been poorly investigated. Our previous experience in animal model studies demonstrated that non-myeloablative treatment, as well as irradiation, can modulate the immune response and enhance the antitumor activity of adoptively transferred tumor-immune lymphocytes through the induction of a well-defined pattern of cytokines ("cytokine storm") occurring during the rebound phase after drug-induced myelodepletion. Further studies in animal models demonstrated that chemotherapy strongly enhances the immune response to antitumor vaccines and renders therapeutic vaccination of established tumors extremely effective. On this basis, we have designed a pilot clinical study for evaluating the effects of the alkylating agent dacarbazine (DTIC) on immune responses to antitumor vaccination in desease-free melanoma HLA-$A2^+$ patients who received vaccination (Melan-A A27L and gp100 210M) one day after chemoterapy. Primary and secondary end-points of the study were the assessment of antigen-specific $CD8^+$ T cell responses and clinical responses, respectively. IFN-g ELISPOT assay and tetramer analysis were used to monitor immune responses before and at different times during vaccination, in either *ex vivo* or *in vitro* expanded $CD8^+$ T cells. A strong *ex vivo* expansion of peptide-specific $CD8^+$ T cells was observed only in patients treated with dacarbazine plus vaccine (4/5 patients). Moreover, *in vitro* stimulation of $CD8^+$ T cells demonstrated that patients receiving the combined treatment developed a peptide-specific immune response and ability of specifically lysing tumor cells consistently higher than the group treated with vaccine alone. Among the five patients treated with vaccine alone, four went into progression. In contrast, 3 out of 5 patients treated with vaccine plus darcabazine are still disease free 4 years after treatment. These results suggest that the efficacy of cancer immunotherapy can be synergistically enhanced by combining cancer vaccines with alkylating antineoplastic agents. Further clinical trials have been designed to prove the efficacy of this combination treatment in a relevant number of cancer patients.

P 2 MOLECULAR IMAGING AND FUNCTIONAL GENOMICS APPROACHES ON CHOLINE METABOLISM IN OVARIAN CANCER

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Epithelial Ovarian Cancer (EOC) is the most lethal gynecological malignancy in industrialized countries. Noninvasive *in vivo* imaging approaches with high sensitivity and specificity would improve early diagnosis and timely assessment of response to therapy of recurrent EOC. By detecting abnormal choline phospholipid metabolism in tumors, Magnetic Resonance Spectroscopy (MRS) fosters investigations to identify novel genetically-driven biomarkers of tumor progression and endpoints of therapy response.

Aims of the study were to:

- investigate gene expression and activity of enzymes of EOC choline metabolism;
- evaluate the effectiveness of 1H MRS in EOC diagnosis in patients;
- identify novel targets of EOC therapy at pre-clinical and clinical level. To this end we used MRS, micro-array-based genomics, immunofluorescence microscopy on cells and biopsies and *in vivo* MRS on animal models and patients.

We showed that Phosphocholine (PCho) accumulation in EOC cells is mainly due to upregulation/activation of choline kinase α and increase in PC-plc activity; PC-plc accumulates on the outer plasma membrane of EOC cells, colocalizing with β1 integrin in nonraft domains; PC-plc inhibition results in decreased cell responsiveness to mitogens, suggesting a role of this phospholipase as target for anticancer therapy.

Pilot 3D chemical shift imaging examinations on 9 patients with unilateral or bilateral ovarian masses showed that the total choline peak (tCho, 3.2 ppm) represented a tumor biomarker with a sensitivity 93% (14/15 masses); specificity 88% (15/17); positive predictive value 88% (14/16); negative predictive value 94% (15/16) and overall accuracy 91% (29/32). Emerging knowledge on selective genomic/biochemical regulation of PC-cycle enzymes may open new ways to targeted anticancer therapies in EOC. Preliminary clinical examinations suggest that 1H MRS can be added to imaging of ovarian masses, to allow *in vivo* metabolic evaluation of these tumors.

P 3 SOMATICALLY ACQUIRED JAK1 MUTATIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Aberrant signal transduction contributes substantially to leukemogenesis. The Janus Kinase 1 (JAK1) gene encodes a cytoplasmic tyrosine kinase that noncovalently associates with a variety of cytokine receptors and plays a nonredundant role in lymphoid cell precursor proliferation, survival and differentiation. Here, we report that somatic mutations in JAK1 occur in individuals with Acute Lymphoblastic Leukemia (ALL). JAK1 mutations were more prevalent among adult subjects with T-cell precursor ALL, where they accounted for 18% of cases, and were associated with advanced age at diagnosis, poor response to therapy and overall prognosis. All mutations were missense, some predicted to destabilize interdomain interactions controlling the activity of the kinase. Three mutations that were studied promoted JAK1 gain of function, and conferred interleukin 3-independent growth in Ba/F3 cells and/or interleukin 9-independent resistance to dexamethasone-induced apoptosis in T cell lymphoma BW5147 cells. Such effects were associated with variably enhanced activation of multiple downstream signaling pathways. Leukemic cells with mutated JAK1 alleles shared a gene expression signature characterized by transcriptional upregulation of genes positively controlled by JAK signaling. Our findings implicate dysregulated JAK1 function in ALL, particularly of T-cell origin, and point to this kinase as a target for the development of novel anti-leukemic drugs.

P 4 INVOLVEMENT OF DENDRITIC CELL SUBSETS DURING CYCLOPHOSPAMIDE-INDUCED IMMUNE ACTIVATION: IMPLICATIONS FOR THE DESIGN OF COMBINATION THERAPY PROTOCOLS AGAINST CANCER

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The combination of chemotherapy with anticancer vaccination or immunotherapy has been considered to be inappropriate for years based on the belief that the efficacy of cancer vaccines would be inhibited by the immunosuppressive effects of chemotherapy. Recently, new knowledge has been generated on the immunomodulatory properties of some chemotherapeutic agents, such as Cyclophosphamide (CTX), leading to a renewed interest in combination therapy regimens for cancer.

In previous studies we observed that CTX can exert, on one hand, a direct effect on the tumor mass leading to the control of tumor growth and, on the other hand, an immunostimulatory activity through the homeostatic expansion of T and B lymphocyte pools and the modulation of the expression of various soluble factors (cytokine storm). Since the induction of an effective antitumor response requires the active participation of host APCs, responsible for adequate antigen presentation and lymphocyte priming, we investigated the effects of CTX treatment on Dendritic Cells (DCs) *in vivo*. We show that in mice implanted with EG7.OVA thymoma, CTX treatment induced a transient reduction of total bone marrow cells, but not of DC precursors, which, instead, proliferate displaying enhanced DC generation capabilities *in vitro*. Accordingly, in secondary lymphoid organs, conventional CD8α+ DCs, the key DC subset specialized in the cross-presentation of cell-associated antigens, undergo a transient and selective depletion followed by a rebound phase, in a way similar to what previously observed for lymphocytes. In addition, plasmacytoid DCs, the main type I IFN producers, progressively accumulate in the spleen and in the lymph nodes of CTX-treated tumor-bearing mice. Interestingly, the percentages of myeloid–derived suppressor cells dramatically decrease in tumor-bearing mice, early after CTX administration, and remain at low levels for up to 10 days after treatment.

Current studies are aimed at clarifying the function and the role of the different DC subsets during chemotherapy, with the final aim of optimizing combination therapy protocols against malignancies.

P 5 MICROARRAY ANALYSIS TO STUDY THE IMMUNOMODULATORY ACTIVITY OF ANTICANCER DRUGS: IMPLICATIONS FOR CHEMO-IMMUNOTHERAPY STRATEGY DESIGN

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Several studies have shown that, in order to induce a consistent antitumor response, immunotherapy needs to be combined with chemotherapy. Presently, Cyclophosphamide (CTX) represents the gold standard immunomodulatory chemotherapeutic agent. Although the antitumor efficacy of its combination with immunotherapeutic strategies has long been studied in preclinical models as well as in clinical trials, some of the mechanisms underlying its ability to potentiate the immune response still need to be clarified. An high throughput technology, such as microarray, can represent an invaluable tool to gain insights into the molecular mechanisms associated with the efficacy of antitumor combined therapies.

In a previous study we proposed that the immunomodulatory properties of CTX depend on the induction of a "cytokine storm", occurring primarily in the bone marrow of treated mice. To deepen our knowledge of this phenomenon, we analyzed the global gene expression profiles of bone marrow cells at different times after CTX treatment by microarray analysis. This analysis showed that CTX profoundly affects gene expression in bone marrow cells at early time points (already 1 day after treatment), inducing, on one side, the up-regulation of several genes involved in cell differentiation, cell migration and regulation of the immune response, and, on the other hand, the decrease of biological functions, such as biosynthetic and metabolic processes, ribosome assembly and cell cycle.

In the attempt to enlarge the panel of drugs that can be successfully combined with immunotherapy, we compared the ability of different anticancer agents (CTX, Dacabazine, Docetaxel, Paclitaxel, Doxorubicin and Cisplatin) to synergize with adoptive immunotherapy in the rejection of established tumors. The combination of immunotherapy with alkylating agents, such as CTX or Dacarbazine, resulted in complete tumor regression respectively in 100% and 40% of mice. Conversely, the other chemotherapeutic agents were devoid of any immunopotentiating effect in this experimental setting. We are currently analyzing the gene expression profiles induced by the other chemotherapeutic drugs (either efficacious in combination with immunotherapy or not) with the purpose of identifying the mechanisms associated to the ability of certain drugs to synergize with immunotherapy and with the final intent of defining novel strategies for cancer treatment.

P 6 NOVEL SERUM PROTEOMIC ANALYSIS IN A METASTATIC MELANOMA MURINE MODEL AND IN CUTANEOUS MELANOMA PATIENTS FOR NEW BIOMARKER DISCOVERY

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Electrophoretic analysis of the serum-proteome represents a powerful tool for biomarker discovery and early diagnosis of cancer and identification of new pathogenetic mechanisms. 2D- electrophoresis, although able to discriminate large number of proteins, does not allow the easy evaluation of a large number of patients and could not be suitable when a difference between the proteoma from healthy and cancer sera is due to a faint qualitative or structural protein modification. Therefore we developed and tested a novel electrophoretic method, coupled to mass spectrometry, with sera from a murine metastatic melanoma model and human sera from cutaneous melanoma patients.

Sera from C57BL/6 mice injected with B16F10 cells or with saline solution as control (7 mice per group), were subjected to different denaturation pre-treatments identified during the optimization methodological step, then electrophoresed in SDS-PAGE 16x18 cm gradient gels followed by silver staining and densitometric analysis. The results were compared with those obtained from 15 melanoma and 15 healthy subjects. Protein bands were identified by MALDI-TOF MS. Student's two tails t-test was performed: $p \leq 0.05$ was considered statistically significant.

A methodological study to optimize SDS-PAGE of serum proteins was carried out, testing more than 20 pre-treatments under different denaturing conditions. The three protocols showing the highest efficiency were selected and more than 110 protein bands were discriminated, with <5 ng/band sensitivity. The differentially detectable bands in murine melanoma vs controls sera and in human neoplastic vs healthy sera were identified. Six protein bands, with Mr ranging between 15-150 KDa, were found reproducibly (>80%) de-regulated in cancer vs control sera. The identified peptides include: members of apolipoproteins, complement factors, cytoskeleton and protease inhibitors. A preliminary validation study was carried out using immunological techniques and an *in vitro* invasion assay with human melanoma cells. Both approaches confirmed the proteomics findings, showing a significant down-regulation of a member of proteases inhibitor family in sera from melanoma patients, which therefore could be related to the highly invasive behaviors of cutaneous melanoma. Further studies are ongoing to confirm whether the identified proteins may represent novel biomarkers for early detection of human melanoma.

P 7 BIOLOGICALLY TARGETED THERAPIES IN THYMIC EPITHELIAL TUMORS (TET): PRESENT STATUS AND DEVELOPMENT OF A CLINICAL AND MOLECULAR STUDY

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Thymic Epithelial Tumors (TET) represent a unique model among epithelial cancers, as a variety of immune dysfunctions/autoimmune diseases are related to the thymic epithelial cell proliferation. Although a wide proportion of TET exhibit a benign behaviour, advanced chemorefractory and metastatic TET represent a challenge in clinical oncology. The rarity of TET add difficulties in setting clinical trials and in developing new therapeutical strategies. Over the last years, our multicenter cooperative group promoted clinical and tissue-based studies to address critical issues in the diagnosis and management of TET. The Octreotide-responsiveness has offered a significant therapeutical approach to the treatment of relapsing TET. Recently, among ERBB Tyrosine Kinase (TK) growth factor receptors, the Epidermal Growth Factor Receptor (EGFR) has been shown to be often overexpressed in TET. EGFR overexpression correlated with different grades of malignancy and stages of the disease. We and others have reported the efficacy of Cetuximab, an anti-EGFR mAb, in cases of metastatic and chemorefractory TET. Erlotinib, an EGFR-TK Inhibitor (TKI), was also reported to induce a response. Imatinib, a tyrosine-kinase inhibitor of c-KIT and PDGFRα/β, was shown to induce a partial response in a case of c-KIT expressing thymic carcinoma harboring an activating kit mutation. Recent literature data confirm that somatic mutations of EGFR and KIT, although rare, occur in TET. Interestingly, Imatinib mesylate

shows indirect antitumoral effects, stimulating Natural Killer (NK) cells and thus promoting an immune response to tumor cells. Therefore, a Clinical Phase II trial, approved by AIFA among Clinical studies for Rare Diseases, has been opened to test the efficacy, evaluated by tumor response and immunological parameters, of Imatinib mesylate as second line treatment of Thymic carcinoma. Relapsing and chemorefractory Thymoma and Thymic Carcinoma cases have been evaluated for the tissue expression, by immunohistochemical methods, of EGFR and c-KIT (CD117). Moreover, a molecular study (mutational analysis by direct sequencing) has been very recently developed to evaluate the c-KIT, EGFR and KRAS genes in TET. The purpose of the study is to evaluate the efficacy of targeted therapies, in association with immunological response-predictive parameters, and to correlate the clinical data to TET genomic status for relevant oncogenes.

P 8 THE miR-15A/miR-16-1 CLUSTER CONTROLS PROSTATE CANCER PROGRESSION BY TARGETING MULTIPLE ONCOGENIC ACTIVITIES

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Prostate cancer is the second leading cause of death from cancer among men. Despite its high incidence, the molecular and genetic events involved in prostate cancer development and progression remain poorly understood.

MicroRNAs (miRNAs) are non-coding, single-stranded RNAs of ~ 22 nucleotides that constitute a novel class of gene regulators likely involved in a wide range of cellular functions. The first indication that miRNAs can have a role in tumorigenesis came from a recent study showing an association between B-Cell Chronic Leukaemia (B-CLL) and the deletion or downregulation of two clustered miRNA genes, miR-15 and miR-16, located at 13q14 and targeting Bcl-2 mRNA. Deletion within this locus has been found in different tumor types, including prostate cancer, in which it correlates with tumor progression and metastases.

Here we report that 80% of the 35 tumors analyzed lost the miR-15a/miR-16-1 cluster, particularly in advanced stages. We found that Cyclin D1 and Wnt3a are new targets of miR-15a and miR-16, and together with Bcl2 are inversely correlated with the miR cluster expression in prostate cancer cells. Knock-down of miR-15a/miR-16 increases the expression of Bcl-2, cyclin D1 and Wnt3a, promoting survival, proliferation and invasiveness of untransformed prostate cells, which became tumorigenic in immunodeficient NOD/SCID mice. More importantly, *in vivo* delivery of antagomir for miR-15a/miR-16 results in prostate hyperplasia with disruption of glandular acini associated with cyclin D1 and Wnt3a upregulation, indicating that loss of miR-15a/miR-16 could be a relevant pathogenic event even in the early phases of tumorigenic process.

Conversely, reconstitution of miR-15a/miR-16-1 expression in prostate cancer cells resulted in growth arrest, apoptosis and dramatic regression of prostate tumor xenografts. We propose that miR-15a and miR-16 act as tumor suppressor genes in advanced prostate cancer through the control of cell survival, proliferation and invasiveness. These findings have considerable therapeutic implications and may be exploited in the future for novel treatments of prostate cancer.

P 9 F_2-ISOPROSTANE IN UMBILICAL CORD PLASMA AS BIOMARKER OF OXIDANT INJURY IN HIGH RISK NEWBORNS

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The development of early clinical indices that could predict the neurological outcome in high risk newborns, including pre-term births and multiple gestations, remains a major issue in child neurology. The availability of reliable biomarkers easily measurable in newborns is instrumental to adopt interventions during the narrow period of time in which neuroprotective strategies could be effective. Inflammation and oxidative stress are probably the major mechanisms for brain lesion and poor neurodevelopmental outcome. The high content of lipids renders lipid peroxidation the key feature of oxidant damage in the developing brain tissue. Among lipid peroxidation products, F_2-Isoprostanes (F_2-IsoPs) are currently regarded as the most accurate method to assess *in vivo* oxidant stress status. Their evaluation has been extensively used to monitor the occurrence of oxidative stress in adult neurological diseases and, more recently, infant brain diseases. Increased F_2-IsoP levels were reported in CSF of premature infants with periventricular leukomalacia. In amniotic fluid the levels of 15-F_{2t} -IsoP, a major F_2-IsoPs formed *in vivo*, were predictive of foetal growth restriction. Furthermore, using an experimental model of perinatal asphyxia, we found that 15-F_{2t}-IsoP brain levels were predictive of delayed behavioural alterations. Recently, we have optimized an analytical method to measure 15-F_{2t}-IsoP in small samples (less than 0.2 ml) of cord plasma and shown that it is a sensitive biomarker of fetal oxidative stress during labor. Since prematurity and growth abnormalities are common risk factors contributing to higher perinatal morbidity and mortality in multiple than singleton births, in the present study we evaluated the 15-F_{2t}-IsoP cord plasma levels in 38 twins and 12 triplets. We found that 15-F_{2t}-IsoP levels are higher in: 1) preterm newborns (less than 31 Gestational Weeks (GW) than in babies born at 32-36 GW; 2) very low birth weight (less than 1500 gr) than in low birth weight newborns (1500-2500); 3) lighter weight twin of pairs with moderate to severe weight discordance (more then 25%) at birth. Although observation on larger groups of subjects are needed, these results suggest that 15-F_{2t}-IsoP levels in cord plasma may represent an useful indicator of fetal distress in pregnancies complicated by multiple gestations.

P 10 THE 3Rs MODEL AND THE USE OF NON-HUMAN PRIMATES IN PARKINSON'S DISEASE STUDIES

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The aim of this paper is to discuss the use of non-human primates in the study of Parkinson's Disease (PD), looking at both the scientific and the ethical implications that such use implies. We will refer, as theoretical framework, to the model of the "3Rs" originally proposed by Russell and Burch in 1959. The experimental work conducted on non-human primates to study PD, is a powerful and appropriate case-study to inspire fruitful discussion on the use of animals as models in biomedical research. PD is a widespread and very serious illness. Therefore, Parkinson's Disease is an important pathological condition which affects a large part of the population, for which to understand its causes and to find a possible cure, is very desirable. However, important information related to this aim come from invasive experiments performed on a particular animal, that is, the non-human primate, whose use is reason for great ethical concern. Rodents are, of course, very useful models for the study of PD, but non-human primates can help us to understand mechanisms, which rodents cannot. Therefore, this scenario poses the conditions for a potentially interesting and stimulating cost-benefit analysis on the issue of animal experimentation. It is not the aim of this paper to give answers to very complicated questions, but rather to underline some critical points worth of a deeper discussion, also in the light of the more general issue of the relationship between humans and non-humans in research laboratories.

P 11 MASS SPECTROMETRY DETERMINATION OF THE QUANTITATIVE RATIO OF PRP^{TSE} ALLOTYPES IN INDIVIDUALS WITH GENETIC TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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Transmissible Spongiform Encephalopathies (TSEs), or prion diseases, are neurodegenerative disorders affecting both humans and animals and are characterized by the accumulation, in the Central Nervous System (CNS), of fibrillar structures mainly composed of the pathological isoform (PrP^{TSE}) of a host encoded protein, the cellular prion protein (PrP^{C}). Whereas it is certain that PrP^{TSE} formation has an essential role in the development of prion diseases, a number of important aspects of these disorders, like the factors driving PrP^{C} to PrP^{TSE} conversion, remain poorly defined. In particular, since 10-15% of human TSEs occur in people with mutations of the PrP gene, there is a great interest in elucidating the role played by mutated residues in the pathological conversion.

The aim of this work was to gain information about the relative propensity of mutant and wild type PrP to acquire the pathological conformation by determining the relative amounts of PrP^{TSE} allotypes in the brains of patients affected by different forms of genetic Creutzfeldt-Jakob Disease (gCJD).

We applied a quantitative LC/MS (Liquid Chromatography/Mass Spectrometry) protocol, based on the use of appropriate synthetic deuterated (internal standards) and non-deuterated (calibrants) peptides, to perform quantitative analysis of PrP^{TSE} allotypes recovered in extracts purified from brains of patients with gCJD associated with R208H and V210I mutations.

Our analyses showed that the amount of mutant allotype is 1.5-3 folds higher than that of wild type allotype suggesting that, in the genetic TSEs under examination, the mutation is likely involved at a very early stage of the process of production of PrP^{TSE} aggregates, such as the formation of the first nuclei of polymerization. Further studies are in course to better elucidate the amyloidogenic behaviour of PrP allotypes bearing other TSE-associated mutations: if the mutant allotype will be proven to have an essential role to start the pathological cascade, a genetically-mediated therapy might be planned to modulate, or even turn off, the expression of the corresponding mutant, disease-triggering PrP allotype in carrier individuals.

P 12 NON-INVASIVE NEAR INFRARED OPTICAL TOPOGRAPHY: A NOVEL STRATEGY FOR TESTING AND MONITORING CORTICAL ACTIVATION/OXYGENATION IN TRANSLATIONAL NEUROSCIENCE

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Clinical imaging is incorporating methods that are able to define brain function. Functional Near-Infrared Spectroscopy (fNIRS) is a not harmful, non-invasive and safe optical technique allowing the simultaneous acquisition of oxygenated and deoxygenated hemoglobin concentration changes (Δ[O2Hb] and Δ[HHb], respectively) from an array of optical fibers on the scalp to construct maps of cortical activity. Based on the tight coupling of neuronal activity and oxygen delivery, Δ[O2Hb] and Δ[HHb] as measured by fNIRS are quantified and taken as indicators of cortical activation. The hemodynamic response typically observed over an activated cortical area consists of a decrease in [HHb] accompanied by an increase in [O2Hb] of two or threefold of magnitude, resulting in an increase in the total hemoglobin concentration ([tHb]=[O2Hb]+[HHb]). This hemodynamic pattern is representative of a localized increase in regional blood flow (rCBF). Concentration changes in [HHb], the paramagnetic form of hemoglobin, are assumed to be the basis of functional magnetic resonance imaging (fMRI). fNIRS reconstructs two dimensional images of cortical [O2Hb] and [HHb]. The resolution of the images is in the order of one cm. The generated spatial maps of the hemoglobin concentration changes correspond to specific regions of the cerebral cortex. Several fNIRS instrumentations/imagers are commercially available and utilized in cognitive neuroscience and clinical medicine. As an example, changes in prefrontal cortex oxygenation (measured by an 8-channel fNIRS system, Hamamatsu Photonics, Japan) during motor performance from non-fatigued to moderately fatigued to severely fatigued conditions in healthy volunteers will be reported and discussed.

The most recently available NIRS technology for monitoring cerebral oxygenation (oximetry) can contribute to the identification of deficits in cerebral oxygenation. Monitoring such deficits supports certain forms of therapy in reversing cerebral oxygenation issues and thereby preventing long-term neurological sequelae. It has been demonstrated that quantitative thresholds for cerebral oxygenation led to the identification of cerebral ischemia in the adult brain and thus increased the scope of clinical use of NIRS.

In conclusion, although fNIRS is still in the earliest stages of translation from research laboratories to clinical applications, NIRS is already having some impact in specific clinical applications (*i.e.* brain oxygenation monitoring in cardiothoracic surgery and neonatal intensive care).

P 13 BLOOD TITERS OF AUTO-ANTIBODIES TO DAT AS BIOMARKERS FOR THE RISK OF ALCOHOLISM: THE CASE OF POSITIVE FAMILIARITY

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Possible interactions between nervous and immune systems in neuro-psychiatric disease and drug dependence remain elusive. Auto-antibodies (aAbs) targeting neuro-receptors may generate disruptive effects onto behavioral domains. In animal models, circulating aAbs to specific neuro-receptors have been shown to affect both spontaneous behavior and response to psychoactive drugs, suggesting that they may act as determinants of vulnerability to psychiatrically-relevant symptoms in humans. Elevated levels of aAbs to opioid mu/delta receptors were demonstrated both in animal models, following chronic morphine exposure, and in heroin addicts. We investigated the levels of aAbs to selected components of neurotransmitter systems in blood samples from alcohol-dependent individuals.

Blood samples and psychometric interviews were collected from a total of 26 alcoholists (frequenters of the Alcohol Liver Disease Unit). Four samples were excluded, the remaining 22 samples were belonging to two conditions (alcoholism familiarity or not, n=11 each). Sera were analyzed with routine blood analyses for immune cell content and other standard haematic parameters. Also, sera were screened with ELISA techniques for content of aAbs targeting the dopamine transporter (DAT, both DAT-Asp and DAT-C epitopes), Serotonin Transporter (SERT), NMDA receptor subunit NR2, and AMPA receptor subunit GluR1. Titers of all aAbs were analyzed for a possible correlation with indices of alcohol consumption and with results of standard haematic parameters.

The levels of DAT aAbs significantly correlate with alcohol consumption, but only for subjects with positive familiarity for alcohol dependence. Interestingly, DAT protein plays a key role in brain motivational systems, and its levels are found to be altered in alcoholic patients, as measured with *in vivo* imaging techniques.

In the presence of a genetic predisposition, excessive consumption of alcohol does elevate the blood titers of DAT aAbs, a finding which might be also reflect efficiency and/or density of brain DAT protein. Thus, blood titers of DAT aAbs are here proposed as peripheral biomarkers for alcohol consumption, at least in case of a positive familiarity, and might hence be used in human patients to assess the efficacy of therapeutic strategies.

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P 14 PECULIAR RESPONSE TO METHYLPHENIDATE IN ADOLESCENT COMPARED TO ADULT RATS: A PH-MRI STUDY

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Adolescent rats and mice differ markedly from adults in several neuro-behavioral parameters. In addition, behavioral response to psychostimulant drugs may be dampened and "paradoxical" effects are often reported. Methylphenidate (MPH) is a psychostimulant elective drug therapy for the Attention Deficit Hyperactivity Disorder (ADHD). We aimed to investigate brain function peculiarities in the response of adolescent rats to MPH, using pharmacological imaging (Ph-MRI).

Adolescent (PND 34 to 43) and adult (PND>60) Sprague-Dawley rats were anaesthetised with isoflurane and examined by a 4.7 T Varian Inova MRI system (USA). After anatomical MRI, axial gradient echo images were collected continuously. After baseline recording (30 min), animals received MPH (4 mg/kg) or saline (SAL) and were recorded for further 30 min. The image sequences were realigned, restored through a Bayesian MCMC approach and detrended. Firstly, for each animal, blood-oxigenation level dependent (BOLD) data were collected from specific ROIs (Prefrontal Cortex, PFC; Nucleus Accumbens, NAcc; Hippocampus, Hip) and analysed with three-way ANOVA (age x drug x time design). Secondly, images were coregistered to one brain template for each group. A parametric Random Effect Analysis was performed on the averaged BOLD signal of the templates.

Region-specific changes in the BOLD signal were evident as a function of age. As expected, among adults MPH induced an increase of BOLD signal in nucleus NAcc and prefrontal PFC, with no effects in the Hip. Notably, among adolescents, MPH induced a marked and generalized decrease of BOLD signal which occurred earlier in NAcc and PFC. Any bias in the BOLD responses was excluded by the measurement of physiological parameters.

Present findings highlight the utility of Ph-MRI to detect brain functional changes following psychoactive agents in animal models. The peculiar negative BOLD effect found in adolescent rats may be suggestive of a reduced cerebro-vascular feedback and/or an increased MPH-induced neuronal activation. Data seem in agreement with age-related rearrangement in the function of central nervous system, and are relevant for a better understanding of brain/behavioral regulations during adolescence.

We wish to acknowledge Mr. C. Martino for help with the haemogas analyses.

P 15 UNUSUAL REPERTOIRE OF VOCALIZATIONS IN THE BTBR T+TF/J MOUSE MODEL OF AUTISM

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BTBR T+ tf/J (BTBR) is an inbred mouse strain that displays social abnormalities and repetitive behaviors analogous to the first and third diagnostic symptoms of autism. Here we investigate ultrasonic vocalizations in BTBR, to address the second diagnostic symptom of autism, communication deficits. As compared to the commonly used C57BL/6J (B6) strain, BTBR pups called more loudly and more frequently when separated from their mothers and siblings. Detailed analysis of ten categories of calls revealed an unusual pattern in BTBR as compared to B6. BTBR emitted high levels of harmonics, two-syllable, and composite calls, but minimal numbers of chevron-shaped syllables, upward, downward, and short calls. Because body weights were higher in BTBR than B6 pups, one possible explanation was that larger thoracic size was responsible for the louder calls and different distribution of syllable categories.

To test this possibility, we recorded separation calls from FVB/NJ, a strain with body weights similar to BTBR, and 129/SvJ, a strain with body weights similar to B6. BTBR remained the outlier on number of calls, displaying low numbers of complex, upward, chevron, short, and frequency steps calls, along with high harmonics and composites. Further, developmental milestones and growth rates were accelerated in BTBR, indicating an unusual neurodevelopmental trajectory. Overall, our findings demonstrate strain-specific patterns of ultrasonic calls that may represent different "dialects" or "languages" in genetically distinct strains of mice. Particularly intriguing is the unusual pattern of vocalizations and the more frequent, loud harmonics evident in the BTBR mouse model of autism that resemble the atypical vocalizations seen in some autistic infants.

P 16 CATHEPSIN D C224T POLYMORPHISM IN ITALIAN SPORADIC CREUTZFELDT-JAKOB DISEASE

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The lysosomal aspartyl protease Cathepsin D is involved in the pathogenesis of several neurodegenerative disorders like Alzheimer's Disease (AD), Parkinson's Disease (PD), and Creutzfeldt-Jakob Disease (CJD).

In particular, Cathepsin D is implicated in Amyloid Precursor Protein (APP) processing and Tau protein degradation.

In sporadic Creutzfeldt-Jakob Disease (sCJD) the lysosomal concentration of Cathepsin D is abnormally high, and associates with an increased neuronal vulnerability. In variant CJD the 224 Ala/Val polymorphism (produced by a C-to-T transition) within exon 2 of the Cathepsin D gene (CTSD) seems to influence the development of the disease.

The objective of this study was to investigate whether the CTSD codon 224T allele increases the risk of developing sporadic CJD in Italy, and whether it influences survival and disease progression.

Blood samples from 103 sCJD and 122 control samples without neuropsychiatric disorders were tested for the CTSD codon 224 polymorphism, prion protein gene (PRNP) codon 129 polymorphism (Met/Val), and Apolipoprotein E (APOE) ε alleles. Genetic data were compared with clinical and pathologic records.

We found that CTSD genotype frequencies in sCJD were not significantly different from those of control subjects. There was no gene interaction between the CTSD 224, PRNP 129 and APOE alleles. We observed a trend for shorter duration of illness (36% reduction) in sCJD patients with CTSD T genotype. Significant reductions of age at onset and death were observed for individuals carrying CTSD codon 224T allele. Moreover, we observed a significant longer duration of disease in the APOE ε2 allele carriers.

In contrast to variant CJD, the examined CTSD polymorphism is not associated with higher risk of developing sCJD. However, in Italian sCJD cases, the 224T genotype seems to carry to a worse prognosis. Combined evaluation of polymorphisms and haplotypes in PRNP, APOE and CTSD genes might be useful to predict survival and disease progression, which may be useful to design and interpret the results of clinical trials.

P 17 CHARACTERIZATION OF THE GENE EXPRESSION PROFILE IN THE SPLEEN OF MICE INTRAPERITONEALLY INFECTED WITH SCRAPIE

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Transmissible Spongiform Encephalopathies (TSEs) are fatal neurological diseases of humans and animals that are caused by transmissible agents called prions. Prions replicate in the Central Nervous System (CNS) inducing characteristic neurodegenerative changes and the accumulation of the TSE-specific, marker protein PrPTSE. Infectivity and PrPTSE may also be found in other body districts particularly those of the Lymphoreticular System (LRS) where, at variance with the CNS, no pathological changes are detectable. This makes the LRS fundamental to study the factors involved in prion replication without the noise produced in the brain by the aspecific activation of pathological cascade.

We have studied the expression pattern of genes, in a mouse model of TSE, in order to identify genes that are specifically activated in the course of the infeciton. At 7, 28, 45, 60 days after inoculation, and when clinical symptoms appear, animals were killed and the spleens collected for gene expression analysis by Differential Display. The data obtained were then evaluated by Real-Time quantitative PCR.

We found that a number of genes are deregulated in the course of the disease: some of them are specific of the immune system, such as the CD40 antigen and the TNF (Tumor Necrosis Factor) receptor; other have a common endoretroviral nature: LTR (Long Terminal Repeat) of ERV K (Endogenous Retroviruses), IAPE (Intracisternal A-Particle-related elements coding for Envelope). The former factors may be useful for the development of new and, hopefully, preclicnical diagnostic test for TSEs, whereas the latter may hypothetically represent potential targets for the preparation of anti-TSE drugs.

Before moving into the development of practical applications, however, additional studies are in course to understand whether these genes are specifically activated during TSE and if they are involved also in other TSE forms.

P 18 THE RAC GTPASE-ACTIVATING BACTERIAL PROTEIN CNF1 INDUCES ANALGESIA UP-REGULATING μ-OPIOID RECEPTORS

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A number of bacterial protein toxins, or portion of them, are increasingly used in the clinical practice. CNF1 is a protein toxin from *Escherichia coli* with a marked neurotrophic activity that constitutively activates the Rho- GTPases, a family of proteins that oscillate between a cytosolic GDP-bound inactive form and a membrane-linked GTP-bound active form, orchestrating the actin cytoskeleton assembly and dynamics. Since the actin cytoskeleton, besides other activities, has been suggested as involved in the mechanical transduction of pain, a cell biology and behavioral study was undertaken with the aim of verifying if the manipulation of Rho GTPases by CNF1 could permit the control of inflammatory pain in experimental animals.

CNF1 induced analgesic effects in mice after peripheral as well as after central administration. This analgesic response required both the sustained activation of the Rho-GTPase Rac, with consequent cerebral actin cytoskeleton remodeling, and the up-regulation of the μ-Opioid Receptors (MOR), the most important receptors controlling pain perception. The crucial role of Rac was proved by the lack of analgesic activity in mice challenged with a recombinant CNF1 lacking the enzymatic activity, whereas the importance of MOR was demonstrated by the inability of CNF1 to induce any analgesic effect in MOR knockout mice.

Altogether, these findings point at CNF1 as a useful tool in studies aimed at the comprehension of the fine mechanisms of pain and, more in general, disclose a new scenario for the pharmacological control of inflammatory pain in translational medicine: the possibility to skip out the "classical" receptor ligation-mediated pain control and the introduction of a new, intracellularly-regulated path aimed at the modulation of pain perception.

P 19 EARLY SOCIAL ENRICHMENT SHAPES ADULT BRAIN FUNCTION AND INCREASES RESILIENCE TO DEPRESSION-LIKE RESPONSES IN THE MOUSE

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During the early postnatal phase, important processes that shape the human brain take place. This highly plastic period offers the possibility for epigenetic factors to affect brain development. An intact, natural-type, early social environment is crucial for brain and behavioral development, as shown by the disrupting effects of its impoverishment or deterioration. In order to study the consequences of the early experiences on adult brain function and vulnerability to psychiatric disorders, we investigated, in the mouse, the long-term effects of being reared in an early social enriched condition: the Communal Nest (CN). CN, which consists in a single nest where three mothers keep their pups together and share care-giving behavior from birth to weaning, mimics the natural ecological niche of the mouse species. In the CN, maternal behavior and peer interactions during early ontogeny are markedly increased. At adulthood, mice reared in CN display higher propensity to interact socially and better social skills when compared to mice reared in standard laboratory conditions. They show also a more pronounced hedonic response in the sucrose preference test. Furthermore, they show a reduced activation of the hypothalamic-pituitary-adrenal axis after acute or chronic exposure to social challenge. These behavioral and neuroendocrine modifications are accompanied by higher NGF and BDNF levels in selected brain areas, including hippocampus and hypothalamus, and increased rate of newly generated brain cells. Overall, these neuroendocrine and behavioral results suggest that mice reared in a social enriched environment are less vulnerable to display depression-like responses. The present findings confirm the crucial role played by the early social experiences in shaping adult brain and behavior. In addition, they indicate the communal nest as a valuable mouse model to evaluate the relevance of epigenetic factors on brain plasticity and to identify new tools to prevent or reverse pathological conditions such as neurodegeneration and psychiatric disorders in humans.

P 20 NON-MOTOR SYMPTOMS IN PARKINSON'S DISEASE: INVESTIGATING EARLY PHASE ONSET OF BEHAVIORAL DYSFUNCTION IN THE 6-OHDA LESIONED RAT MODEL

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In order to investigate the psychiatric symptoms accompanying the early phases of Parkinson's Disease (PD), we injected adult rats with 10.5 µg of 6-hydroxydopamine (6-OHDA) bilaterally into the dorsal striatum. The resulting neurodegeneration led, 12 weeks after injection, to a mild (36%) reduction of striatal dopamine. We tested the behavioral response of SHAM and 6-OHDA-lesioned animals at different time-points after injection, to evaluate the onset and progression of behavioral abnormalities. The results showed that such a mild reduction of dopamine levels was mainly associated with a decrease of anxiety-like behavior, an increase in "depression"-like behavior and a marked change of social behavior. Learning and memory abilities were not affected. Overall, the PD rat model used here displays behavioral alterations having face validity with psychiatric symptoms of the pathology and thus appears as a valuable tool to investigate the neural bases of the early phases of PD.

P 21 MATERNAL DEPRIVATION STRESS AS A RISK FACTOR FOR MENTAL HEALTH: NEUROTROPHINS AS NEUROENDOCRINE SIGNALS IN RODENTS AND NON-HUMAN PRIMATES

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Early adverse events can enhance stress responsiveness and lead to greater susceptibility for neurodegenerative disorders at adulthood. The epigenetic factors involved in transducing specific features of the rearing environment into stable changes in brain function and behavior have only begun to be elucidated. Neurotrophic factors, such as Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF), are good candidates for mediating the effects of early adverse experiences since they are affected by stress and play a major role in brain development and in the functionality of specific neuronal networks involved in cognitive function. Disruption of the mother-infant relationship has been used to model early adverse experiences from rodents to primates. Data obtained from animal models will be discussed to indicate that early maternal deprivation stress is able to affect neurotrophin levels both in the central nervous system and in the peripheral circulation. Changes in the levels of NGF and BDNF following maternal deprivation represent neuroendocrine signals involved in the response to early adversity, which appear as novel target to be exploited to effectively prevent or reverse glucocorticoid-induced damage to the brain. Animal models of early stress provide an important avenue for translational research aimed at developing prevention, intervention and treatment strategies for human psychiatric and neurodegenerative disorders.

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P 22 BIRTH SPACING IN THE COMMUNAL NEST AS A MAJOR FACTOR IN SHAPING ADULT EMOTIONAL RESPONSE AND SOCIAL SKILLS IN THE MOUSE

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Early experiences such as maternal deprivation or handling have been reported to produce persistent changes in brain function and behavior. In addition to these very commonly used paradigms, we recently exploited a novel one that provides the developing pup with a highly stimulating social environment: the Communal Nest (CN). CN consists in a single nest where three mothers keep their pups together and share care-giving behavior from birth to weaning and mimics the natural ecological niche of the mouse species. In order to investigate in detail the complexity of the nest social environment, we analyzed the effects of being reared in three different CN conditions characterized by a birth spacing (interval between two consecutive deliveries) of 3, 5 or 7 days (CN±3, CN±5, CN±7). Birth spacing exerted relevant effects on social skills and emotional response at adulthood. In the social interaction test, the strategies to manage interactions with conspecifics and the establishment of a hierarchy differed among the three groups. In the CN±3 group, differences in behavior associated with social status concerned mainly offensive/defensive behavior. By contrast, in the CN±5 and CN±7 mice, such differences concerned mainly social investigation and affiliative behaviors. In the plus maze test for the evaluation of emotional behavior, birth spacing was inversely related with anxiety-like behavior, CN±3 mice showed less head dipping and more SAP compared to CN±5 and CN±7 mice. Overall, these findings suggest birth spacing as major factor affecting the development of social skills and emotional response and confirm the crucial role played by early social environment in shaping adult behavior.

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P 23 BEHAVIOURAL PHENOTYPING IN A MOUSE MODEL OF RETT SYNDROME: FOCUSSING ON EARLY NEONATAL PHASE AND ON EFFECTS OF CHOLINE SUPPLEMENTATION

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Rett Syndrome (RTT) is a rare neurodevelopmental disorder primarily affecting girls caused by mutations in the X-linked gene MeCP2. RTT causes severe cognitive, social, motor and physiological impairments. In a RTT mouse model which expresses a truncated form of MeCP2 (MeCP2-308), we performed a longitudinal neuro-behavioural evaluation across the lifespan. On postnatal days (pnds) 3, 6 and 9, spontaneous general movements and emotional communicative behaviour (ultrasonic vocalizations) were measured. As early as pnd 3, subtle anomalies in spontaneous general movements were evident in Mecp2-308 male mice: mutant mice exhibited more intense curling responses (righting reflex attempts) and more side responses (righting reflex failures) than wild type (wt) littermates. On pnd 9 Mecp2-308 male pups showed more pivoting and head rising behaviours than wt controls. A significant decrease in ultrasonic vocalizations was found in MeCP2-308 male pups on pnd 6 suggesting a communication deficit in mutant pups. On pnd 60, Mecp2-308 male mice displayed increased anxiety-like behaviours in the light-dark test (more time spent in the dark associated with decreased locomotor activity). These results suggest that an accurate behavioural phenotyping starting from early phases of development provides relevant precocious biomarkers in translational models of human neuro-developmental disorders with neurological and emotional-communicative symptoms emerging during infancy.

In the Mecp2-308 mice we also evaluated the efficacy of a dietary perinatal supplementation with choline (a vitamin of the B-complex and acetylcholine precursor in neurons). Choline supplementation (25 mM) was provided to lactating dams from delivery till offspring weaning (pnd 25). When tested at adulthood, mutant males exhibited reduced locomotor activity and increased emotionality when compared to wt controls. Choline treatment compensated both these behavioural alterations, restoring wt-like levels. Present findings suggest that early choline supplementation improves behavioural symptoms in the mutant offspring, specifically for motor and emotional domains. To probe the functional status of central cholinergic system, mice were challenged with the specific cholinergic muscarinic antagonist, scopolamine (2 mg/kg): the expected hyperactivity profile was evident in wt subjects, not in mutant mice, thus revealing an underlying reduced cholinergic tone. Altogether these results could be exploited for innovative therapeutic approaches targeted at cholinergic pathways.

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P 24 PPAR-GAMMA AGONISTS AS POTENTIAL AGENTS FOR THE TREATMENT OF DEMYELINATING DISORDERS: EFFECTS ON ANTI-OXIDANT DEFENCES AND MITOCHONDRIA IN CULTURED OLIGODENDROCYTES

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The Peroxisome Proliferator-Activated Receptor-gamma (PPAR-g) belongs to a large group of nuclear receptors controlling reproduction, metabolism, development and immune response. Among the members of this receptor family, PPAR-g is the most studied, in part due to its therapeutic potential for treatment of diabetes and related consequences such as metabolic syndrome. Several lines of evidence suggest that PPAR-g natural and synthetic agonists may control brain inflammation and be of potential therapeutic use in human brain diseases. The beneficial effects of PPAR-g agonists are mediated by several mechanisms involving anti-inflammatory activities as well as direct effects on functions and survival of neural cell types, including neurons and astrocytes. The aim of the present study was to unravel the potential beneficial action of PPAR-g agonists on Oligodendrocytes (OLs), the myelin forming cells of the CNS, in order to set the grounds for fully exploiting the therapeutic potential of synthetic PPAR-g agonists, some of which are currently used in clinical practice (type 2 diabetes) or undergoing clinical trials for the treatment of demyelinating disorders. In particular, we evaluated the capability of PPAR-g agonists to strengthen the intrinsic cellular mechanisms protecting OLs from inflammatory and oxidative insults, by using molecular and functional *in vitro* approaches. We found that PPAR-g agonists are able to increase the expression of enzymes and molecules deputed to the defense against oxidative insults (*e.g.* SOD, catalase, UCP2, glutathione). In addition, biochemical and fluorescence dynamic video-imaging studies indicated that PPAR-g agonists might sustain myelination through their activities on mitochondria by favoring Ca2+ waves propagation in differentiating OLs. In a perspective closer to clinical purposes, these data contribute to validate the utilization of selected PPAR-g agonists to treat de-myelinating diseases in which inflammatory events and redox unbalance occur. In a more general perspective, the *in vitro* combined molecular/functional approaches adopted in the present study, can provide useful information to be conveyed and integrated in a broader frame of investigations, aiming at drug validation and, ultimately, public health benefit.

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P 25 DEVELOPMENT OF AN EXTERNALLY VALID MOUSE MODEL OF DEPRESSION THROUGH L-TRYPTOPHAN DIETARY DEPLETION AND NEONATAL CORTICOSTERONE ADMINISTRATION

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Depression, a pathology characterised by mood and neuro-vegetative disturbances, depends on a multi-factorial contribution of individual predisposition (*e.g.* diminished serotonergic transmission) and environmental factors (*e.g.* neonatal abuse or neglect). Despite these evidences, most animal models of depression only address one or the other factor thereby resulting of poor face validity. Here we describe a novel animal model of depression aimed at holding construct, face and predictive validity. In order to mimic the aforementioned factors in the development of depressive-like alterations, mouse pups have been reared by dams exposed to corticosterone (the "stress hormone") in the drinking water, and/or to an L-tryptophan (serotonin precursor) deficient diet. Four groups of CD1 mouse dams (N=12-13 per group) were exposed to Animal Facility Rearing conditions (AFR group), given access to the L-tryptophan restricted diet between postnatal day (P)0-8 (T group), to corticosterone between P1-8 (C group) or both (TC group). Maternal behaviour was observed between P0-10 (3 daily, 75-min sessions); daily water intake and pups' body weight were also measured. Active maternal care steadily declined in all groups throughout lactation and was significantly higher in AFR dams compared to C, T and TC dams. Thus, AFR dams displayed significantly more active nursing (arched back nursing and licking) than all treated dams. Time-budget wise, while T dams showed more activity out of the nest, C and TC dams showed increased resting time. Additionally, C dams showed increased water intake compared to T, AFR and TC; T dams had the lowest water intake. Pups' body weight increased steadily in all groups between P 11-23. However, C and TC pups were significantly lighter than both T and AFR. Additionally, data on anxiety- and depressive-like behaviour in adolescent and adult offspring will be presented. Although preliminary, these data support the hypothesis that a combination of natural predispositions and environmental stress, be the latter in the form of corticosterone administration, reduced maternal care or both, contribute to induce disturbances isomorphic to human depression. The route of administration and the possibility to control the independent variables predisposing to depressive-like symptoms disclose novel avenues towards the development of valid animal models.

P 26 THE "STRANGE" CASE OF AN ITALIAN NEUROSURGEON FASCINATED BY BASIC SCIENCES: AN ABNORMALITY?

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I report my personal experience of italian neurosurgeon interested in basic research. I was graduated at Catholic University of Rome discussing an experimental thesis on spinal cord injury. Since my second year of medical school I was involved in experimental research of neurophysiology. This experience has marked my further medical formation, because it has stimulated my attitude to deepen clinical data and to speculate about underneath biological phenomena.

When I started my residency in Neurosurgery at Verona University, I met Prof Suzuki of the Biochemestry Departement. I asked and obtained to spend a full semester working with him on my personal project about the role of nitric oxide in spinal cord injury. Surprisingly, this choice was considered abnormal by my collegues, who could not understand my interest for biochemestry and basic sciences. However, the meeting of our two different experiences and cultures, was so fertile to produce several projects and publications. Togheter with him, we set a laboratory of biochemical research applied to neurosurgery, studing the role of nitric oxide in spinal cord trauma and in glioma biology and opening further studies of Verona University on cerebral vasospasm.

I am working now as assistant Professor of Neurosurgery at La Sapienza University of Rome and I continue to pursuit my interst in both clinical and basic research, trying to keep alive my relations with basic science researchers.

I am convinced that to improve our medical knowledge through the newest pharmacological and technological applications, we physicians have to reconsider our relationship with basic research. In fact, especially in italian universities, there is often a deep fracture between clinical and basic research, which needs to be healed to share results and to improve our knowledge each other.

All medical students and residents should be involved in basic research, to feel the pleasure and sometime the exaltation as I did, to share an idea, to plan experiments and to shape their intuitions in concrete results. These feelings would be impessed in their minds and we would probably gain a generation of reasoned researchers.

P 27 NITRIC OXIDE AND OEDEMA IN BRAIN INJURY: NEW THERAPEUTIC TARGETS. A PROPOSAL OF COOPERATION BETWEEN NEUROSURGEONS AND BASIC SCIENCE RESEARCHERS

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The main purpose of this project is to find new therapeutic strategies for prevention and treatment of oedema formation and secondary cell death in injured brain, based on inhibition or modulation of Xanthine Oxido-Reductase (XOR)-dependent NO synthesis. In models of focal and diffuse brain injury, has been experimetally demonstrated that NO largely diffuses in injured tissue. In ischaemic tissues, such as the injured brain, large amount of NO would cause secondary cell death by reacting with free radicals. Large amount of NO would induce necrotic and apoptotic mechanisms of secondary cell death. Furthermore large amount of NO, would contribute to the brain swelling, which is a severe clinical condition sustained by an irreverisble relaxation and permeabilisation of brain vessels. Our hypothesis is that in anaerobic and acidotic conditions, such as the post-injured brain, when there is a reduced tissue oxygen tension and local acidosis, NO is mainly produced by XOR rather than NO synthase. In fact, NO synthase has been demonstrated to reduce its activity at these conditions. For *in vitro* experiments, coltures of glial and endothelial cells will be tested. A model of diffuse axonal injury in rats will be prepared. Furthermore a clinical database of patients with severe brain injury, both operated or not-operated, undergone to cerebral and systemic monitoring, will be created. Samples obtained form cell coltures, and samples of brain and cerebral spine fluid (CSF), collected from rats and from the patient group, will be used for biochemestry, proteomic, histochemestry and histo-enzymatic experiments, to demonstrate that, in injured brain: i) NO is largely produced, concurring to the secondary cell death phenomena; ii) NO derives mainly from the XOR. In the latter part of our study, inhibitors of XOR-dependent NO production will be tested *in vitro*. They will be then used on injured rats, to demonstrate their efficacy in preventing NO synthesis and so tissue nitrosilation, oedema extension and secondary cell death. These data, through a development of a model for further pre-clinical studies, could open new therapeutic strategy to prevent and treat secondary cell death and brain swelling in patients with severe brain injury.

P 28 REDUCED LEVELS OF OXIDATIVE STRESS AND GREATER RESISTANCE TO IMMUNOGENIC STIMULI IN A MOUSE MODEL OF EXTENDED LONGEVITY

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Numerous evidence suggest that both stressful life events and oxidative stress may contribute to accelerate the aging process and to promote age-related neurodegenerative disorders. Identification of genetic as well as environmental factors involved in the etiology of these pathologies, and the associated biomarkers, is of primary importance to elaborate and test new interventions to favor successful aging. Deletion of the $p66^{Shc}$ gene in the mouse increases resistance to Oxidative Stress (OS) and extends life span. A growing body of evidence suggests that OS and the neuroendocrine response to stressful stimuli might interact, resulting in greater vulnerability to aging and neurodegeneration. We tested whether $p66^{Shc-/-}$ mice, which are exposed to lower levels of ROS throughout lifespan, might be less susceptible to the effects of a psychophysical stress (restraint) or an immunogenic challenge (Lipopolysaccharide, LPS). The Hypothalamic-Pituitary-Adrenal (HPA) axis negative feed-back was assessed by means of a Dexamethasone-Suppression-Test (DST). Corticosterone levels were assayed as a measure of adrenal functionality. Hippocampal levels of the neurotrophin Brain-Derived-Neurotrophic Factor (BDNF), and of Prostaglandine E2 (PGE_2) and Isoprostanes (15-F_{2t}-IsoP), respectively markers of inflammation and of oxidative stress, were assessed following LPS treatment. Corticosterone levels did not differ in the two genotypes following the restraint stress or as a result of the LPS challenge. By contrast, mutant mice were characterized by a slight escape from suppression in the DST, resulting overall in higher corticosterone levels, and by reduced levels of PGE_2 upon treatment with LPS. These data, taken together, strongly suggest that $p66^{Shc-/-}$ mice might be protected from sudden variations of the internal milieu due to inflammation or infections by means of a more efficient cross-talk between the neuroendocrine and the immune system. This conclusion is further supported by the fact that levels of BDNF and of 15-F_{2t}-IsoP were not affected upon treatment with LPS in the mutants, while were increased in the controls. These data will be discussed also in relation to the healthier behavioral phenotype of the experimental subjects.

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P 29 INNOVATIVE TRIAL DESIGNS FOR RARE DISEASES WITH POOR SURVIVAL: USEFUL TOOLS FOR CLINICAL EXPERIMENTATION IN HUMAN PRION DISEASES

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No effective therapies are available for human prion diseases and few trials are recruiting patients or have been completed in the world. Differences between these studies highlight difficulties arising in this research area, as happens in other rapidly progressive and invariably fatal very Rare Diseases. There was disagreement concerning a randomized, double blind, placebo controlled design to assess efficacy of a drug when limited preclinical data were available. In UK, the "PRION-1: Quinacrine for human prion disease" trial started as a partially randomized patient preference trial to allow patients to decide for treatment, no treatment or being randomized, whereas, in US, the "CJD Quinacrine study" was designed as a randomized, double blind, placebo controlled trial, with the option of delayed treatment starting after 2 months. The uncertainty principle and the concept of clinical equipoise used to justify randomised clinical trials - and to recruit patients - were not univocally interpreted. The enrolment of homogeneous patients, useful to lower the needed sample size, was constantly missed. Multinational networks rather than single centre trials allow the recruitment of homogeneous patients, defined according to the type of the disease (diagnosis, stage and clinical manifestations) and even according to known predictors of survival (or of selected surrogate markers). The primary endpoint and the clinically significant delta differed between trials, and sometimes were subjective and not clinically relevant.

Finally, modern statistical approaches to trial designs that may improve knowledge in the treatment of Rare Diseases are rarely adopted in clinical experimentation. Among them there are sequential trials, which continuously monitor the available evidence and may interrupt the trial earlier; adaptive designs, that use the increasing available data to better allocate new patients in the trials; and bayesian theory, which calculates probabilities that may justify changes in clinical practice on the basis of less definitive information compared to that normally required, with additional collection of efficacy and safety data after the study has been completed.

These novel designs and statistical techniques can accelerate the process of evaluation of drugs for very Rare Diseases, and regulatory authorities should surely have a role in standardizing and implementing them.

P 30 GAUCHER DISEASE: FROM BASIC RESEARCH TO THERAPY

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Gaucher Disease (GD) is a rare genetic disorder caused by mutations in the gene encoding the lysosomal enzyme, Glucosylceramidase (GCase). The consequent accumulation of Glucocerebroside (GC) in the lysosomes of reticuloendothelial cells leads to hepatomegaly, splenomegaly, bone crisis, anemia and pulmonary problems. Neurological involvement also occurs in some patients.

In the past the biological properties of GCase and its mechanism of action have been extensively studied and the role of its physiological activators, namely Sap C and anionic phospholipids, has been elucidated by us and other authors. The knowledge of the physiological behaviour of GCase has lead to several options for the treatment of GD such as Enzyme Replacement Therapy (ERT), substrate reduction therapy and chaperone-mediated therapy. Up to now the best results have been obtained with ERT, based on periodical intravenous infusion of mannose-terminal GCase. ERT relieves most of the clinical symptoms and signs of visceral involvement. However, the delivery of the infused enzyme to the target macrophages (the cells that accumulate GC) is far from optimal due to the high susceptibility of exogenous GCase to denaturation in plasma and tissue fluids.

In order to improve the efficacy of ERT it is of paramount importance to stabilize the enzyme utilized for the infusion. Starting from our past findings on the stabilization and activation of GCase by its physiological activators, we have thus encapsulated GCase and Sap C into anionic phospholipids-containing liposomes. Actually the use of liposomes as a delivery system has been recently shown to offer enhanced pharmaceutical advantages over free drugs. The optimal conditions for encapsulation of GCase were evaluated by enzymatic activity, Western blot analysis and immunofluorescence. Our results show that the half-life in serum of the liposomal bound enzyme dramatically increases in comparison with that of free GCase.

These findings might have important implications for development of more efficient ERT. The stabilization of the internalized GCase might dramatically reduce the quantity of the infused enzyme. Moreover, liposomes might be carriers for the targeting of GCase to specific tissues, such as brain and bone marrow, that the free enzyme cannot reach.

P 31 GAIN-OF-FUNCTION RAF1 MUTATIONS CAUSE NOONAN AND LEOPARD SYNDROMES WITH HYPERTROPHIC CARDIOMYOPATHY

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Noonan and LEOPARD syndromes are developmental disorders with overlapping features, including cardiac abnormalities, short stature and facial dysmorphia. Increased RAS signaling owing to PTPN11, SOS1 and KRAS mutations causes approximately 60% of Noonan syndrome cases, and PTPN11 mutations cause 90% of LEOPARD syndrome cases. Here, we report that 18 of 231 individuals with Noonan syndrome without known mutations and two of six individuals with LEOPARD syndrome without PTPN11 mutations have missense mutations in RAF1, which encodes a serine-threonine kinase that activates MEK1 and MEK2. Most mutations altered a motif flanking Ser259, a residue critical for autoinhibition of RAF1 through 14-3-3 binding. Of 19 subjects with a RAF1 mutation in two hotspots, 18 showed Hypertrophic Cardiomyopathy (HCM), compared with the 18% prevalence of HCM among individuals with Noonan syndrome in general. Ectopically expressed RAF1 mutants from the two HCM hotspots had increased kinase activity and enhanced ERK activation, whereas non-HCM-associated mutants were kinase impaired. Our findings further implicate increased RAS signaling in pathological cardiomyocyte hypertrophy, and point to this kinase as a target for the development of novel therapeutic strategies to treat HCM progression in Noonan and LEOPARD syndromes.

P 32 NEW PERSPECTIVES IN MOLECULAR IMAGING OF CARDIOVASCULAR DISEASES

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Atherosclerosis is the main cause of death in western countries. Therapies are not able to reverse the destructive cascade that occurs after acute myocardial infarction. Nuclear imaging has the potential to provide invaluable information on the composition of the plaque. Cell therapy for cardiac repair is one of the most promising in cardiovascular medicine. Non-invasive techniques to assess the fate of transplanted stem cells and detectors with high sensitivity and submm spatial resolution are needed. We designed and implemented a Single Photon Emission Computed Tomography (SPECT) system for molecular imaging of cardiovascular diseases with spatial resolution = 500 μm, sensitivity=0.3 cps/kBq, active area of 100×100 mm2, made of a tungsten collimator, a scintillator and Position Sensitive Photomultiplier Tubes:

- pinhole collimator (0.3 mm hole) with magnification factor 3 (reasonable compromise for the FOV, sensitivity and spatial resolution (~ 500 μm));
- scintillator: pixilated CsI (Tl);
- photo detector: high segmentation;
- readout electronics: individual channel;
- measurements have been performed at Johns Hopkins with (APOE +/-) mice and AnnexinV labeled with 99Tc.

Results: a young mouse (6 weeks) didn't show plaques, a control mouse (25 weeks) didn't show uptake, ApoE (+/-) mouse (25 weeks) showed suspicious hot spots. The spatial resolution is ~ 0.8 mm. It can be improved (0.3 mm) by reducing the dimension of the hole of the collimator. We will study mice with infarction by using SPECT techniques both the diffusion of stem cells injected on mice heart and the effect of therapy. The radiotracer (MIBI labeled with 99mTc) has to be injected repeatedly, so it would be impossible to use tail vein to deliver the radiotracer. Perfusion measurements have been performed on mice with two routes of delivery: tail vein and peritoneum. Results: injecting the radiotracer in the peritoneum is possible. The uptake is reduced, so the sensitivity of the system has to be increased. In conclusion a powerful microSPECT detector system has been designed and implemented for molecular imaging study of cardiovascular diseases. The performances of the detector are as expected. Further calculations and measurements results will be presented at the Conference.

P 33 MANIPULATING THE CELL CYCLE IN FAVOR OF TISSUE REPAIR AND REGENERATION

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In adult vertebrates, the overwhelming majority of cells are not in the cell cycle at any given time. The ability of these cells to enter the mitotic cycle and proliferate on appropriate cues is of paramount importance in regeneration and tissue repair. Indeed, many tissues and organs are endowed with poor self-repair capabilities due to the limited ability of their constituent cells to proliferate.

Recent work by our group has shown that it is possible to reactivate the cell cycle in any non-proliferative cellular state (quiescence, senescence, terminal differentiation), by temporarily suppressing the expression of specific cell cycle inhibitors (cyclin-dependent kinase inhibitors, CDKIs). Such manipulation induces lasting, reversible proliferation of quiescent and senescent cells, even in the absence of growth factors.

In initial *in vivo* applications of our findings, we now show that the same cell cycle-reactivating effect can be achieved in mouse tissues such as the skeletal and cardiac muscle, by transduction of shRNAs targeting specific CDKIs.

In principle, our findings could find wide application in biotechnology and tissue repair in all instances in which cell proliferation is a limiting factor. As CDKIs appear to be pivotal inhibitors of the cell cycle in all cases tested, their suppression could even be exploited to induce proliferation of cell types that cannot be currently cultured *in vitro*. Our preliminary *in vivo* studies support this contention.

P 34 POST-PRANDIAL TRIACYLGLYCEROL RICH LIPOPROTEINS INDUCE MONOCYTE CHEMOATTRACTANTS SECRETION BY MACROPHAGES

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Atherosclerosis is an inflammatory disease. Macrophages actively participate to the atherogenesis, either by accumulating lipids and by the secretion of several cytokines. Nowadays, despite there is a strong evidence that post-prandial Triacylglycerol Rich Lipoproteins (ppTGRL) are atherogenic and induce macrophage lipid accumulation, little is known on the role of ppTGRL on inflammatory macrophage functions. With this study we aimed to investigate whether ppTGRL influenced chemokines macrophage secretion.

ppTGRL were isolated by sequential ultracentrifugation from plasma of normo-lipidemic subjects 150min after the consumption of a standard breakfast (energy intake 1,050 kcal, 63% fat). Human Monocyte-Derived Macrophages (HMDM) were incubated for 24hours either with 10 and 30 μg/ml cholesterol carried by ppTGRL or an equivalent amount of cholesterol transported by Fetal Bovine Serum (FBS). Control incubations were performed without lipids (w/o FBS), and for positive control, HMDM were stimulated with 1μg/ml LPS. The supernatants were used for the determination of MIP-1alpha, MIP-1beta, IL8, TARC, RANTES, EOTAXIN and MDC by SearchLight™ Proteome Arrays.

LPS stimulated the secretion of analysed chemokines. We found that both concentrations of ppTGRL induced a significant and similar increase ($n=3$, $p<0.05$) in the secretion of the main macrophage effectors chemokines. MIP-1alpha (195±100 and 156±89 pg/ml) and MIP-1beta (1282±662 and 1112±571 pg/ml) were increased by about 3 and 5 fold with respect to HMDM incubated w/o FBS (53±16 and 240±61 pg/ml for MIP-1alpha and MIP-1beta, respectively). The IL8 secretion (1100±187 and 1139±37 pg/ml with 10 and 30 μg ppTGRL /ml, respectively), a strong chemoattractant for monocytes as well as for leukocytes, was also increased ($n=3$, $p<0.05$) in comparison to HMDM incubated w/o FBS (667±113 pg/ml). RANTES, EOTAXIN and MDC secretion was not affected, while ppTGRL significantly reduced in a dose-dependent manner the release of TARC, a platelet activator which lacks the capability to recruit monocytes. These effects were specifically induced by ppTGRL but not from FBS-lipids. Present results suggest that postprandial phase is associated with an increased production of monocyte/macrophages chemoattractant factors, which could be related to the higher cardiovascular risk found in diseases with a delayed clearance of ppTGRL, such as diabetes, obesity and chronic renal failure.

P 35 A MURINE MODEL OF ARTERIAL HYPERTENSION TO STUDY THE ASSOCIATION BETWEEN SPORADIC ALZHEIMER'S DISEASE AND CARDIOVASCULAR RISK FACTORS

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Genetic Alzheimer's Disease (AD) accounts for only few AD cases and is almost exclusively associated to increased production of the beta-amyloid peptides (Abeta) in the brain. Instead, the majority of patients is affected with the AD sporadic form and typically has an altered Abeta clearance from the brain. The identification of factors that influence the onset and progression of sporadic AD is a key step toward understanding its mechanism(s) and developing successful therapies. Increasing epidemiological studies describe a strong association between AD and cardiovascular risk factors, particularly hypertension. Besides its well known peripheral outcomes, hypertension exerts detrimental effects on the cerebral circulation, favouring chronic brain hypoperfusion, a condition associated to impaired energy substrate delivery to brain tissue, probably resulting in alterations typical of AD and vascular dementia. However, a clear demonstration of a pathophysiological link between cardiovascular risk factors and AD aetiology is still missing. To deepen our knowledge of the mechanisms involved in brain response to hypertension and their possible role in promoting Abeta deposition in the brain, we studied a mouse model in which permanent Transverse Aorta Coartaction (TAC), results in a persistent increase in blood pressure and, in the long term, in chronic hypoperfusion of both cerebral hemispheres and in Abeta deposition, clearly detectable as early as 4 weeks after hemodynamic disruption. In this model we have evaluated, at different time points, the extent of glial activation and the expression of several genes involved in the inflammatory response, in relation to the observed parenchymal and perivascular Abeta deposits. Our results indicate cellular and molecular alterations that occur in time and brain region specific fashion, possibly related to hemodynamic changes and/or hypometabolism, and that might represent targets for novel therapeutic approaches.

P 36 RAS SIGNALLING BLOCKADE AS A THERAPEUTIC APPROACH AGAINST EARLY NEUROGLIAL DYSFUNCTION IN DIABETIC RETINOPATHY

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Pharmacological manipulation of Renin-Angiotensin System (RAS) is a well-known approach to treat cardiovascular disease and it has been proven also useful in reducing the progression of vascular diabetic complications such as nephropathy and retinopathy. Recent evidence on Diabetic Retinopathy (DR), the leading cause of blindness in working people, demonstrate that neuroglial alterations may precede overt vasculopathy and could be responsible for earlier functional deficits in vision.

Aim of this study was to verify the involvement of RAS signalling pathways in diabetic retinal neuroglial dysfunction in an *in vitro* experimental model of DR and to test if RAS signalling blockade may contrast neuronal and glial alterations.

Retinal explants from adult Sprague-Dawley rats were cultivated in normal glucose (NG: 5.5mM), High Glucose (HG: 30mM), or iso-osmolar mannitol (M: 5.5mM glucose+24.5mM mannitol) for 48 hours. Treatments were conducted in the presence or absence of Angiotensin-Converting Enzyme-inhibitor enalaprilat (EPR: 200 μM), which inhibits the conversion from Angiotensin I to Angiotensin II, thus blocking RAS signalling. Molecules involved in RAS signalling and glial markers were detected by Western Blot analysis (WB) and Immunohistochemistry (IHC).

We found that HG increased tyrosine phosphorylation of proteins identified as proline-rich tyrosine kinase 2 (pPyk2-Tyr402) and as phospholipase Cγ1 (pPLCγ1-Tyr783), with respect to NG or M. IHC confirmed the increased pPyK2-Tyr402 throughout the retinal layers, in both glial and neuronal cells. Glial activation was demonstrated by increased Glial Fibrillar Acidic Protein (GFAP) in HG-treated explants by WB and HIC. We also observed an increase in c-src and lyn kinase activities and an activation of both MAPKs (p44/42MAPK) and the transcription factor cAMP-Responsive Element Binding protein (S133 CREB) in HG vs NG. Phosphorylated CREB was detected in neuronal nuclei in inner nuclear and ganglion cell layers.

EPR inhibited HG-induced PLCγ1 and S133 CREB phosphorylation, confirming that RAS signalling pathway was actually activated in HG. However, HG-induced GFAP increase was not reverted by EPR, suggesting that a different signalling pathway was responsible for glial activation. These results highlight the need of a combined therapeutic approach, targeting different HG-activated signalling pathways, to exert efficient protection against neuroglial dysfunction in DR.

P 37 IFN-β AS A NATURAL ADJUVANT TO IMPROVE BCG VACCINATION

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Tuberculosis is still a leading cause of death worldwide despite the availability of the Bacillus Calmette-Guerin (BCG) vaccine and specific antibiotic treatment. Attempts over the years to design a vaccine more protective than BCG have been unsuccessful and, therefore, development of new vaccines is now a priority. Given the role played by DC in initiating and regulating a protective T-cell response against Mtb, to identify the reasons for the ineffectiveness of BCG in the induction of a long-lasting protective immune response, we investigated the impact of BCG infection on the immune functions of Dendritic Cells (DC). Therefore, we characterized the immune properties of human Monocyte-derived DC (MoDC) after infection with BCG in comparison with *Mycobacterium tuberculosis* (Mtb) H37Rv. Having found that Mtb and BCG are similarly taken up by MoDC, the comparative analysis was extended to MoDC maturation and cytokine expression. Overall, the results indicated that BCG was a less efficient inducer of MoDC maturation than Mtb. In particular, BCG-infected MoDC did not express IFN-β and produced less IL-12 than Mtb-stimulated counterpart.

Noteworthy, when BCG-infected MoDC were exposed to IFN-β a fully mature phenotype was displayed and significant amount of bioactive IL-12p70 was released. with a consequent enhanced capacity to induce a Th1 response.

In conclusion, our comparative studies indicate that IFN-β may improve BCG immunogenicity by acting as powerful adjuvant at a critical cross-road of innate and adaptive immunity. Our data warrant consideration to improve BCG vaccination strategies.

P 38 TRANSLATING NEW DATA ON MULTIPLE SCLEROSIS PATHOGENESIS INTO NOVEL THERAPEUTIC CONCEPTS

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Multiple Sclerosis (MS) is a chronic inflammatory disease of the Central Nervous System (CNS) that affects young adults and leads to permanent neurological disability. Due to prominent demyelination in the CNS, MS is thought to be caused by an autoimmune response targeting myelin components and eliciting subsequent neurodegeneration. Conclusive evidence supporting this pathogenic model is however still missing.

To date, the possibility that infectious agents trigger and/or sustain a CNS-targeted immune response in MS has been explored but remains highly debated. On this poorly defined background of knowledge, MS still awaits a cure as the efficacy of currently used immunosuppressive and immunomodulatory drugs is limited. Based on results obtained in preclinical models and neuropathological analyses performed in post-mortem brain tissues from MS patients, we have recently proposed that Epstein-Barr Virus (EBV), a *ubiquitous* B-lymphotropic γ-herpesvirus, plays a key role in driving the intrathecal humoral immune response and the T-cell mediated inflammatory process that are characteristic of MS. The model involves intracerebral migration, expansion and activation of latently EBV-infected B cells/plasma cells, periodic EBV reactivation in the CNS, and virus-induced immunopathology as the main mechanism responsible for myelin and neuronal damage. This scenario is compatible with epidemiological observations associating EBV seroconversion to MS and with immunological studies showing increased immune reactivity to EBV in MS patients compared to healthy seropositive individuals.

The recognition of an infectious component in MS pathogenesis paves the way to the search of new clinical markers and to the development of preventive and therapeutic anti-herpesvirus strategies.

P 39 VACCINATION WITH RECOMBINANT MHV68 PRODUCING IFNα EFFECTIVELY PROTECTS MICE AGAINST INFECTION WITH WT MHV-68 AND DRAMATICALLY REDUCES LONG-TERM SPLEEN LATENCY

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Human gammaherpesviruses such as Epstein-Barr Virus cause lifelong infections and associated diseases, by virtue of their ability to establish latent infection. Mice infected with murine herpesvirus 68 (MHV-68) represent a versatile experimental setting to study the biology of gammaherpesviruses and to test vaccination strategies against them. A number of studies published in the past years suggested that the limited immunity generated against isolated viral components by subunit vaccines cannot counteract the multiple immune evasion strategies operated by MHV68. Indeed, a significant inhibition of the establishment of long-term latency could be observed in mice vaccinated with strains of MHV68 genetically modified to cause a defect in reactivation or establishment of latency.

We recently observed that a clone of recombinant MHV-68 carrying the mouse IFNα1 gene (MHV-68mIFNα1) shows a significant *in vivo* attenuation, which is mediated by the cytokine released during the course of the infection, and affects both the acute replication and spleen latency.

These results prompted us to evaluate the efficacy of MHV-68mIFNα1 in a prophylactic vaccination regimen aimed at inhibiting the symptoms of acute virus infection and the establishment of MHV-68 long-term latency after the infection. Here we show that mice vaccinated with MHV-68mIFNα1, administered as a live-attenuated or partially inactivated (by psoralen and UV exposure) vaccine, were protected against the challenge with wt MHV-68 from the acute infection and long-term spleen latency.

In general, the results presented herein confirm the efficacy of live attenuated latency-defective viruses in eliciting protection against all phases of MHV68 infection. In particular, our study proved the adjuvant activity of type I IFN, produced at the site of the infection by the vaccinating virus, in stimulating an anti-MHV68 immune response stronger than the one induced by the wild type virus. We believe that the ability of MHV-68mIFNα1 to produce IFNα, thus efficiently stimulating the immune system whenever it reactivates from latency, makes this recombinant virus a safer live-attenuated vaccine as compared to the latency-deficient clones previously described by others.

P 40 SERUM TGFβ1 AND NATURAL HISTORY OF TYPE 1 DIABETES IN NOD MICE AND HUMANS

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Type 1 Diabetes (T1D) is an organ-specific autoimmune disease characterized by the presence of Islet-related Autoantibodies (IrAbs) and the presence of insulitis with progressive loss of beta cells that ultimately leads to overt diabetes. The immunomodulatory cytokine TGFβ1 seems to have a relevant role in the protection from autoimmune diabetes. In fact, recent data show that regulatory cells with TGFβ1 dependent activity are able to restore self-tolerance in overtly diabetic NOD mice.

These develop a spontaneous form of autoimmune diabetes that mimics many features of the human disease. Our aim was to investigate the possible significance of serum TGFβ1 measurement in the natural history of diabetes in NOD mice, as well as in children positive for at least 1 IrAbs. Methods. Serum TGFβ1 (both total and active) was measured at monthly intervals in 26 NOD mice during the spontaneous development of diabetes, in a cohort of 60 schoolchildren from general population, and in 9 siblings of subjects with T1D with a follow-up of 4 years.

Results. Serum TGFβ1 fluctuations occurred in the prediabetic period both in NOD mice and humans and diabetes diagnosis followed a sustained reduction of active TGFβ1 (aTGFβ1) levels.

Moreover, in mice different increments in aTGFβ1 during the first weeks of life associated with severity of insulitis and percentage of insulin positive cells. Conclusions. Our data strongly suggest that in NOD mice fluctuations of serum aTGFβ1 mirror the course of insulitis during the natural history of diabetes.

The observed increase in aTGFβ1 is probably the expression of regulatory mechanisms put in place to counteract the auto-aggressive insulae inflammation and its progressive decrease marks the shift from controlled to destructive insulitis.

Furthermore, the preliminary observations reported in humans suggest that similar mechanisms might be operating also in prediabetic phase of T1D. Therefore, the possibility to follow the course of insulitis by measuring serum aTGFβ1 might represent

a valuable tool for deciphering the natural history of insulitis process during the silent phase of beta cell loss in subjects at risk for T1D. It might also be valuable in testing the effect of therapeutic intervention aimed at increasing immune-regulation involving TGFβ1.

P 41 THE SPECIAL ATTITUDE OF IFN-DC IN INDUCING T CELL PRIMING AND TH1 POLARIZATION: UNRAVELLING OF THE MECHANISMS

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We previously demonstrated that Dendritic Cells (DC) generated from human monocytes after a 3-day culture with IFN-alpha/GM-CSF (IFN-DC) are highly efficient in inducing a TH-1 type of immune response and $CD8^+$ T cell responses against defined antigens in different models, and are superior to CD40L-matured IL-4-DC, in inducing cross-priming of $CD8^+$ T cells against viral antigens. This property correlates with enhanced potential of IFN-DC to express cytokines of the IL-12 family, namely the specific subunits of the IL-23 and IL-27 cytokines.

In order to gain insight into the mechanisms of TH1 polarization by IFN-DC, we have recently studied the interactions between IFN-DC and autologous naïve $CD4^+$ T cells. Furthermore, based on IL-23 role in the generation and expansion of the newly discovered TH17 CD4 T lymphocytes, we have evaluated the capability of IFN-DC of promoting IL-17 production and the expansion of TH17 polarized autologous naïve CD4 T lymphocytes. In cultures of naïve CD4+ T cells exposed to the *Staphylococcus aureus*-derived superantigen SEA in the presence of autologous IFN-DC higher percentages of cells co-expressing CD25/CXCR3 and CD25/C212 (activated TH1 cells) and producing IFN-gamma have been detected, as compared to the cultures with conventional DC. These features were further enhanced when IFN-DC were pre-treated with the TLR7 ligand Imiquimod. On the contrary, CD4 T cells activated with IFN-DC have been found to produce very low levels of the cytokine IL-13 (TH2-associated cytokine) as compared to conventional IL-4-DC.

Interestingly, IFN-DC, when used to stimulate autologous CD4 naïve T lymphocytes in the presence of SEA, and particularly of anti-CD3 antibodies, favoured the appearance of CD4 T lymphocytes expressing the TH17 phenotype-associated markers CCR4 and CCR6, and promoted the production of significant levels of IL-17 in culture supernatant.

Altogether, these results suggest that IFN-DC, in addition to be directly licensed for an efficient $CD8^+$ T cell priming and to possess a special attitude to bias the T cell response toward a TH1 type, may also promote the expansion of TH17 CD4 lymphocytes. These features render IFN-DC particularly attractive candidates as cellular adjuvants for therapeutic cancer vaccines.

P 42 DIFFERENTIAL ACTIVITY OF *M. TUBERCULOSIS* ANTIGENS IN THE STIMULATION OF NEONATAL DENDRITIC CELL-MEDIATED IMMUNE RESPONSES

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Mycobacterium Tuberculosis (MTB), the causative agent of Tuberculosis, produces a disease which is generally limited to the primary infection stage. Host control of MTB relies heavily on the activation of CD4+ T cells by IL-12-producing APC, although the mechanisms that underlie the initiation of adaptive immune responses to MTB are still unclear. Priming of naïve T lymphocytes occurs in draining lymph nodes and is thought to be mediated by Antigen (Ag)-bearing Dendritic Cells (DCs) migrating from infected sites. Vaccine immunogenicity largely depends upon DC activation capacity, which can be enhanced by delivering the antigenic compounds in the presence of immunomodulators that act as adjuvants for DC activation.

Here, we have examined the immunomodulatory effects of several MTB antigens on DCs from neonatal mice. We show that among all Ag tested, a secreted MTB protein (hereafter: MTB secreted protein (MTBsp)) alone was found to promote both the differentiation and activation of neonatal DC. Exposure of BM-derived or splenic neonatal DC to MTBsp induced significant up-regulation of CD40, CD86 and MHC molecules which was accompanied to enhanced capacity to stimulate allogeneic CD4 and CD8 T cells. Similar responses were observed in DC derived from adult mice. Ag85B showed no stimulatory capacity on DC function, whereas HBHA slightly promoted MHC class-II-restricted T cell stimulation by DC. In addition, co-administration of MTBsp with Ag85B or HBHA strongly promoted DC activation, at levels comparable with other natural adjuvants, such as LPS or type I IFN. Of interest, injection of MTBsp, alone or in combination with Ag85B, but not of Ag85 alone, induced selective expansion of CD8α+ DC in the spleen. Our data suggest that MTBsp, unlike other MTB antigens, may function as an adjuvant for DC-mediated immune responses in both adult and neonates. We hypothesize that its co-administration with other immunodominant MTB Ag may be usefully exploited to augment DC immunogenicity especially in a system, such as the neonates, where these cells are hampered.

P 43 ASSESSMENT OF A PRELIMINARY PROTOCOL OF THERAPY AGAINST HEPADNAVIRUS CHRONIC INFECTION BASED ON A COMPLEX OF ANTI-PRES/S AND VIRAL PARTICLES (IGC)

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Immunomodulatory approaches as vaccine therapy, interleukins and antigen pulsed dendritic cells administration, applied in clinical trials, are promising but have produced non conclusive results. To this end the WHV/Marmotta Monax is a useful pre-clinical model on the study of treatment of chronic HBV infection.

The objective of the present work was to evaluate the antiviral effect of IGC administration in WHV DNA chronically infected woodchucks.

Woodchucks were vaccinated against HBV envelope with IGC, intravenously or intradermally administered. Sera samples were collected every two weeks. The follow-up schedule was 24-26 weeks. Levels of serum antibodies, to WHV core antigen (anti-WHc), WHV "e" antigen (anti-WHe), [WHV "e" antigen (WHeAg)] and anti-HBs, were determined. WHV genome copies were calculated using a TaMan Real-Time PCR.

The serological pattern (WHeAg, WHeAb, WHcAb) and WHV DNA viral load of one of the initially WHV negative woodchucks (w789), at time 0 and during the following 26 weeks after intravenous IGC administration. Compared with WHV DNA level of the WHV infected control woodchuck, lower WHV DNA levels were observed during the whole follow-up period. In particular at week 2 the WHV DNA was undetectable in one animal, and successively, 4 logs lower than the highest value (1 x 1,010 copies/ml, week 10) observed. Similar WHV DNA viral loads were observed during the follow-up in animals that were negative WHV DNA at the beginning of the study.

The IGC (composed of antibodies preS/S of HBV complexed with WHV viral particles) administration in WHV negative woodchucks produced a viral infection which, compared to the WHV infection in the control, was characterised by lower viral load and seroconversion from WHeAg to WHeAb. The use of IGC, in 2 out of 4 WHV chronically infected animals, showed a significant reduction of WHV DNA, WHeAg and WHeAb seroconversion. Further evaluation and better understanding of involved mechanisms could clarify the possible role of adjuvancy in combination therapies of such complexes.

P 44 FROM E-HEALTH TO PERSONALISED MEDICINE

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The research agenda of the TBS group is aiming at offering a complete solution for the management of molecular medicine in the standard care environment. The impact of the different -omics (genomics, proteomics, metabolomics,….) is already representing a very important part of the research effort in medicine and is expected to modify dramatically the model of delivery of healthcare service. The TBS group is facing this challenge through a R&D effort in order to transform its Clinical Information System into a complete suite for the management of clinical research and care pathways, supporting a completely personalised approach. The IT suite allows research to integrate the Electronic Clinical Records with data from technologies such as DNA and protein microarrays, data from diagnostic and molecular imaging, and workflow management solution. In this paper we will discuss the results from the participation to different European and national research projects, sharing this development aim. We provided the IT integration suite for projects for the identification of therapy-relevant mutations of tumor suppressor genes in colon cancer (MATCH EU project), for the genetic base for the impact of metabolic diseases on cardiovascular risk (MULTIKNOWLEDGE EU project) and for the identification of biomarkers for Parkinson Disease (SYMPAR project in Italy).

P 45 ETHICAL ISSUES IN TRANSLATIONAL RESEARCH

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"Knowing is not enough; we must apply. Willing is not enough; we must do" (Wolfgang Goethe): the importance of translational research is unquestionable. Like clinical research however, translational research needs ethical reflection.

In "Strangers at the bedside", a classical in bioethics literature published in 1991, D. Rothman described the shift which occurred in medical ethics during the second half of the Twentieth century: decisions traditionally left to the individual clinician's judgement became relevant in public debate and subject to external control (revision by ethics committees, international codes and national binding regulations). After the Seventies, this transformation was largely due to the nascent bioethics. Today a detailed regulatory system is completely assumed in clinical research. Does "bench science" need a similar mechanism? We are persuaded that the answer is affirmative. Episodes of ethical concerns about "bench science" fill the pages of bioethics and basic science's journals. Not all research raise weighty problems such as, for example, research on human embryos. Ethical problems however arise in the transition from laboratory science to research on human subjects: pursuit of beneficent purposes, safety, informed consent, therapeutic misconception, conflicts of interests in public/private collaborations, resource allocation, personal data protection and most of all risks due to attempts to move basic research too quickly into areas that have a specific therapeutic focus. The scientific community may still perceive public inputs as an undue intrusion in individual scientists' and physicians' domains. Both levels however are necessary: collective control is essential to ensure the fulfillment of ethical standards in translational research; but regulatory systems (ethical guidelines and procedures, peer-review and revision by ethics committees) cannot substitute to scientist's integrity, good will and ethical sensibility. The risk with public control is possibly diminishing ethics to a negotiated consensus; at the individual level the risk is awarding collectively relevant decisions to contestualist subjectivism. The ultimate goal of translational research is incorporating scientific discoveries into improved care and population health. This goal is achieved by developing methods and processes: in doing so, science must be accompanied by a honest search of ethical foundations.

P 46 EVALUATION OF MOLECULAR GENETIC MARKERS INVOLVED IN THE PROGRESSION FROM HCV-ASSOCIATED PRE-NEOPLASTIC LESIONS TOWARD HEPATOCELLULAR CARCINOMA BY cDNA MICROARRAY ANALYSIS

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Hepatitis C Virus (HCV) infection is a major cause of Hepatocellular Carcinoma (HCC) worldwide. The molecular mechanisms of HCV-induced hepatocarcinogenesis are not yet fully understood. Besides tissue inflammation and regeneration indirect effects, a more direct oncogenic activity of HCV can be postulated leading to an altered expression of cellular genes by early HCV viral oncoproteins. The aims of this study are the identification of molecular mechanisms involved in the progression from HCV-associated pre-neoplastic lesions toward HCC and the development of early diagnosis systems as well as new biotherapies for the control of progressing cancer lesions. Eleven primary and metastatic HCCs along with normal tissue from the same patients and seven normal tissues from matched non-neoplastic liver patients have been obtained from patients enrolled at the INT in Naples. A cDNA microarray analysis has been performed using chips spotted with 36,000 cDNA clones, representing 90% of the human genes. Of the 6,674 genes selected with an unsupervised Eisen's clustering analysis, a specific set (>100, on average) of genes showing a log ratio higher or lower than 3 has been identified among paired groups and are under further characterization. Informative data on the global pattern of gene expression in HCV-correlated HCC have been obtained. The analysis of gene expression patterns and pathways characterization in HCV-associated lesions, compared to normal liver tissue counterpart, will help in shedding lights on the HCV role in the HCC etiopathogenesis. Moreover, it should allow the identification of possible prognostic/progression markers as well as molecular targets for innovative therapeutic strategies.

P 47 MORPHOGENOMIC IMMUNE RESPONSIVENESS TO PREVENTIVE/THERAPEUTIC VACCINES

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An innovative *ex-vivo* immune "morphogenomic" strategy has been recently developed and validated by our group to screen the immune effectiveness of preventive/therapeutic vaccines on fresh human cells, as a surrogate of a pre-clinical/clinical model. In the present study, as proof-of-concept, global transcriptional profile of PBMCs stimulated with HIV candidate vaccine (VLPs) has been evaluated in HIV-infected patients with low/high viral load in comparison with healthy volunteers. PBMC from HIV infected patients showed signatures consistent with baseline profound activation of chemokine production. Immune stimulation with HIV-VLPs was not blunted by ongoing HIV-1 chronic activation but further enhanced. However, unlike the immune-activator LPS, neither *in vivo* HIV replication, nor *ex-vivo* stimulation with HIV-VLPs stimulates the activation of Interferon-Stimulated Genes (ISGs). This innovative *ex-vivo* "morphogenomic" strategy represents a highly efficient screening tool of vaccine immune effectiveness for guiding modifications/optimizations of vaccination strategies and understanding failures in individuals enrolled in clinical trials. In particular, it allowed to discover that pro-inflammatory stimuli, targeting the activation of ISGs, should be introduced in vaccine approaches to amplify the innate and adaptive anti-HIV-1 responses aiming to an effective cell-mediated viral clearance. We propose that future studies should take into account this findings for the discovery of potentially correlative patterns associated with the natural history of the disease and/or it response to treatment.

P 48 MICRORNA AND RARE TUMORS: THE HEPATOBLASTOMA EXAMPLE

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Hepatoblastoma (HB) is a rare liver cancer with an incidence of 1.5 cases per million children aged 6 months and three years.

The importance of Wnt/β-catenin signaling pathway in regulating liver cell proliferation, development and essential functions in the adult liver has been widely reviewed. Epidemiologists revealed an extremely high prevalence of HB in families affected by FAP, due to inactivation of the APC tumour suppressor gene, whose activity controls the down-regulation of β-catenin.

Deregulation of Wnt/β-catenin signaling pathway cause accumulation of β-catenin in the cytosol and in the nucleus, where interacts with various transcription factors of the TCF family to modulate the transcription of genes as c-Myc, Cyclin D1, etc. Overexpression of these genes is due to the progression HB.

An additional growth factor frequently implicated in human HB is Igf2 gene. Its up-regulation has been described in a high percentage of human HB and although the exact mechanism for this alteration is not entirely certain it appears that loss of imprinting and/or promoter demethylation are at least partly involved.

In 2005 microRNAs were cloned from human fetal liver. Five new microRNAs have been identified, one of these, the miR-483, is among more expressed in this tissue. miR-483 locates in chromosome 11p15 in one intron of the igf2 gene. Since HB patients show alteration in locus 11p15, it is therefore possible an involvement of this microRNA in the disease.

The up-regulated expression of Igf2, suggest that the IGF-axis may be involved in development of the tumor, therefore regulated by the same transcription factors. One of these is the β-catenin, which is translocated in the nucleus by the overexpression of IGF2. Using prediction algorithms of Microrna we discovered that β-catenin has a binding site for miR-483 in his 3'utr. We report that transcriptional activation of Igf2 also generates miR-483 from Igf-2 gene intron that regulates β-catenin protein expression. We suggest that miR-483 expression could contribute to fine tune Wnt/β-catenin signaling antagonizing the positive effect of IGF2 on β-catenin. Moreover, patients analysis show a peculiar pattern of microRNA in hepatocarcinogenesis compared to other liver cancer.

P 49 GSTM1, GSTT1, CONSTITUTIONAL FACTORS, SUN EXPOSURE AND THE RISK OF MELANOMA

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Cutaneous melanoma may result from a multi-factorial process involving both genetic predisposition and exposure to environmental factors. The Gluthathione S-Transferases (GSTs) are a family of enzymes known to play an important role in cellular protection against oxidative stress (*e.g.* UV radiation). This study was focused on a possible involvement of GST T1 and M1 polymorphisms in the risk modulation of cutaneous melanoma.

Within a case-control study conducted in the inpatient wards of the hospital IDI-San Carlo Rome, aimed to study risk factors for melanoma, individual patterns at two polymorphic genes (GSTM1 and GSTT1) belonging to Gluthathione S-Transferases family (GSTs) were investigated in 340 subjects (188 cases of cutaneous melanoma and 152 controls) by the Italian Workers' Compensation Authority. Information on socio-demographic characteristics, medical history, sun exposure and pigmentary characteristics was collected for all subjects. The polymerase chain reaction was used to identify the presence or absence of the GSTM1 and GSTT1 gene in DNA samples. Logistic regression was the method used to estimate odds ratio and 95% confidence intervals.

The frequency of GSTT1 null genotypes was higher in cases (55%) than controls (45%) but not for GSTM1 (56% and 54% respectively). Forty-four subjects (17 controls and 27 cases) out of 340 were carriers of both GSTM1 and GSTT1 null genotypes. In a multivariable model including age, sex, education, number of nevi, pigmentary characteristics, sun exposure and GSTM1 and GSTT1 genotypes, increased risks for melanoma were found for subjects with light brown (OR: 1.95; 95% CI: 1.11-3.44) and blonde and red hair colour (OR: 4.52; 95% CI: 1.83-11.1), increasing number of common acquired nevi (OR: 3.71; 95% CI: 1.89-7.31 for 25-59 nevi and OR: 5.94; 95% CI: 3.07-11.5 for 60 nevi or more), sunburns in childhood (OR: 2.22; 95% CI: 1.21-4.07) and for subjects carrying both GSTM1 and GSTT1 "null" genotype (OR: 1.55; 95% CI: 0.69-3.46).

Our results show an increased risk for melanoma for subjects with high number of nevi, light pigmentary characteristics, reported sunburns in childhood and with both GSTM1 and GSTT1 "null" genotypes. Further research is necessary for confirming the findings.

P 50 MODULATION OF MUCOSAL IMMUNE SYSTEM BY PROBIOTICS AND BACTERIAL PRODUCTS IN INFLAMMATORY BOWEL DISEASES

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In the last few years, we explored the possibility to modulate the mucosal immune-response in Inflammatory Bowel Diseases (IBD) by the use of Probiotic Bacteria (PBT) during the remission phase of the disease, or a microbial product (Cholera Toxin B subunit: CTB) during the active phase of the disease. To this end we performed both *in vivo* studies in experimental murine colitis and *in vitro* studies involving intestinal tissue from IBD patients. We found that:

- PBT administration over a 3-week period to mice that have recovered from an initial induction of TNBS colitis reduces the severity of a second induction of TNBS-colitis. This protection was associated with the appearance in the lamina propria (LP) of $CD4^+$ $CD25^+$ regulatory T cells and IL-10 -dependent $CD4^+$ Latency Associated $Peptide^+$ (LAP) T cells bearing cell-surface TGF-beta in its latent form as observed in normal mucosa after a mild and/or transient breach in epithelial barrier function. $CD4^+$ LAP^+ T cells proved to be the protective cells since LP cell populations from probiotic treated mice depleted of these cells failed to transfer protection from colitis in recipient mice. We then conducted an open-label study, in which patients with ileal pouch-anal anastomosis for ulcerative colitis at different periods from surgery without signs and symptoms of pouchitis were randomized to PBT or no treatment for 12 months. Patients treated with PBT showed a significant reduction in the pouchitis disease activity index score associated with a significant reduction of tissue IL-1 beta, a significant increase in the percentage of mucosal $CD4^+$ $CD25^{high}$ and $CD4^+$ LAP^+ cells compared with baseline values.
- Oral administration of CTB was able to prevent and cure TNBS-colitis and this effect is associated with reduction of mucosal IL-12 and IFN-gamma production. We observed similar effects also in tissue cultures of human intestinal specimens obtained from patients with IBD. CTB is also able to reduce IL-12 production by human Monocyte Derived Dendritic Cells (MDDC) and CTB-pretreated MDDC are able to reduce IFN-gamma production of cocultured naïve T cells. In conclusion, probiotic bacteria and microbial products might represent therapeutic options in IBD.

P 51 MECHANISMS OF ACTION OF *MALALEUCA ALTERNIFOLIA* (TEA TREE) OIL ON *CANDIDA ALBICANS* ISOLATED FROM HIV$^+$ PATIENTS

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The essential oil of *Melaleuca alternifolia* (TTO) exhibits broad spectrum activities such as antimicrobial, antifungal and anti-inflammatory functions. In fact there are many susceptibility data on a wide range of gram-positive and negative bacteria and the antifungal activity of TTO against yeasts and dermatophytes is reported in several papers.

TTO is composed of approximately 100 composed, mainly monoterpenes, sesquiterpenes, and their alcohol derivatives, among with major components include terpinen-4-ol.

In our study we investigated the effects of TTO treatment against the yeast *Candida albicans*. Proliferation study showed that TTO was effective both on fluconazole-susceptible (3153) and on -resistant (AIDS68) strains of *C. albicans* isolated from HIV$^+$ patients. The cells of the two strains were grown in the presence of TTO at the concentration ranging from 0.25% to 1% for 2, 10, 30 and 60 min. The analysis by fluorescence microscopy after Hoechst staining revealed an evident chromatin condensation in 3,153 cells at 2 min, while in AIDS 68 cells it appeared at 30 and 60 min. Other morphological alterations typical of apoptosis, such as nuclear fragmentation, was never observed at any time of treatment. In order to clarify the mechanisms of action of TTO, we analyzed the cell cycle of the same samples by flow cytometry (FACS) after propidium iodide staining. DNA histogram analysis revealed that TTO treatment induced an accumulation in G1 phase. Moreover, antifungal effect was detected by FACS analysis of PI-treated cells. The results obtained revealed that TTO was able to induced membrane alterations as observed by an increased of PI-positive yeast cells. These preliminary findings seem to indicate this natural product as a new antimycotic agent with a special mechanism of action.

P 52 m-THPC-MEDIATED PHOTODYNAMIC THERAPY OF MALIGNANT GLIOMAS: ASSESSMENT OF A NEW TRANSFECTION STRATEGY

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Malignant gliomas represent the most common primary brain tumor: more than 50% of them are Glioblastoma Multiforme (GBM). Photodynamic therapy may offer a very good chance of targeted destruction of infiltrating GBM cells, thus increasing the survival time and recurrence-free interval of GBM patients. Among photosensitizing agents, meta-Tetrahydroxyphenylchlorin (m-THPC) is promising for the treatment of brain tumors. In this study we investigated the transfection activity of Dimyristoyl-sn-glycero-phosphatidylcholine (DMPC) liposomes, containing a cationic gemini surfactant, loaded with m-THPC on human (A172, U118, DBTRG, LN229) and murine glioblastoma cell lines (C6). The uptake (flow cytometry) and the intracellular distribution (confocal microscopy) of m-THPC, loaded in several formulations of cationic liposomes, were analyzed by making a comparison with those obtained by employing the same chlorin in the pharmaceutical form (Foscan®). Moreover, by cloning efficiency assay the potential therapeutic efficiency of chlorin delivered by liposome formulations was compared to that of the pharmaceutical compound, before and after irradiation with laser light at 652 nm.

The results obtained by *in vitro* tests indicated that cationic liposomes: i) transferred m-THPC in glioblastoma cells more efficiently than pharmaceutical formulation; ii) significantly ($p << 0.001$) increased the m-THPC cytotoxic effect after laser irradiation; iii) seemed to exert their cytotoxic action in the early phase of interaction with the cells, during adhesion to the plasma membrane.

P 53 CROSS-KINGDOM VACCINES: DOGMA AND HERESY

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A vaccine made up by an algal β-glucan (laminarin; β-1-3 glucan with occasional β-1-6 single glucose side chains), conjugated with diphtheria toxoid as a carrier protein component, protects against infections by different fungi and induces antibodies capable of inhibiting fungal growth. This is a sort of "cross-kingdom" vaccine because the immunizing antigen and the vaccination target belong to two different kingdoms, and this is certainly the first case in the field of human vaccines, which are generally based on the dogma "one or more specific antigens against one disease or syndrome". Thus, it is "heretically" possible to convey in a single immunological tool the potential to protect against multiple infections, in our case all those caused by β-glucan-expressing microorganisms (practically all human pathogenic fungi and some bacteria). The generation of antibodies with the potential of directly inhibiting the growth of, or killing the fungal cells also opens an exciting perspective for both active and passive vaccination in immunocompromized subjects.

The above approach could be theoretically extended to non-fungal infections by selecting the appropriate molecular pattern shared by a given microbial group (*e.g.* peptidoglycan for Gram positive bacteria). Noteworthy, the molecular patterns are those microbial molecules which foster innate immunity through their binding to the pattern-recognition structures on host cells. Thus, single-component, molecular pattern-based vaccines would merge the broad target range typical of innate immunity with the highly focussed specificity of the adaptive immunity.

P 54 ROLE OF LOX-1 FOR UPTAKE OF APOPTOTIC CELLS AND ANTIGEN CROSS-PRIMING OF CD8+ T CELLS IN PLASMACYTOID-LIKE IFN-α-CONDITIONED DENDRITIC CELLS

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Dendritic Cells (DCs) are potent antigen-presenting cells whose phenotype and functions are shaped by cytokines. In this study, we have correlated the phenotype and gene expression profiles of IFN-α-conditioned monocyte-derived DCs (IFN-DCs) with their special attitude in the uptake of apoptotic cells and antigen cross-priming of $CD8^+$ T cells. IFN-DCs showed the phenotype of activated $BDCA2^+$ plasmacytoid DCs and over-expressed an ensemble of genes belonging to DC-mediated immunological pathways and to Scavenger Receptor family, including the Hsp70 binding receptor LOX-1, as compared to IL-4-derived DCs. In IFN-DCs, mAbs to LOX-1 inhibited: i) Hsp70 binding; ii) Hsp70-induced functional activation; iii) uptake of apoptotic bodies. The LOX-1-mediated capture of apoptotic cells by IFN-DCs was associated with subcellular rearrangements of MHC class I and LAMP-2 antigens. Notably, IFN-DCs pulsed with allogeneic cell-derived apoptotic bodies induced a marked cross-priming of autologous $CD8^+$ T cells reacting against MHC class-I restricted antigens. This induction was inhibited by anti-LOX-1 antibody. The overall results demonstrate that LOX-1 represents a key molecule of IFNα-conditioned-DCs for the uptake of apoptotic bodies as well as for subsequent antigen cross-priming of $CD8^+$ T cells, unraveling a potentially novel mechanism by which IFN-α can shape the immune response in physiological and pathological conditions.

P 55 PROTON PUMP INHIBITORS INDUCE DEATH OF HUMAN METASTATIC MELANOMA THROUGH A CASCADE OF EVENTS FOLLOWING PROFOUND DISTURBANCE OF CELLULAR pH GRADIENTS

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The acidic tumor microenvironment has an important role in cancer progression and malignancy. The alteration of tumor acidification following treatment with Proton Pumps Inhibitors (PPI) underlies two different antitumor effects: i) sensitization of drug-resistant tumors to chemotherapeutics in solid tumors; ii) direct apoptosis-inducing activity on human lymphoblastic tumors. We tested here the *in vitro* and *in vivo* molecular and biochemical mechanisms underlying the antineoplastic effects of PPI on human metastatic melanoma. First, we observed that melanoma cells derived from metastatic lesions are more sensitive to the antiproliferative effects of PPI when cultured in acidic medium conditions. Consistently, PPI-induced apoptotic cell death in human melanoma was clearly increased by the acidic pH of the culture medium. The pro-apoptotic effects of PPI were dependent on Reactive Oxygen Species (ROS) - and activated caspases since both ROS scavenging and caspases inhibitors completely abrogated apoptosis. Acidification of cytosolic pH and alkalinization of lysosomal vesicles were observed after treatment with PPI. The pro-apoptotic effect of PPI was associated with the accumulation of autophagic vacuoles and a significantly increased PPI-induced apoptosis when autophagy genes were knocked-down, suggesting that PPI may inhibit the autophagic flux.

The antineoplastic activity of PPI was clearly demonstrated *in vivo* in SCID mice engrafted with human melanoma cells. PPI treatment significantly delayed the growth of human melanomas and such effect was associated with a profound modification of the intra/extracellular pH gradients as measured by magnetic resonance spectroscopy analysis. Indeed, PPI induced a rapid increase of the tumor extracellular pH associated with acidification of cytosolic pH, causing a substantial normalization of pH gradient across the plasma membrane. The data presented underline the importance of pH regulation in tumor malignancy and provide insight into the identification of tumor acidity as a potential novel target for antitumor therapy.

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