

GENE AND CELL THERAPY

▷ MODIFYING THE FATE OF HUMAN EMBRYONIC STEM CELLS

Human embryonic stem (ES) cells hold considerable promise for deciphering the processes of human embryonic development, elucidating the mechanisms of oncogenesis, and providing a source of differentiated, functional cells for use in regenerative medicine. The key to each of these opportunities lies in understanding the molecular mechanisms that regulate the decisions made by ES cells between self renewal and commitment to differentiation. Our own research is focused on exploring the nature of these molecular processes in a way that will allow eventual manipulation of the fate choices made by ES cells, and the discovery of approaches to directing the cells towards specific lineages of differentiation.

Peter W. Andrews, *University of Sheffield, UK*

▷ HUMAN EMBRYONIC STEM CELLS – A POTENTIAL PLATFORM FOR CELL AND GENE THERAPY OF THE CNS

Human embryonic stem cells (hESCs), may serve as a renewable source of neural precursors (NPs) for cell and gene therapy of the CNS. In the presentation we will show that hESCs may be induced to differentiate, under defined culture conditions, into highly enriched cultures of developmentally competent NPs. Following transplantation to the developing mouse brain, the human NPs participate in host brain development. When transplanted into the striatum of Parkinsonian rats, the animals exhibit a significant partial correction of behavioral deficits. These data encourage further developments that may eventually allow the exploitation of hESCs for cell and gene therapy in neurodegenerative disorders.

Benjamin E. Reubinoff, *The Hadassah Human Embryonic Stem Cell Research Center, The Goldyne Savad Institute of Gene Therapy, Hadassah University Medical Center, Jerusalem, Israel*

▷ STEM CELLS AND THE ADVANCE TOWARDS GENE THERAPY FOR HEMOGLOBINOPATHIES

Lentiviral based vectors incorporating complex globin expression cassettes have enabled dramatic advances toward gene therapy of hemoglobinopathies. Proof of principle “cures” have now been demonstrated using such vectors for gene transfer to hematopoietic stem cells in both murine models of thalassemia and sickle cell disease. Promising data is also recently emerging for human hematopoietic stem cell targets assessed in immunodeficient mouse models. Safe and effective application of gene therapy however will require further development of effective strategies to achieve high level repopulation with engineered stem cells in non-myeloablative conditioned patients. Advances on this front include new approaches to achieve high level *ex-vivo* expansion of hematopoietic stem cells using Hox transcription factors (eg HOXB4).

R. Keith Humphries, *British Columbia Cancer Agency, Vancouver, Canada*

▷ GENETIC MARKING STUDIES IN NON-HUMAN PRIMATES: INSIGHTS INTO HEMATOPOIESIS AND LEUKEMOGENESIS

The rhesus macaque provides an excellent preclinical model with direct relevance to human biology for the investigation of hematopoiesis and gene transfer outcomes. We have used this model to optimize gene transfer utilizing a series of integrating retroviral and lentiviral vectors. Via mapping of individual vector insertion sites, we can study clonal behavior of transduced cells *in vivo*. There are striking patterns of integration with relevance to leukemogenesis, and we report on the development of a myeloid tumor 6 years following transplantation with retrovirally-transduced CD34+ cells in one animal.

Cynthia E. Dunbar, *National Institutes of Health, Bethesda, USA*

▷ GENE THERAPY OF SEVERE COMBINED IMMUNODEFICIENCIES (SCID)

SCID are genetically determined conditions that offer an attractive setting to assess the efficacy of gene therapy. Indeed, knowledge of molecular pathophysiology of the various SCID, associated with the highly proliferative capacity of T cell precursors and the very long life span of mature T lymphocytes, tells us that transduction of a small number of precursors could enable to restore a functional immune system. This has indeed been shown for the X-L form of SCID, by using *ex vivo* retrovirally mediated gene transfer into CD34+ cells. However, a significant toxicity is observed that requires modifications in vector design in order to reduce the enhancer activity of the viral LTR.

Alain Fischer, *Inserm U429, Hôpital Necker-Enfants Malades, Paris, France*

▷ GENE THERAPY FOR SEVERE COMBINED IMMUNODEFICIENCY

Primary immunodeficiencies (PID) are a heterogeneous group disorders in which inherited genetic defects compromise host immunity. Although often clinically severe, bone marrow transplantation is highly successful if a genotypically matched family donor or unrelated donor is available. However, for the majority of individuals, this is not the case, and survival from mismatched transplants is substantially lower and associated with predictable toxicity. The difficulties associated with conventional HSC transplantation have therefore driven the development of novel gene therapy strategies which have recently produced remarkable clinical effects.

Adrian J. Thrasher, *Institute of Child Health, London, UK*

▷ GENE THERAPY OF ADA-DEFICIENT SCID

Gene therapy for adenosine deaminase (ADA)-deficient SCID has progressed from the early pilot trials of safety and feasibility to the recent demonstration of efficacy and clinical benefit. We showed that gene therapy with bone marrow hematopoietic stem cells, combined with low dose busulfan, resulted in multilineage, long-term engraftment, correction of both immune and metabolic defect, and proven clinical benefit, in the absence of enzyme replacement therapy. Overall, no adverse effects or toxicity have been observed in the patients treated worldwide with ADA gene therapy. These results have

important implications for future applications of stem cell gene transfer in other immune disorders.

Alessandro Aiuti, *San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milano, Italy*

▷ GENE THERAPY OF CHRONIC GRANULOMATOUS DISEASE

Chronic Granulomatous Disease (CGD) represents a group of primary immunodeficiencies characterized by recurrent, life-threatening bacterial and fungal infections. CGD is caused by mutations in any one of four genes encoding for the subunits of the phagocytic NADPH oxidase complex. Based on our preclinical work, a Phase I/II gene therapy trial was started in 2004. Two X-CGD patients, 26 and 25 years old, were treated with gene modified cells at the Department of Hematology, University Medical School, Frankfurt. A significant correction of the defect was achieved in both patients. Both patients are well and free of bacterial and fungal infections since transplantation.

Manuel Grez, *Institute for Biomedical Research, Georg-Speyer-Haus, Frankfurt, Germany*

▷ APPROACHES TO THE GENE THERAPY OF THE β -HEMOGLOBINOPATHIES

Sickle cell disease and β -thalassemia are among the most prevalent genetic diseases worldwide. Long-term disease correction was achieved in mouse models by *ex-vivo* lentiviral transfer of a regulated therapeutic globin gene to hematopoietic stem cells. Prevention of vector leukemogenesis was tackled by the use of a self-inactivating vector and chromatin insulators with a satisfactory safety profile in experimental models. Large scale, clinical grade vector production and CD34+ cell transduction, clinical protocol design and quality controls have been established towards the initiation of human trial. Complementary and alternative approaches involving stem cell selection/amplification, RNAi and gene correction will be discussed.

Philippe Leboulch, *Harvard Medical School, Boston, USA*

▷ GENE THERAPY IN THE SKELETAL MUSCLE

Gene-based therapeutic interventions are considered in the skeletal muscle, not only in the context of diseases directly affecting muscle function, but also in cases where the systemic distribution of a therapeutic protein is needed. Depending on the type of application, local or widespread modification of the tissue must be achieved. Recent progresses in gene transfer technologies and improved methods for delivery to the muscle, bring about the possibility of successful proofs of concept in the clinic.

Olivier Danos, *CNRS UMR 8115, G n thon, Evry, France*

▷ MESOANGIOBLASTS FOR THE CELL THERAPY OF MUSCULAR DYSTROPHIES

Mesoangioblasts are vessel-associated stem cells that cause a dramatic functional amelioration of the dystrophic phenotype when delivered in a mouse model of muscular dystrophy. We are currently investigating: 1. the effect of mdx mesoangioblasts, genetically corrected with different lentiviral vectors, in the mdx mouse to compare the efficacy of micro-dystrophin with exon-skipping small nuclear RNA; 2. the effect of mesoangioblasts in a dog model of muscular dystrophy; 3. the growth and differentiation potential of human mesoangioblasts, isolated from patient muscle biopsies. The results obtained will be discussed.

Giulio Cossu, *Stem Cell Research Institute, HSR, Milano, Italy*

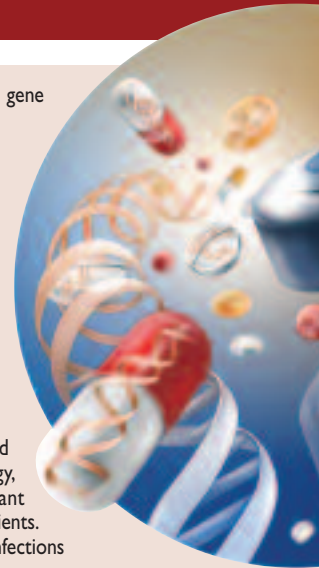
▷ RETINAL DEGENERATIONS: FROM CELL THERAPY TO CELL SIGNALLING; PRE-CLINICAL AND CLINICAL ISSUES

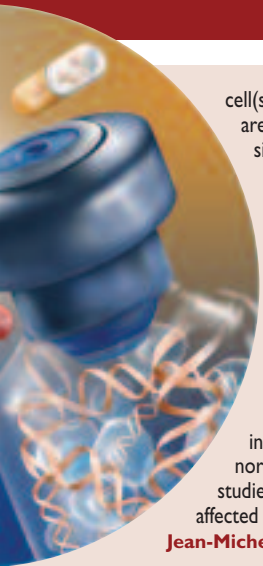
Retinal degenerations are among the most heterogeneous group of inherited diseases. The appropriate targeting of gene and/or cell based therapies needs to take into account not only the causative mutation but also factors such as functional versus structural loss of retinal cells therefore implying a clear definition of 1) the therapeutic window (i.e. timing, functional studies...) 2) the target cells (e.g. cones) 3) of the appropriate patient cohort (e.g. genotyping) 4) the primary and surrogate outcome measurements ... Strategies to rescue central vision (i.e. cone cells) in rod-cone dystrophies take into account these key issues.

Jos -Alain Sahel, *Inserm U592, Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France*

▷ GENE AND CELL THERAPY IN CRITICAL LEG ISCHEMIA

The stimulation of angiogenesis by gene or cell therapy is a promising approach in critical leg ischemia. The phase I or II trials published to date demonstrate that pro-angiogenic growth-factors or cell therapy by bone-marrow mononuclear cells can develop new collateral vessels and increase blood flow. Several questions are pending: can a single growth-factor allow persistent angiogenesis or are synergism of factors require? Bone marrow contains stem cells that have pro-angiogenic properties. Among the mononuclear cells used in clinical trials it remains to demonstrate which type(s) of





cell(s) are responsible for revascularization, and which mechanisms are involved: is it a paracrine mechanism that stimulate angiogenesis or a vasculogenic process with incorporation of vascular stem cells within the arterial wall?

Joseph Emmerich, *Inserm U428, Université Paris 5, France*

▷ CORRECTION OF NEUROPATHOLOGY IN THE DOG MODEL OF HURLER SYNDROME

Hurler syndrome is a lysosomal storage disease responsible for severe and intractable neurological disorders in young children. We corrected the disease in the brain of affected mice and dogs. Adeno-associated vector-mediated gene delivery allowed the creation of intracerebral sources of the missing enzyme α -L-iduronidase, from which spreading was documented in the entire brain. Histopathology and biochemical disorders were normalised up to one year after vector administration. Ongoing studies will determine the feasibility and modality of a clinical trial in affected children.

Jean-Michel Heard, *Inserm U622, Institut Pasteur, Paris, France*

▷ TRANSFER AND REPROGRAMMING OF NEURAL STEM CELLS IN SPINAL CORD INJURY

Spinal cord injury affects 11.000 Europeans/year and there are 300.000 victims in Europe. Two decades ago, repair of the spinal cord was regarded as an impossible task. This gloomy outlook is slowly yielding to cautious optimism. To protect from secondary damage, replace and repair what has been lost, cell and tissue grafting strategies have shown promise. Such therapies will be discussed, focusing on neural stem cells. When naive cells are grafted in rodents, we recently detected that positive effects were accompanied by allodynia. Transduction of Neurogenin-2 before engraftment changed the fate of grafted cells, alleviated allodynia, and improved sensory recovery. Thus, while uncritical implantation of pluripotent cells carries risk, genes may be used to control cell fate leading to better and safer future treatment protocols.

Lars Olson, *Karolinska Institutet, Stockholm, Sweden*

▷ HEMATOPOIETIC STEM CELL GENE THERAPY FOR X-LINKED ADRENOLEUKODYSTROPHY USING AN HIV-DERIVED LENTIVIRAL VECTOR

Based on the efficiency of allogeneic bone marrow transplantation (BMT), we have designed a gene therapy strategy for Childhood Cerebral Adrenoleukodystrophy (ALD), a devastating demyelinating disease. Using a HIV-derived lentiviral vector, we have demonstrated the preclinical feasibility and safety of this approach and submitted an application to the French AFSSAPS concerning autotransplantation of ALD patient CD34+ cells genetically modified with an HIV-derived vector. This clinical trial will include children with cerebral ALD, candidates for allogeneic BMT, with no matched donor.

Nathalie Cartier-Lacave, *Inserm U561, Hôpital Saint Vincent de Paul, Paris, France*

▷ SPECIFICITY OF RETROVIRAL DNA INTEGRATION

Retrovirus vectors for gene therapy pose a risk of oncogenesis due to effects of the integrated provirus on host cell gene expression. Therefore, we have been studying the specificity of retroviral DNA integration at two levels. First, meta-analysis of published libraries of target sequences for integration of ALV, MLV, and HIV DNA has revealed an unexpected symmetrical preference for bases around the integration site, different in sequence and location for each virus. Second, we have directly examined the effect of transcriptional activation on integration into a specific (metallothionein) gene, finding that 100-fold increases in transcription are associated with significant decreases in integration. The implications of these observations will be discussed.

John M. Coffin, *Tufts University, Boston, USA*

▷ CONTROL OF RETROVIRAL DNA INTEGRATION BY CELLULAR FACTORS

We have investigated the mechanisms by which HIV targets DNA integration in the human genome. Previous genome-wide studies of HIV DNA integration revealed that transcription units are favored targets. Avian sarcoma-leukosis virus (ASLV) and murine leukemia virus (MLV), in contrast, showed different favored integration targets. MLV favors sequences encoding transcription start sites, while ASLV targets sites sprinkled around the genome in a near-random fashion. Analysis of the effects of gene activity by transcriptional profiling revealed that HIV integration is particularly favored in active transcription units. Thus each of the three retroviruses analyzed showed unique target site preferences, suggesting that each type of integration complex makes virus-specific interactions with chromatin that guide site selection. We report the identification of a human cell transcription factor that, when mutant, results in reduced HIV DNA integration in transcription units. Genes responsive to this factor could be identified by transcriptional profiling, and these genes could be shown to be preferential targets for HIV DNA integration. Further progress in this area will be discussed.

Frederic Bushman, *University of Pennsylvania, Philadelphia, USA*

▷ INTEGRATING VECTOR SYSTEMS - CHALLENGES TO BASIC, PRECLINICAL AND CLINICAL DEVELOPMENT

Integrating vector systems offer the opportunity to add therapeutic transgene information to a patient's somatic stem cells, potentially for a lifetime. Current retrovirus-derived insertional vector systems, long thought to integrate semi-randomly, are now found to seek the context of expressed genes in their target cells, rendering their

unwanted interaction with their genomic target context more likely than previously thought. Like other pharmaceutical agents, gene vectors apparently have a therapeutic window and may exhibit excessive toxicity if overdosed. A summary of our current knowledge on the characteristics of particular vector systems, the distribution and side effects of their genomic insertion and possible consequences for ongoing and future vector and trial design will be discussed.

Christof von Kalle, *Cincinnati Children's Hospital Research Foundation, USA*

▷ MODULATION OF TRANSGENE IMMUNE RESPONSE IS A REQUIREMENT FOR SUCCESSFUL GENE THERAPY TRIALS

Stable gene replacement by *in vivo* or *in vitro* gene transfer has important therapeutic application for genetic and acquired diseases. However, successful gene therapy is often limited by the immune response to the transgene products, which result in elimination of transduced cells. The induction of transgene specific tolerance offers a novel approach for the prevention of immune responses directed towards genetically modified cells. Tolerance can be achieved through induction of T regulatory (Tr) cells, including the CD4+CD25+ natural Tr cells and the type I acquired Tr cells. These regulatory T cells can be induced *in vitro* or *in vivo* using tolerogenic antigen presenting cells and can suppress antigen specific responses.

Maria Grazia Roncarolo, *San Raffaele Telethon Institute For Gene Therapy (HSR-TIGET), Milano, Italy*

▷ MEGANUCLEASE: FROM DNA DOUBLE-STRAND BREAKS TO GENE THERAPY

Meganucleases are sequence specific endonucleases with large recognition sites, which can stimulate homologous gene targeting up to 10,000-fold. Thus, meganucleases may represent a universal tool for genome engineering, including the correction of mutations involved in monogenic inherited diseases. Now, therapeutic applications depend on our ability (i) to develop novel meganucleases with dedicated specificities and (ii) to use meganucleases to trigger gene correction in stem cells or somatic tissues, with minimal toxicity. Our recent progresses in both directions will be presented.

Frédéric Pâques, *Collectis S.A., Romainville, France*

▷ GENOME EDITING: ITS PROMISE AND LIMITATIONS FOR GENE THERAPY

For the treatment of monogenic disorders, direct correction of the mutation by editing the genome has important theoretical advantages over the transgenic approach of randomly adding a normal copy of the gene to the chromosomes of the patient's cells. Until recently the technology available to directly correct genomic mutations has been very inefficient, but new strategies to produce specifically targeted double strand DNA breaks have shown promise for moving genome editing toward the clinic. As was true with the development of gene therapy using transgenes, use of genome editing for patients with primary immunodeficiency diseases will be an early application.

R. Michael Blaese, *Institute for Inherited Disease Research, Newtown, USA*

▷ NEW DEVELOPMENTS IN LENTIVIRAL VECTORS

The presentation will be centered on the development of two types of lentiviral vectors. The first are vectors which are unable to integrate into the genome but nonetheless display a great efficiency with no adverse effects. These vectors will be discussed in the context of the gene therapy of quiescent cells such as neurons or retinal cells. The second are vectors which integrate but for which the integration site is directed toward a "neutral locus" thus making them ideal for gene transfer in dividing cells such as adult stem cells.

Jacques Mallet, *CNRS UMR 7091, Hôpital de la Pitié-Salpêtrière, Paris, France*

▷ PHILOSOPHICAL AND ANTHROPOLOGICAL QUERIES ABOUT GENE/CELL THERAPY

Autotransplantation of skin cells or of genetically manipulated blood cells is already in use and is considered ethically unproblematic. For patients with conditions such as diabetes, myocardial infarct or neurodegenerative diseases, the investigation of the properties of stem cells has opened the hope that gene/cell therapy might in the future replace transplantation surgery. The fear has however been expressed that new therapeutic procedures using genetically manipulated human cells, possibly human embryonic cells, or cloned cells, might alter our "vision of humanity". The paper examines the arguments pro and contra current or future research on human cells with a view to therapeutic uses.

Anne Fagot-Largeault, *Collège de France, Paris, France*

▷ REGLEMENTATIONS

Cell and Gene therapies are defined as "advanced therapy medicinal products" according to European directives 2001/83/EC and 2003/63/EC, and follows the current European regulation for medicinal products. In this case, products will be submitted through an European centralized procedure. In France, these directives have been implemented into French law, which has, in addition, extended this framework to "preparation", which are not covered by these directives. In both cases (medicinal products and preparation), AFSSAPS is the French authority in charge of authorization for i) establishments, ii) process/products, iii) clinical trials; and applies the same requirements with regards to quality, safety and efficacy.

Sophie Lucas, *Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS), Saint-Denis, France*