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Animal Models & Translational Medicine: Quality and Reproducibility of Experimental Design

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MAIN LECTURE AND ORAL PRESENTATIONS

The Translational Mouse: From Genetic Models to the Mouse Hospital and Co-Clinical Trials, for a Novel Understanding and Treatment of Cancer

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The Co-Clinical Trial Project is a cutting-edge new platform for clinical trial optimization, which we have developed at our Cancer Center at Harvard. The platform also rests on a "Mouse Hospital" infrastructure, which is equipped as a human hospital, if not better, to perform experimental clinical trials in mouse models of disease, exactly as they would be run in the human hospital. In the "Co-Clinical" Approach for Cancer Therapy optimization, mouse models of cancer, which are representative of the diversity of human cancer, are treated with the same drug, and following the very same clinical protocol offered to human patients enrolled in experimental clinical trials in the human hospital. This allows for "mice-to-human" stratification and cross-validation of response and resistance to specific treatment modalities. Drugs can be tested on "Immune-deficient" mice that are transplanted with human tumors derived from biopsies (Patient Derived Xenographs: or PDX). Importantly, however, "Co-Clinical" Trials can also be run by enrolling "immune-competent" genetically engineered mouse models of cancer (GEMMs) bearing genetic mutations associated to human cancer to assess how the various cancer genetic make ups and therapeutic treatments impact and are impacted by the immune microenvironment. Exciting new data from these platforms and on-going analyses will be presented.

Good scientists and Good Storytellers

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In UK and US to be good scientists it also means being good storyteller. A few years ago, the literary agent of the world's greatest scientists, John Brockman, wrote a book, "The Third Culture," in which great researchers told the public about the meaning of their researches. Italian researchers are now aware of how important it is to disseminate their researches, even for their public acceptance. In his speech, Luca Carra sets out some basic rules for spreading science well, and explains why it is important for scientists to do so.

Information, Disinformation, and Percezione

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Performing good science and providing valuable information on science is critical, among others, for its public perception. A key problem is providing a satisfactory definition of (or re-defining) science, in the light of the enormous changes that have occurred in the second half of XIXth century, e.g., the growth of what we call "big science". And ask ourselves whether a changed science has maintained its fundamental values. Even a cursory analysis of current science seems to indicate that not all the essential components of the extraordinary enterprise we call science are still highly valued, and that too many exceptions, often hidden behind the "global enterprise", are every day under our eyes. With potentially devastating effects on both science prosperity and science perception. Another key problem relates to science dissemination, and how information, bad information or disinformation may shape an altered public perception of science. Several "spectacular" examples of inappropriate communication of science will be illustrated, with an emphasis on some recent cases in the field of neuroscience. This problem will be discussed, and a personal vision presented on a highly debated issue, the role in researchers in science communication.

Experimental Design Optimization: From Design to Reporting—Talk On ARRIVE Guidelines And Experimental Design Assistant (EDA)

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The reproducibility of biomedical research using animals has come under scrutiny in recent years, and quality standards in

the design, analysis and reporting of in vivo research have been flagged as concerns. The NC3Rs has been working in this area over the last ten years and led the development of two key resources to support researchers and improve the design, analysis and reporting of *in vivo* experiments. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines consist in a 20 item checklist, which summarise the minimum information necessary to describe a study in a comprehensive and transparent manner. The guidelines cover the main aspects of a scientific publication and make recommendations on the reporting of the study design, experimental procedures, animal characteristics, housing and husbandry, and statistical analysis. Several studies are investigating the impact of the guidelines and their usability. The Experimental Design Assistant is a web application with a supporting website, which helps researchers design animal experiments, by increasing the transparency of the experimental plan, and providing feedback and dedicated support for randomisation, blinding and sample size calculation. The objective of these resources is to maximise the output of research using animals. Wide dissemination and uptake are essential to ensure the science emerging from animal research is fully exploited.

Systematic Review of Animal Studies: Indispensible for Experimental Design and Translational Value

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Systematic literature reviews (SRs) have become mainstream in evidence-based clinical medicine. For animal studies, SRs are not yet commonplace, but more and more animal researchers perform SRs, and important initiatives are arising to promote them. In the presentation, I will provide a general introduction on SRs. SRs comprise multiple steps: Formulating the research question; Writing a protocol; Defining specific inclusion and exclusion criteria; Systematically searching; Selecting papers; Assessing study quality; Data extraction; Data synthesis; and Interpreting the results. Data synthesis may or may not comprise a form of meta-analysis. Because of their comprehensive search strategy, transparent methodology and explicit inclusion and exclusion criteria, systematic reviews should be more objective than narrative reviews. I will discuss the methodology to perform a thorough SR step by step, focussing on animal studies. Methodology for SRs of animal studies is still less developed than that for clinical trials, but progress is being made. For example, by now specific filters are available for searching animal studies in several literature databases, as well as specific methods for assessing the quality of the included evidence. The applicability of systematic reviews to optimise the experimental design of new animal studies will be illustrated with case studies on cystic fibrosis; microdialysis experiments measuring intracerebral adenosine; and methotrexate in rheumatoid arthritis. I will address the use of SRs for animal model choice and experimental design. With these case studies, examples will be provided of how to manage unexpected problems in the review process to ensure a useful product.

Statistics as optimization tool

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The target function of any experimental approach can be roughly expressed as the maximization of the ratio R = 'variance explained by the model' / 'cost of resources'. Statistical thinking has a great role in the maximization of R, intervening at different levels of definition of the experimental plan. Here I will present both classical optimization procedures linked to the computation of the power of the study (with a special emphasis on often neglected aspects like the definition of 'minimal effect size' and 'pre-study odds') as well as emerging problems linked to the so called 'curse of dimensionality' arising from the huge number of variables the experimenter can get on the same statistical unit thanks to recent high-throughput techniques.

Experimental Autoimmune Encephalitis

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Since its first description, experimental autoimmune encephalomyelitis, originally designated experimental allergic encephalitis (EAE), has been proposed as animal model to investigate pathogenetic hypotheses and test new treatments in the field of central nervous system inflammation and demyelination, becoming, in the last thirty years, the most popular animal model of multiple sclerosis (MS). This experimental disease can be obtained in all mammals tested so far, including non human primates, allowing very advanced pre-clinical studies. Its appropriate use has led to the development of the most recent treatments approved for MS, demonstrating also its predictive value when properly handled. Some of the most exciting experiments, opening new perspectives in immunology and neuroscience, have been performed in this model.

Animal Models of Traumatic Injury of the Spinal Cord for Preclinical Studies

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The poor therapeutic repertory for traumatic injuries of the central nervous system represents a primary medical and economical problem for the society. A major issue in the field is how to establish appropriate scientific standards to ensure reproducibility of preclinical studies aimed to proof-of-concept and efficacy studies. This discussion also coincides with the increasing concern of the scientific community on how to increase data reproducibility for preclinical data. In fact, the poor reproducibility in preclinical studies is producing a substantial dis-investment of big-pharma in the field, and is also impacting on the reliability of translational neuroscience. Starting from lab experience and literature on the traumatic spinal cord injury models, the following points will be discussed:

1. Laboratory *vs* large animals;
2. Pro- and contra- of available methods for traumatic spinal cord injury;
3. Neurological deficit scoring: direct observation and related clinical scales *vs* computerized video-tracking techniques;
4. How to establish the appropriate number of animals to be included in a study
5. How to establish the appropriate primary and secondary end-points;
6. How to quantity the lesion's area *vs* lesion's volume;
7. Multiple site studies, data repository, data sharing and Standard Operating Procedures;
8. In vitro models: something helpful for spinal cord injury?

The 3R rules will be the pivot for the discussion, as follow: Replacement: how to establish the appropriate end-points for in vivo studies? Reduction: not one more, not one less. Refinement: standardization of lesion models, the intra- e inter-labs variability and reproducibility of complex animal models.

Il Ruolo del Modello Animale Nello Studio Della Malattia di Alzheimer: Un Esempio di Ricerca Traslazionale

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The mouse has a high degree of phylogenetic conservation when compared to humans in the structure and function of both hippocampus and entorinal cortex circuits; these brain structures mediate memory function and are particularly vulnerable to Alzheimer's disease. The mouse has a number of genes and a chromosomal organization similar to humans. At the same time, mouse models provide a simplified system that facilitates experimental manipulation. Knowing the limitations that each research tool displays, there are important aspects to keep in mind when evaluating the results obtained in mouse models. During the presentation, experimental observations, carried out on a murine model of Alzheimer's disease, will be discussed. We recently demonstrated that an early degeneration of a brain mesenchephalic area containing dopamine neurons (i.e, ventral tegmental area, VTA) causes a reduced release of dopamine (neurotransmitter produced by dopaminergic neurons in the ventral tegmental area) in areas that regulate some cognitive functions (such as memory) but also some behaviours. This result is of particular interest as even in the early stages of human disease there are cognitive and non-cognitive symptoms similar to those observed in the experimental model. This study opens up to further neuroimaging surveys on patients on a brain area that has not been considered in the context of the initial stages of Alzheimer's disease.

Cancer stem cells and genetic barcoding in murine model to study gliomagenesis

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During gliomagenesis, cells accumulate mutations and gain malignancy. By now, it is unclear how easily this process can be undertaken by the cells, because it is hard to directly measure the probability of a cell to become a glioma in vivo. Combining genetic barcoding and NGS allows to face this task without the need of a huge number of animals, by allowing to follow the fate of thousands individual cells at the same time in each animal. Our study is based on a well-characterized murine model of gliomagenesis, induced by transducing PDGF-B in embryonic neural progenitor cells in utero. Each PDGF-transduced cell is univocally labelled by a degenerated barcode sequence included in the transducing vectors. After their development, gliomas can be harvested and analyzed by NGS. By using in-house developed software, we retrieved barcodes from tumor masses and reconstructed the clonal composition of independent tumors at different stage of progression. The analysis allowed accounting the size of the different clones, estimating their contribution to the whole tumors. Our data show that early stage gliomas, unable to graft when transplanted in adult mice, are typically composed by about one thousand independent clones. In contrast, progressed gliomas, able to root when grafted in adult mice appear much less complex, with a mass being composed mostly by one or two clones and, in total, by less than 100 clones. Our results show that while initial stages of tumorigenesis are rather unselective, the path towards full-blown malignancy is much harder to be undertaken.

An In Vivo Model of the Human Hematopoietic Stem Cell Niche

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The bone/bone marrow microenvironment that maintains the behavior of hematopoietic stem cells (HSC) by controlling stem cell self-renewal and differentiation has been defined as stem cell niche. Within the last few years, several *in vivo* models of bone marrow niche have been generated in order to mimic the native human hematopoietic microenvironment and to reproduce the range of molecules that interact with human HSC. We established a model in which cartilage pellets differentiated *in vitro* from human mesenchymal stromal cells (MSCs) generated complete ossicles upon heterotopic implantation in SCID/beige mice in the absence of exogenous scaffolds, reproducing the human HSC niche *in vivo*. Of note, a near-perfect architecture of a miniature bone organ, including cortical bone, marrow cavity, donor-derived marrow stroma, host-derived sinusoidal circulation, and host-derived hematopoietic tissue developed in a timely manner. In addition, we demonstrated that human HSCs injected into ossicle bearing mice could stably engraft into the generated human niche, showing inside the ossicles the presence of engrafted human HSCs and derived multilineage reconstitution. The novelty of this model allows for the use of a completely humanized system, without the presence of artificial scaffolds, in which the recreated niche is derived from human normal MSCs, patient-derived MSCs and/or MSCs modulated to express or silence key molecules. In particular, this model allows for the generation of a disease-specific humanized niche that mimics the real dynamics of the affected bone marrow microenvironment.

Patient-derived Xenografts (PDX) as Models for Oncology Drug Development

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One of the reasons for high failure rate of new drugs in oncology is the lack of appropriated preclinical models. In vitro cell lines have been extensively used for drug development but their clinical predictive value is modest. The ability to successfully engraft surgical-derived tumors from cancer patients has been established for the majority of tumor types. These preclinical models of patient derived xenografts (PDX) are being consistently characterized and used for drug development in oncology. Although they faithfully recapitulate the genetic and biology of human tumors, debated is their predictively for patient drug response. Opportunities and limitations of the PDX in translational research and drug discovery will be discussed. An integrated platform of ovarian cancer PDX, combining their molecular and biological characteristics with drug response will be presented (Ricci et al. Cancer Res. 2015). Examples on the antitumor activity of target selective novel drugs, the optimization of combination treatments with standard therapy, including angiogenesis inhibitors, and the role of the microenvironment in drug response and malignant behavior will be discussed.

INFRAFRONTIER: the European Research Infrastructure to Study Mammalian Gene Function

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INFRAFRONTIER is the European research infrastructure for the development, phenotyping, archiving and distribution of mouse models for the study of human genetic diseases. The European Mouse Mutant Archive (EMMA), a core activity of INFRAFRONTIER, is a public repository of mouse resources. It preserves scientifically valuable resources that may otherwise get lost due to breeding failures, pathogen infection or genetic contamination, and distributes them upon request. It applies extensive quality control to distribute only genetically standardized mammalian models in specific pathogen free (SPF) condition. EMMA repository is the third largest mouse repository in the world and cryopreserve thousands of models of rare and common human diseases. INFRAFRONTIER thus contributes to improving the quality, validity and reproducibility of research using high quality animal models. It actively promotes the global sharing of mouse resources making them publicly available thtough the website www.infrafrontier.eu. INFRAFRONTIER provides public access to systemic phenotyping in the new mouse clinics. This allows obtaining a truly systemic understanding of the biology of mammalian diseases. The INFRAFRONTIER mouse clinics use operation procedures that are highly standardised on an international level, the resulting phenotyping data are deeply annotated and linked to complementary preclinical and clinical datasets. INFRAFRONTIER also puts a lot of effort into the development of new technologies that refine existing experimental procedures to reduce animal numbers and increase reproducibility of results. The INFRAFRONTIER partners are also major contributors to the International Mouse Phenotyping Consortium (IMPC), which aims to generate a comprehensive open-access catalogue of mammalian gene function.

Biobanks and the Italian Node of BBMRI

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Biobanks are repository created to safeguard our most valuable asset, biological samples, that would be used for research. Imagine the power of thousands or even millions of samples ! Biological resources – living organisms, cells, genes, and related information – are the essential raw material for the advancement of biotechnology, human health, and research and development in life sciences. BBMRI-it, the Italian Node of the European Research Infrastructure for Biobanking and BioMolecular Resources (BBMRI-ERIC), is a research infrastructure involving biobanks and biological resource centres located throughout Italy. BBMRI.it includes 18 universities, 23 IRCCS, 40 hospitals, 290 research groups and 90 biobanks. The main goals of BBMRI.it are i) to take Italian collections of biological resources, Biobanks and networks to a new level of coordination and efficiency, ii) to supply new common services for the community of biobanks, iii) to provide better access for users from public and private sector. To reach these goals BBMRI.it set uo Common Services. The CS IT created the IT infrastructure developing tools to improve interoperability of research databases. The CS Quality has been implemented to monitor biobanks and biomolecular resources, providing information on guidelines/best practices, harmonizing operational procedures, implementing the quality management system criteria, promoting training on the issues of quality. The CS ELSI works at the service of all stakeholders to ensure ethical and legal compliance.

Rodent models to assess the plasticity and the functional role of the human gut microbiome Patrizia Brigidi*

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The metabolic performance of the gut microbiome modulates and regulates several aspects of the host physiology, such as enhancement of the digestive efficiency and modulation of energetic homeostasis, competitive barrier against colonization/invasion, development, education and function of the immune system, central nervous system modulation, and endocrine system modulation. Thus, changes in the phylogenetic and functional profiles of the gut microbiota play a role in our health and disease status. The effects of this complex microbial ecosystem can be studied by functional data, gained from metagenomic sequencing of different physiological/pathological condition, observation of human phenotypes, but one the most powerful way to observe the direct impact of the intestinal microbiota on host health is the use of rodent models, including conventional, germ-free and humanized animals. Indeed, the adoption of these animal models allows to measure the impact of gut microbiota-host mutualism on several organs/tissues, i.e the immune system (Payer patch number, T cell differentiation, mucosal iNKT tollerance), the gastrointestinal tract (intestinal permeability, epithelial cell turnover, mucus thickness), the central nervous system (synaptogenesis, changes in behavior, stress reactivity, anxiety-like behavior), the endocrine system (regulation of sex hormones), besides the effects on nutrition (energy extraction and storage, body fat) and protection (barrier effects, pathobionts increase, SCFA-producers decrease). In this perspective, it is mandatory to optimize the reproducibility of the animal models in preclinical studies, identifying and standardizing the more contributing factors.

POSTER SESSION

An Innovative Translational Approach to Oxaliplatin Induced Peripheral Neurotoxicity in Animal Models: Nerve Excitability Testing

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Oxaliplatin is the cornerstone for colorectal cancer treatment; its use is limited by peripheral neurotoxicity, known as Oxaliplatin-Induced Peripheral Neurotoxicity (OIPN). There is no cure for this condition that affects a large proportion of cancer survivors; thus, OIPN treatment has become a hot topic in Oncology practice. OIPN consists of a chronic condition (sensory loss and neuropathic pain at limb extremities) and an acute one. Transient ion channel dysfunction has been suggested to cause acute neurotoxicity: patients experience transient cold hyperalgesia and cramps/spasms, after administration (lasting 24-72 hours). A worse acute neurotoxicity has been related to a more severe chronic one. Ion channels are mainly studied through *in vitro* techniques. However, also *in vivo* studies should be performed to test new drugs to cure OIPN. Our aim was implementing advanced neurophysiology in animal models to better understand OIPN pathogenesis, pos-

sibly relating acute and chronic phenomena. Nerve Excitability Testing (NET) is suitable to test axonal hyperexcitability induced by Oxaliplatin in *in vivo* experiments. We obtained a full NET profile of significant changes in our animal model of acute and chronic OIPN (Wistar female rats). Thus, we verified NET highly translational potential: the *in vivo* NET changes in animals can be matched, in fact, to findings from *in vitro* experiments focused on ion channels; moreover, NET is easily applied in humans and has yet been used to demonstrate acute phenomena in Oxaliplatin treated patient. Our next step is testing drugs able to modulate acute neurotoxicity as tentative prevention of chronic OIPN.

Linking Sox2 Activity to Its Downstream Effectors in Glioma Maintenance, Towards the Definition of Therapeutic Targets

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We previously demonstrated an essential role of transcription factor Sox2, well known as a "staminality factor" in normal embryo development, for the maintenance of neural cancer stem-like cells using a Sox2 conditional deletion mutant in a mouse model of PDGF-induced high-grade glioma (pHGG). Transplanting wild-type pHGG cells into mouse brain generated lethal tumors, but mice transplanted with Sox2-deleted cells remained tumor-frees. Cultured Sox2-deleted pHGG cells show decreased growth-rate, activation of glial differentiation, and increased cell death compared to pHGG cells that express Sox2. Microarray analysis identifies early gene expression changes following Sox2 deletion. Intrestingly, genes overexpressed following Sox2 loss are enriched in known oncosuppressor factors. In this study, we manipulated the expression of various of these Sox2-target genes within non-Sox2- deleted pHGG cells, to ask if we can reproduce the loss of tumorigenicity obtained with Sox2 deletion, towards the definition of therapeutic targets.

Overexpression of four downstream Sox2 target genes encoding known oncosuppressors (Zfp423, Ebf1, Hey2 and Cdkn2B), though not of others (Hopx, Wif1, Sdc4, Cryab, Rgs2), reproduces, to varying degrees, the Sox2-deleted pHGG cells phenotype. pHGG cells overexpressing these downstream Sox2 targets show growth-rate reduction compared to normal pHGG cells. Moreover, overexpression of Zfp423 and Cdkn2B glial differentiation. These *in vitro* data suggest that we identified four key effectors in the loss of tumorigenicity of Sox2-deleted pHGG cells. One next important step will be the transplantation of pHGG cells overexpressing the four factors in mouse brain to address the consequences on *in vivo* tumorigenicity.

Examples of Pathologies in Rodents that Cause Them to Be Excluded from Experimental Procedures

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The Designated Veterinarian and the Responsible of Animal Welfare, as established by the law decree D.Lgs 26/2014, have the important task of monitoring the health and housing conditions of laboratory animals in order to avoid inflicting avoidable pain and discomfort before, during and after experimental procedures. Sometimes animals show signs of malaise that require intervention to temporarily interrupt the experimentation or completely exclude a subject from the procedures. The Designated Veterinarian and the Responsible of Animal Welfare's role is also to communicate with the researchers and explain to them what problems have emerged so as to decide together what strategy to follow in order to safeguard both the animals' wellbeing and the scientific data. In this organization, the housing facility technicians' role is fundamental since very often they are the first ones to notice the above-mentioned signs of malaise. We will present some examples of pathologies that have caused the temporary or permanent exclusion from experimental procedures of rodents housed in a conventional housing facility. We will describe the diagnostic steps, starting from non-specific initial symptomatology, moving to the general objective examination and to the diagnostic support in the lab (virological, bacteriological, parasitological analysis, etc.), finishing with the possible anatomopathological confirmation.

Research with Farm Animals

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Several large mammals, including sheep and goats are commonly used in research in a variety of fields including orthopedic, as a model of cardiovascular conditions and infectious disease. The main problem using this models is the data standardisation, in fact some study, in particular about the prevalence of patogenous, clinical study or drugs testing effects6, were conducted in commercial farms, and therefore do not fall under the scope of the European Directive (2010/63/EU) on the protection of animals used for scientific purposes, or samples were collected after slaughter. Working as clinician in those rural contest, during routine practices like parasitological surveillance or serologic infectious screening and ultrasound pregnancy diagnosis, we observe that genetic variability and animals management (animal's diet, and endo- ecto parasitosis prevention protocols or vaccinations) are the most important factors that influence ours diagnosis, like for experimental data. We obtain some dates that shown the relationship between genetic variability and animal management in different farms. We observe that genetic uniformity is less important than breeding conditions, in fact clinical dates is more comparable in farmers that use the same management respect farmers with similar genetic animals but different management. We want to provide our practical experience to improve data understanding and improve experimental design, selecting the farmers looking at animals genetic uniformity and similar management conditions, as though in the field of basic research. We hope that clinic applied research communicate with basic research to get more similar experimental designs.

Combinatorial Control of *Spo11* Alternative Splicing by Modulation of RNA Polymerase II
Dynamics and Splicing Factor Recruitment during Meiosis

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Homologous recombination and chromosome segregation in meiosis rely on the timely expression of two splice variants of the endonuclease SPO11, named a and b. However, in spite of its physiological importance, the mechanism underlying *Spo11* alternative splicing in meiosis is still unknown. By screening the activity of factors that are predicted to bind the alternatively spliced region of *Spo11*, we identified hnRNPF and H as key regulators of this splicing event in mouse spermatocytes. Although neither hnRNP was up-regulated in meiosis concomitantly with

the switch in splicing, their recruitment to *Spo11* pre-mRNA was favored by selective modulation of RNA polymerase II (RNAPII) phosphorylation and processivity in proximity of the regulated exon. Furthermore, antisense oligonucleotides masking the hnRNPF/H binding sites recapitulated exon 2 skipping and SPO11a splicing, suggesting that hnRNPF/H act by competing out positive splicing regulators. Remarkably, knock-in mice lacking SPO11a proceeded through meiosis with defective X-Y chromosomes segregation, generating aneuploid gametes. Thus, our work reveals that modulation of RNAPII dynamics in concert with hnRNPF/H recruitment exerts a combinatorial control of the timely-regulated *Spo11* splicing during meiosis, which is essential for gamete ploidy.

Newly Developed Multi-Cistronic Platform for Producing Multi-Genes Genetically Modified In Vitro and In Vivo Models in a Single Transgenesis Experiment

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Oxidative stress is a major and recurring cause of damage during inflammation, as following ischemia-reperfusion injury (IRI) in organ transplantation. Increasing evidences report ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1) and ecto-5-nucleotidase (E5NT), two main ectonucleotidases of purinergic signaling, and heme oxygenase-1 (HO-1) as key molecules in down-regulating inflammatory response in several pathological contexts. Several biomedical applications, such as xenotransplantation, require a multiple genetic-engineering approach to ensure a successful outcome. Advances in genetic engineering technologies have led to the development of efficient polycistronic vectors based on the use of the 2A self-processing oligopeptide. We developed an F2A-based multicistronic system to test the functional effects of co-expression of the three anti-inflammatory proteins, HO-1, E5NT and ENTPD1 and to evaluate whether this novel combination may have a synergic role of protection relevant in IRI and transplantation settings. The novel multicistronic plasmids we designed and produced resulted to drive efficiently the simultaneous expression of the three genes with relevant enzymatic activities of the correspondent proteins in different in vitro models. Moreover, the combined over-expression mediated a cytoprotective effect against both pro-inflammatory and oxidative stimuli. The triple cistronic cassette was used for the production of a multi-gene transgenic mouse model for the over-expression of hHO1, hE5NT and hENTPD1 in a single experiment of transgenesis, thus with the advantage to save time, resources and animals number. Three novel transgenic mouse strains were produced and the proper enzymatic activity of the two ectonucleotidases was detected in the heart of at least two of the three mouse lines.

Angiotensin-(1-7) Effects in a Rat Model of Ventilator-Induced Diaphragmatic Dysfunction (VIDD)

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Ventilator-induced diaphragmatic dysfunction (VIDD) is a frequent event during mechanical ventilation. Recent data show that renin-angiotensin system is involved in diaphragmatic skeletal muscle atrophy after mechanical ventilation (Kwon, O.S. et al. J Appl Physiol 119:1033-41).We evaluated the effects of the treatment with Angiotensin-(1-7) [Ang-(1-7)] in a rat model of VIDD. Moreover we verified if the administration of A-779, natural antagonist of Ang-(1-7) receptor, reverted the effects. Rats underwent prolonged mechanical ventilation (8 hours), while receiving continuously iv sterile saline 0.9% (Vehicle) or Ang-(1-7) or A-779 treatment. At the end, rats were sacrificed and diaphragm removed for ex vivo diaphragmatic contractility measurement (with electric stimulation), histological analysis and quantitative real-time polymerase chain reaction for Myogenin mRNA levels analysis.As shown in the table, muscular fibers cross sectional area were higher and Myogenin mRNA levels were lower in Ang-(1-7) group. Diaphragmatic contractility did not differ. Systemic treatment with Angiotensin-(1-7) during prolonged mechanical ventilation seemed to ameliorate the diaphragmatic function, probably due to the maintenance of the muscular fibers anatomy. This action appears mediated by the Mas receptor, whose blockade reverted the beneficial effects of Ang (1-7).

*p<0.05 vs Vehicle	Diaphragmatic contractility (N/cm2)	Cross Sectional Area in muscular fibers (µm2)	Myogenin mRNA levels (ratio to b-actin)
Vehicle	4.94 ± 3.31	2426 ± 397	7.02 ± 3.02
Ang-(1-7)	6.19 ± 3.33	2990 ± 760 *	2.44 ± 1.22 *
A-779	4.49 ± 2.39	2612 ± 550	6.81 ± 4.78

A Dynamic Splicing Program Modulated by Sam68 Insures Proper Synaptic Connections in the Developing Cerebellum

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Sam68 (Src-Associated substrate in Mitosis of 68 kDa) belongs to the STAR (signal transduction and activation of RNA metabolism) family of RNA binding proteins. Sam68 is involved in the regulation of alternative splicing of pre-mRNAs, acting as repressor or enhancer depending on the specific target. Although expressed almost ubiquitously, Sam68 levels are particularly high in the developing cerebellum at birth and steadily decline over the first three weeks. Notably, many splicing targets of Sam68 encode for synaptic proteins with potential role in the establishment of neuronal circuits. Starting from these observations, we investigated the role of Sam68 during cerebellar development and in mature cerebellar functions. At postnatal days 10, the cerebellum of *Sam68* knockout (*Sam68*-/-) mice displays abnormal foliation in the central zone (lobes VI-VII), accompanied by mislocalization of Bergmann glia cells and Purkinje cells in the molecular layer of the developing cortex. Notably, we also found that Sam68 orchestrates a timely regulated splicing program of genes encoding synaptic proteins during cerebellar development, whose dysregulation in *Sam68*-/- mice leads to functional defects in adult neurons. Indeed, electrophysiological recording revealed reduced frequency and amplitude of spontaneous excitatory postsynaptic current (sESPSCs) in mature Purkinje cells, suggesting dysregulation of synaptic contacts. Accordingly, adult *Sam68*-/- mice exhibit an ataxic phenotype and motor coordination deficit. Thus, our study uncovers a Sam68-dependent splicing program that is required to guarantee the establishment of the correct spatial/temporal neuronal circuitry during cerebellar development.

Pre-clinical Efficacy of Novel Kinase Inhibitor Nintedanib on PAX5 Fusion Gene in Pediatric Ph-like B-Cell Precursor Acute Lymphoblastic Leukemia

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Despite the current risk-based stratification protocol, about 20% of pediatric patients with B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) experience relapse. In the large subset of intermediate-risk patients, Ph-like (or BRC/ABL-like) is a novel high risk subgroup, which represents 15% of BCP-ALL patients. The transcriptional factor PAX5 is frequently involved in several translocations in Ph-like patients, determining the formation of fusion genes encoding for aberrant proteins. This project aims to functionally characterize *PAX5* fusion genes, elucidating the involved signaling pathways and to test the efficacy of the inhibitor Nintedanib/BIBF1120. Ex-vivo treatments with Nintedanib on primary BCP-ALL samples demonstrated its significant efficacy both in monotherapy and in combination with standard chemotherapy (Annexin V viability assay of leukemic cells in co-culture on human bone marrow stroma). In addition, the efficacy of Nintedanib was evaluated in an in vivo humanized NSG mouse model, obtained by i.v. transplantation of human primary BM leukemic cells into sub-lethally irradiated immunodeficient mice. Efficacy of BIBF1120 has been confirmed in vivo, especially in bulk disease setting (high level engraftment), especially in BM, spleen, PB and CNS. In addition, the combination with Dexamethasone synergized the treatment efficacy. Finally, Phosphoflow analysis showed the involvement of Akt pathway before and after treatment by Nintedanib (FACS). Overall, this study proposed PAX5 fusion proteins as a good target for novel approaches in BCP-ALL treatment. Furthermore, these data suggested use of Nintedanib, in clinic for solid tumour, to improve the outcome of this specific subgroup of patients.

Urine Proteome and Fe and Zn Excretion in a Porcine Model of Fe-deficiency: Effect of Fe-fortified Breads

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Fe-deficiency is common in piglets, indeed, it is mandatory to supplement them with exogenous Fe. The aim of the present study was to evaluate the impact of Fe-fortified bread on urine proteome and Fe and Zn excretion in a porcine model of Fe-deficiency. Twenty-four piglets (44 days old) were divided in four groups and fed microencapsulated iron (G1), iron sulphate (G2) or normal bread (G3; G4) for seven days. In order to induce Fe-deficiency, G1, G2 and G3 piglets did not receive Fe-dextran supplementation. Urinary proteins were separated by SDS-PAGE. Fe and Zn concentrations, total proteins, creatinine and urine protein to creatinine ratio (UPC) were also determined. At the end of the trial, SDS-PAGE resulted in similar profiles among groups characterised by the presence of few high and medium molecular weight (MW) protein bands and many bands at MW<20 kDa. Overall, G3 Fe-deficient piglets showed a faint band of transferrin that was not visible in G4 (control) piglets and a more consistent band of albumin, though not significantly different. Urine Fe concentrations ranged from 25.3±18.3 ng/ml (G4) to 51.8±39.8 ng/ml (G2), while Zn from 0.20±0.10 (G1) to 0.39±0.12 µg/ml (G2). Zn concentrations were similar to those reported in children fed iron fortified flours. This research reports the first preliminary data regarding urinary Zn, Fe and proteins in a porcine model of Fe-deficiency. Further studies are needed to clarify the relation between urine proteome and Fe and Zn excretion since the identification of biomarkers in urine of biomedical animals is of great relevance for the Refinement of the procedures.

Increase Education to Reduce Intolerance

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Tolerance is "the virtue that makes peace possible [and] contributes to the replacement of the culture of war by a culture of peace". (UNESCO, 1995). It can prevent prejudices and stereotypes and is mainly based on knowledge for understanding. The public debate about animal experimentation is still a "war territory" with public concerns frequently transformed in propaganda. Education can prevent intolerance in particular by giving the opportunity to face different points of view among students. Introducing the basic Laboratory Animal Science in scientific higher education can open up the mind to students, even if not directly involved with animal experimentation, by offering them the opportunity to discuss and reflect on the ethical aspects together with the scientific ones. Since 2006 a Laboratory Animal Science basic course was offered to students of Biotechnology Degree of Bologna University; the course starts with the production of a text on a personal opinion on Animal experimentation and ends with a reflection on the above-mentioned text. A series of educational activities are offered to the students, firstly on the 3Rs then on the application of ARRIVE checklist to analyse research papers of different quality and finally a peer to peer activities (PlayDecide) that is particularly useful to reveal some hidden deep emotion-based beliefs the resulting debate allow students to come to an informed decision about the issue. The final reflection attests that, during the course, the students acquire a more structured belief and are prepared to face the argumentation of animal experimentation antagonists without stereotypes and prejudices.

Theta-burst Stimulation in Early and Late Parkinsonian Animals Exerts Therapeutic Effects through Neuronal and Non-neuronal Mechanisms

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Animal models of Parkinson's disease (PD) have helped to demonstrate that in patients, basal ganglia alterations are associated with the loss of synaptic plasticity in response to dopaminergic pharmacological treatment. Finding new therapeutic strategies that combine pharmacological and non-pharmacological approaches may alleviate symptoms of PD with limited side effects. Repetitive transcranial magnetic stimulation (rTMS) has been used in PD patients with mixed results on its therapeutic outcome, and the mechanisms by which it exerts beneficial effects

are still unclear, limiting its potential. Here we show that acute rTMS, with intermittent theta-burst stimulation (iTBS) protocol, induces a rescue of corticostriatal plasticity with recovery of akinesia associated with selective increase of dopamine in dorsolateral striatum of late parkinsonian animals, together with a reduction of astrogliosis and microgliosis. Using intracellular recordings from corticostriatal slices from low-dose 6-hydroxydopamine (6-OHDA)-lesioned rats, modelling early PD, we show that a single session of in vivo cortical iTBS rescued motor functions and corticostriatal long term potentiation in early-stage parkisonism. This effect was mediated by GluN2B subunit-containing NMDA receptors, as selective inhibitor ifenprodil could abolish TMS-mediated plasticity. Further support to our hypothesis is provided by molecular analysis showing a marked decrease in GluN2B in the postsynaptic compartment after TMS, pointing to a possible recruitment of extrasynaptic GluN2B in this form of TMS-mediated plasticity. Taken together, these data suggest that rTMS exerts different effects in late- and early-stage PD, alleviating symptoms also through non-synaptic mechanisms and through intracellular pathways classically associated with excitotoxicity.

Fludarabine Greatly Enhances Engraftment of Human Normal and Malignant Hematopoiesis in Immunodeficient Mice

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Despite the variety of immunodeficient mice, there is still an unmet need for xenotransplantation models that recapitulate normal and malignant human hematopoiesis. The addition of fludarabine to irradiation in the conditioning regimen could make recipients more permissive for human cells engraftment. SCID/beige mice were sublethally irradiated and treated with fludarabine, 48 h before injection of human cells. Mice irradiated not receiving fludarabine form control group. For normal reconstitution, we transplanted $CD34^+$ progenitors purified from human cord blood, whereas for leukemic reconstitution, AML cell line KG-1 or freshly thawed AML blasts were transplanted. Human engraftment was evaluated in peripheral blood (PB), bone marrow (BM) and spleen by flow cytometry. The group given fludarabine with irradiation showed a higher proportion and absolute number of normal huCD45+ cells in BM compared to control at 6 weeks. Such an increment was also observed in PB and spleen. Engrafting cells in the BM included myeloid, B-lymphoid and HSC. The addition of fludarabine did not significantly worsen the mice survival over 6 weeks. Moreover, this model is useful also for the reconstitution of malignant myeloid haemopoiesis, showing a massive engraftment of KG-1 cell line, accompanied by signs of disease. Strikingly, primary AML samples from 8 patients engrafted within the first 8 weeks after transplantation in 50% of pre-conditioned animals, infiltrating primary sites of clinical AML. The treatment with fludarabine provides an efficient approach for the xenotransplantation of human normal and AML cells in immunodeficient mice, resulting in a rapid and straightforward in vivo model.

Intravenous Immunoglobulin Preparation Attenuates Pain Behaviors in a Wistar Rat Model of Bortezomib-Induced Peripheral Neuropathy

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Intravenous immunoglobulin (IVIG) preparations consist of purified antibodies from thousands of healthy human donors. Although there are studies in literature evaluating their effectiveness in different autoimmune and inflammatory diseases, there are no data about their possible role on bortezomib (BZ)-induced neuropathic pain. In order to elucidate the benefits of IVIG, we intravenously infused Wistar rats with a preventive (BTZ and IVIg co-treatment for 8 weeks) and therapeutic (4 weeks of BTZ treatment followed by a 4 week IVIg-BTZ co-treatment) schedules. Neurophysiological and behavioral tests were performed at different time points. Animals were sacrificed after 3ws (acute phase) and 8ws (chronic phase) and tissue samples (DRG, sciatic nerve, caudal nerve, skin) were collected.BZ induced a painful peripheral neuropathy that was associated with an increased number of CD68 positive macrophages in the DRG and peripheral nerves. In both preventive and therapeutic schedule, co-treatment with IVIG was not able to rescue neurophysiological alteration caused by BZ. Mechanical allodynia and cold hyperalgesia evaluations showed that IVIG injection protected from BZ effect in both treatment schedules. In contrast, only preventive IVIG schedule was effective in protecting the caudal nerve from BZ damage and in preserving the intra-epidermal nerve fibers density induced by BZ. Furthermore, morphological improvement of peripheral nerves was obtained and DRG macrophage infiltration levels tended to be reduced after IVIG injection. In conclusion, we were able to demonstrate for the first time that preventive IVIG therapy is able to relieve painful symptoms in BZ-induced neuropathic pain.

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Opossum (*Monodelphis domestica*): A Captive-bred Research Colony Housed at the University of Trieste, Italy

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The grey short-tailed opossum *Monodelphis domestica* is a useful laboratory animal for its small size, non-seasonal breeding and ease of care. Our opossum colony belongs to the Department of Biotechnology, University of Rijeka and is bred at the University of Trieste through an agreement of scientific cooperation signed in 2014 and prolonged up to 2017. Here we present an overview of the colony management rules, the health monitoring procedures and the main aspects of the use in biomedical research of this species. During a 20 years period (1998-today), basic care and welfare, breeding techniques and embryo manipulation were standardized at the Animal Facility resulting in a stable and long-term colony of captive-born animals. Although opossums are exceptionally healthy animals, we tested the colony once a year for detecting potential pathogenic nasopharyngeal and cecal flora. *M. domestica* is a useful animal model since it is the first marsupial whose genome has been sequenced. The pups are born very immature, with the unique possibility to successfully regenerate spinal cord after injury during the first two weeks of their life. After that, the regenerative capacity is abruptly lost: at 14 days

in cervical spinal segments and at 17 days in less mature lumbar spinal segments. Thus, neonatal opossums represent the unique opportunity to achieve and study mammalian central nervous system that can regenerate, without a need of invasive intrauterine surgery of pregnant females (like necessary for other mammalian laboratory animals, such as mouse or rat).

Animal Models for the Study of Human Central and Peripheral Nervous System Disease

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In our country the use of animals for scientific purpose is regulated by the D.Lgs.vo 26/14 and it is focused on the issues of the 3 Rs (Reduction, Replacement and Refinement). Its guiding principle concerns the protection of the walfare of animals, the address to substitution of procedures, the reduction of animals number, the limitation of pain, suffering, stress and damage resulting from experiments. While it is desirable to replace the use of animals in procedures by alternative methods, their use continues to be necessary to study human neurology diseases. Chemotherapy-Induced Peripheral Neurotoxicity (CIPN) is a frequent, potentially severe and dose-limiting side-effect of cancer treatment. Diabetes is a metabolic disease with increasing incidence worldwide and with important social and economic effects. Increased life expectancy and improved survival rates are often associated with long-term treatment-related neurological complications that severely compromise the quality of life and the functional status of patients so also the study of Central Nervous System (CNS) disorders is important. For these reasons, we have developed animal models for studying chemotherapy-induced peripheral neurotoxicity, diabetic neuropathy and multiple sclerosis. All was done selecting species and methods that ensure the use of the minimum number of animals that would provide reliable results causing the minimum pain, suffering or distress.

Probing Atherosclerotic Plaque Permeability Using Fluorescent Blood Pool Agents in Apoe$^{(-/-)}$ Mouse Model

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Atherosclerosis is an artery degenerative disease resulting in plaques leading to stenosis, embolization and thrombosis. Currently, several imaging techniques are able to identify plaques in humans but not to clearly define composition as a predictor of an acute event, causing difficulties on the definition of a proper treatment. A diagnostic tool aimed to stratify plaques with respect to different permeability (i.e. different dangerousness) could help clinicians to predict the response to a drug-loaded nanosystem based therapy. The aim of this study was to investigate the plaque endothelial local permeability in the ApoE(-/-) mouse model with Optical Imaging using fluorescent blood pool agents. Methods: a human serum albumin conjugated with Cy5 (HSA-Cy5) and an albumin binder conjugated with IrDye800 (B26170) were administered to ApoE(-/-) mice at different weeks of feeding with high fat diet. Arterial trees were removed, imaged with a fluorescence microscope system and then histologically processed. Results &Conclusions: plaques developed in different districts of the arterial tree were classified through a grading index (between 1 and 3) with respect to their morphology and displayed a higher macrophage content at the early stage of development. Both the fluorescent probes showed higher permeation in early plaques than in more advanced ones, thus correlating with a high inflammatory state. The proof of concept that nano-based systems are able to probe permeability of atherosclerotic plaques, defining which ones are suitable for an anti-inflammatory therapy based on drug-loaded nanoparticles, was reached through OI and could be relatively easily translated in a clinical tool for MRI with the use of a proper Gd-based blood pool agent.

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Identification and Functional Characterization of Sox2-Target Genes Involved in the Self-Renewal and Differentiation of Neural Stem Cells Cultured from the Mouse Brain

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The Sox2 gene encodes a transcription factor active in neural stem/progenitor cells (NSC) in the developing vertebrate central nervous system (CNS). Heterozygous Sox2 mutations in humans cause a characteristic spectrum of CNS abnormalities. To understand the role of Sox2 in neural development, we previously generated Sox2 conditional KO mutations in mouse, that allowed us to observe an important function for Sox2 in the maintenance of NSC self-renewal, in long-term *in vitro* cultures derived from P0 mouse forebrain, as well as *in vivo* (in the hippocampus). In *in vitro* cultures, Sox2-ablated NSC self-renew for several passages like the wild-type ones, but then undergo progressive exhaustion. I found that, upon differentiation, they also generate reduced numbers of neurons, with reduced arborisation. Sox2 can regulate its targets by controlling long-range interactions between genes and distal enhancers, that regulate gene expression; indeed, many of these interactions are lost in mutant cells. By RNAseq, we identified genes are downregulated following Sox2 ablation. To test their role as mediators of Sox2 functions, I am re-introducing them into Sox2-deleted cells via lentiviral vectors, to test if they rescue long-term self-renewal, and neuronal differentiation. The most downregulated gene in mutant cells, Socs3 (Suppressor of cytokine signalling 3), rescues the ability of mutant cells to grow long-term, and may partially rescue the neuronal differentiation defect. We are presently testing an additional small number of genes, found downregulated in Sox2-mutant cells, which include key regulators of cell proliferation, by transducing them into mutant cells, individually and in combinations.

Biobank of Chimeric Tumors: The Missing Link between Human and Animal Biobanks, Mouse Models of Genetics, Epigenetics and Translational Medicine

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Biobanca is a facility for the collection, storage and distribution

of human biological samples and the associated data for research and diagnosis. It's officially recognized by the competent health authorities, applies a quality system and guarantees the rights of the subjects involved. Nowadays biobanks are mainly of three types: a) biobanks of oncological, genetic, cardiovascular, and multispeciality diseases; b) population biobanks including random cohorts and genetic isolates; c) biobanks of archive tissues. In recent years, veterinary biobanks of synantoin animal diseases have been developing. In this area of continuous research, data sharing and innovation in the medical / diagnostic field, our in-vivo preclinical oncological research biobank is located. The aim is to create and maintain this biobank in order to store biological material from experimental murine models:

- chimeric models (man-to-mouse)
- murine models of oncological pathologies
- biological samples from murine OGM colonies

The materials present in the in Chimeric tumors biobanks can be considered as the link between human and animal biobanks. It can be used by doctors and researchers to implement translational medicine, and is a great opportunity to improvement biomedical research. All samples are accurately cataloged and have detailed storage, in order to make them easily traceable and use immediate and correct. Chimeric tumors Biobank can also be used as a tumor registers interface, to verify on a scientific basis the data reported in the same registers. The products of this biobank will be: create an ever larger and interactive network among stakeholders, implement patents, knowledge of new risk factors.

Neonatal Umbilical Cord Blood Transplantation Halts Disease Progression in the Murine Model of MPS-I

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Umbilical cord blood (UCB) is a promising source of stem cells to use in early haematopoietic stem cell transplantation approaches for several genetic diseases that can be diagnosed at birth. Mucopolysaccharidosis type I (MPS-I) is a progressive multisystem disorder caused by deficiency of the lysosomal enzyme α-L-iduronidase, and patients treated with allogeneic HSCT at the onset have improved outcome, suggesting to administer such therapy as early as possible. Given that the best characterized MPS-I murine model is an immunocompetent mouse, we here developed a transplantation system based on murine UCB. With the final aim of testing the therapeutic efficacy of UCB in MPS-I mice transplanted at birth, we first defined the features of murine UCB cells and demonstrated that they are capable of multi-lineage haematopoietic repopulation of myeloablated adult mice similarly to bone marrow cells. We then assessed the effectiveness of murine UCB cells transplantation in busulfan-conditioned newborn MPS-I mice. Twenty weeks after treatment, iduronidase activity was increased in organs of MPS-I animals, glycosaminoglycans storage was reduced, and skeletal phenotype was ameliorated. This study explores a potential therapy for MPS-I at a very early stage in life and represents a novel model to test UCB-based transplantation approaches for various diseases.

Therapeutic Approaches in a Murine Model of ALS

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The animal models are essential to understand pathogenic mechanisms of neurodegenerative diseases. Several animal models have been created but only few are able to reproduce the features of human pathology. Concerning Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disease characterized by motoneuron cell death, different transgenic mice have been developed. These animals carry mutated genes that are correlated to the familial form of ALS. In particular, SOD1(G93A) mice, a transgenic mouse carrying the human SOD1 gene with G93A point mutation, is the mostly used for ALS study. This animal reproduces the clinical and the histopathological features of ALS. In the early phase of the disease the animal shows protein aggregations and mitochondrial dysfunction as reported in ALS patients. Moreover, at the onset of the disease, animal reduces their body weight, muscle strength and shows lower limbs tremors that leads to muscular atrophy and paralysis, as in the human pathology. In our research group we used this murine model in order to test possible therapeutic approaches for ALS. Currently we are studying two different therapies: the first based on the administration of two epigenetic drugs (Resveratrol and MS-275), the second regard the use of exosomes obtained from adipose stem cells. In both approaches animals improved their motor performance and postpone lifespan, indicating that these two different approaches could be used as possible therapy in ALS.

Characterization of the Role of the Sox2 Transcription Factor in the Development of the Hippocampus and the Visual System by Conditional Knock-outs in Mice

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Heterozygous mutation of the Sox2 transcription factor gene in humans leads to a characteristic spectrum of defects affecting the nervous system, including defects of the hippocampus (important for memory formation) and the visual system. Defects in the postnatal hippocampus and the eye have been reproduced in mice by conditional ablation of the Sox2 gene. We found that Sox2 ablation at different time points during development of the telencephalon results in abnormal hippocampal embryonic development and the earlier the deletion, the stronger the phenotype. The dentate gyrus of the hippocampus is the region the most sensitive to Sox2 ablation and we find a drastic reduction of neurons and of neural progenitors in this location when Sox2 is ablated. The visual system is composed of the eyes, the dorsolateral geniculate nucleus (dLGN) in the thalamus, and the visual cortex. Sox2 is expressed in the eyes, the dLGN and the developing cortex. We generated cortical and thalamic Sox2 conditional knock-outs (cKO) in mouse. In the Sox2 cortical cKO the primary visual area (V1) is slightly reduced in size. On the other hand, in the thalamic cKO the dLGN is greatly reduced in size. We are currently trying to understand what genes are directly regulated by Sox2 in the hippocampus and the visual system to better understand how

Sox2 functions and to get insight into how this transcription factor could regulate development of the nervous system in humans.

In Vivo Imaging Service for Basic Research and Preclinical Studies

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"Castel Romano" Plaisant Animal Facility, Roma, IT

Allevamenti Plaisant s.r.l. has developed a new activity that offer expertise on live animals to assist researchers on experimental procedures. Plaisant "Castel Romano", the animal facility of Allevamenti Plaisant, has a well trained staff to conduct experimental procedures on different research areas. One of our top services is the In Vivo Imaging on live animals. The bio-imaging is a very powerful system to monitor molecules and drugs distribution and to observe kinetic of tumour growth. The same mouse can be observed many times, instead of sacrifice it and collect tumours at different kinetic times, this is according to the 3R's (reduction) rule. The IVIS (In Vivo Imaging System) gives a good comparison of manual measurement methods (calibration) and photon release. This can reduce distress in mice due to the restrain and give a major accuracy of data, minimising risk of errors. The mice are inoculated with solid tumour cells and leukaemia cells, genetically modified to express luciferase. 10 minutes before acquisition, the mice are inoculated with luciferase substrate that is metabolised giving luminescence to the cancer cells. Mice are anesthetised by gas anaesthesia (isoflurane) for a maximum of 3-5 minutes and observed. Isoflurane is quickly metabolized and doesn't give any suffering to the animals. Leukaemia cells and solid tumour cells administration is performed by intravenous or intraperitoneal injection and by subcutaneous or orthotropic (Colon, liver etc.) injection respectively. In addition Plaisant has a Transgenic Facility active in production of transgenic, KI and KO mice, cryopreservation and re-derivation of mice lines.

SPERA Reasons for Research: "Research Goes to School"

ABCD (Associazione di Biologia Cellulare e del Differenziamento), AINI (Associazione Italiana di Neuroimmunologia), AISAL (Associazione Italiana per le Scienze degli Animali da Laboratorio), SIBBM (Società Italiana di Biofisica e Biochimica Molecolare), SIBE (Società Italiana di Biologia Evoluzionistica), SINS (Società Italiana di Neuroscienze), SIVAL (Società Italiana Veterinari Animali da Laboratorio), VITARES (Veterinary Immunotherapy and Traslational Research), UZI (Unione Zoologica Italiana)

What is research? What's its purpose? What do scientists do in their labs? Is science far from our everyday life? Answering these questions is the first step in order to give new momentum to scientific research in Italy. The dissemination of scientific information is crucial to ensure that citizens use critical thinking to form a personal opinion about various aspects of research, without prejudice. This is extremely important because common citizens, even if they may feel distant and even extraneous to the world of science, are actually the ultimate recipients of all research. This is why the Federation "SPERA - Reasons for Research" was founded, bringing together several scientific associations. Our main tool is the direct contact with new generations, taking scientists' experience and knowledge into schools to encourage students to interpret scientific results based on real data. Researchers affiliated with SPERA go to elementary, middle and high schools to talk about subjects ranging from zoology to neuroscience, and addressing delicate topics such as the use of the animal model in biomedical research, vaccines and addictions. According to the students' age, different approaches have been adopted to present these topics: games, debates and open discussions were extremely interesting and promising. It will also be important to involve university and doctoral students. SPERA's activities in schools will continue for the incoming school year, and an increasing number of researchers will be involved to explain their daily work.