Société Française de Thérapie Cellulaire et Génique

French Society for Gene and Cell Therapy

Annual Congress - Abstracts

© ISTOCK.COM/EFESENKO

9-11 March 2016

Palais des Arts, Marseille, France

www.sftcg.fr @SFTCG #SFTCG2016

Invited Speaker Abstracts

INV01

Steps and challenges involved in gene therapy drug development

A Galy [1]

1: Généthon

Abstract not available.

INV02

Data integration and bioinformatics for precision medicine in oncology

P Hupé [1]

1: Institut Curie, PSL Research University, CNRS UMR 144, INSERM U900, Mines ParisTech

Life science has entered the so-called "big data era". The field of clinical research is no exception to this rule. Indeed, the use of high-throughput experiments (e.g. next-generation sequencing) and medical imaging produces a large amount of very complex and heterogeneous data. Storing, querying and analyzing this tsunami of data raises many new challenges at the technical, organisational and scientific levels.
Using this data requires a strong bioinformatics environment capable of (i) guaranteeing the integration and the traceability of data, (ii) ensuring the correct processing and analyses of genomic data, and (iii) applying well-defined and reproducible procedures for workflow management and decision-making. To address these issues, we will present an information system we developed at Institut Curie. This information system named KDI (Knowledge and Data Integration) able to handle the heterogeneity and the complexity of the data. It facilitates the data integration and tracks in real-time the processing of individual samples.
We will show how the system has been used to support precision medicine in oncology. We will used as an exemple the SHIVA clinical trial. It is a multicentric randomized phase II trial which compares targeted therapy based on tumor molecular profiling versus conventional therapy in patients with a refractory cancer (Servant et al., 2014).

INV03

Faecalibacterium prausnitzii, one of the most promising next-generation probiotics to prevent and to treat gastrointestinal disorders and diseases in Humans?

P. Langella [1] R. Martin-Rosique [1] S. Miquel [1] C. Michon [1] C. Bridonneau [1] F. Chain [1] M. Thomas [1] J.M. Chatel [1] L. Bermudez [1] H. Sokol [1]

1: Commensal and Probiotics-Host Interactions Laboratory, Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France

In 2008, we have identified F. prausnitzii as the first anti-inflammatory commensal bacterium detected on the basis of human clinical data and validated in acute high-dose TNBS colitis model. Since this finding, diminished prevalence and abundance of Faecalibacterium prausnitzii have been reported in gastrointestinal disorders as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Today, it is well established that the absence of F. prausnitzii is associated with several human dysbiotic diseases and can be considered as a biomarker of human health.
We will thus describe its beneficial effects in i) a novel chronic inflammation model; ii) in a novel chronic low-grade inflammation model to mimic the disorders observed in IBS patients;- and iii) in acute stress models which are neomaternal separation mice model and partial restrain stress in rats. Recent data on the mode of action will be also described including i) the novel gnotobiotic model which led us to the identification of anti-inflammatory metabolites; and ii) the identification of a potential anti-inflammatory F. prausnitzii MAM (for Microbial Anti-inflammatory Molecule) protein.
All these recent results confirm the high potential of F. prausnitzii as a potential next-generation probiotic for both IBS and IBD patients.

INV04

Going viral against cancer

N Boisgerault [1 2 3 4]

1: Centre de Recherche en Cancérologie Nantes-Angers 2: Inserm U892 3: CNRS 6299 4: Université de Nantes

Recent developments in the field of cancer virotherapy have confirmed the clinical potential of oncolytic viruses. Two decades of research recently led to the approval, in the USA, of the oncolytic herpesvirus T-VEC for the treatment of patients with unresectable melanoma. Other oncolytic agents, such as vaccinia virus in hepatocellular carcinoma or measles virus in multiple myeloma or ovarian carcinoma, have shown promising results in clinical trials and could be approved by public health agencies within a few years. Pharmaceutical companies are also showing growing interest for this therapeutic approach, which

could foster further developments in the field. The large choice of therapeutic viruses available offers numerous possibilities to treat aggressive malignancies that are still resisting to conventional anticancer therapies. Indeed, oncolytic viruses are not only capable of infecting and killing specifically tumor cells, but are also able to induce strong and specific anti-tumor immune responses by promoting both immunogenic death of tumor cells and efficient priming of immune cells. Strategies of 'oncolytic immunotherapy' are thus being developed to combine the efficacy of oncolytic viruses with novel immunotherapies. This includes viruses engineered with genes encoding immunostimulatory cytokines or tumor-associated antigens, but also combination strategies with immune checkpoint inhibitors such as anti-PD-L1 antibodies. As oncolytic viruses have already shown a remarkable safety profile in preclinical studies and clinical trials, we can hope that these new therapeutics will open a new era in the treatment of cancer.

INV05

Towards the commercial manufacture of ex vivo gene modified cells: CAR-T cells.

B Dropulic [1]

1: Lentigen Technology Inc., A Miltenyi Biotec Company

Several successful clinical trials have demonstrated the safety and efficacy of using
Lentiviral-vector modified cells for the potential treatment of several important diseases. However, for these therapies to become widely available to the patients that need them, a method for robust manufacture of such patient-specific cells at the commercial scale is required. We are developing scaled-up vector manufacture and automated cell processing to develop an integrated work-flow solution for the manufacture of patient-specific gene-modified cells. Preclinical studies of an anti-CD22 CAR-T cell product that is currently being evaluated in a phase I clinical trial will be shown as a case study.

INV06

Development of therapeutic proofs of concept in Progeria and Defective Prelamin A Processing associated Syndromes

P Cau [1 2] A De Sandre-Giovannoli [1 2] K Harhouri [2] N Lévy [1 2]

1: AP-HM, Département de Génétique Médicale et Biologie Cellulaire, Hôpital d'Enfants La Timone, Marseille 2: AMU-Inserm UMR_S 910, « Génétique médicale et génomique fonctionnelle » Faculté de Médecine, Marseille

Progeria is a rare and severe premature ageing disease and is mainly caused by the toxic accumulation of Progerin, a truncated form of Prelamin A.
In 2003, we identified a recurrent dominant mutation causing most HGPS cases in the LMNA gene encoding ubiquitous nuclear A-type lamins. This de novo mutation activates a cryptic pre-mRNA splicing site leading to the production of a truncated Lamin A precursor called "progerin". Progerin cannot be fully post-translationally processed, remains aberrantly prenylated and accumulates in cells' nuclei, where it exerts several toxic effects. We have demonstrated a link between other nosologic entities classified as progeroid syndromes and defective prelamin A processing, either caused by mutations in LMNA or its main post-translational processing enzyme FACE-1/ZMPSTE-24. Preclinical studies provided proofs-of-principle that the combined use of prenylation inhibitors, statins and amino-bisphosphonates (N-BPs), could improve the natural course of the disease, including growth, bone density and survival. The beneficial effects of these drugs could be ascribed to the reduction of Progerin prenylation levels, as well as, probably, to their specific pharmacological activities. This allowed to launch a phase II trial on 12 European children affected with Progeria that favorably impacted some of the most severe symptoms. Beside, using our KI progeria mouse model, we validated Antisense oligonucleotides for Splicing directed intervention towards a future trial.
Finally, we have recently shown that progerin is sequestered into abnormally shaped Promyelocytic-Nuclear Bodies (PML-NB) and subsequently identified a class of proteasome inhibitors to drive its specific elimination. These molecules induce both progerin degradation through macroautophagy, and strongly reduce its production through downregulation of a protein controlling prelamin A splicing. Cellular senescence is reduced and viability and proliferation enhanced in HGPS fibroblasts. In vivo, injection in skeletal muscle also reduces progerin. While encouageing, these results will be translated in clinics only after the pre-clinical and systemic validation steps are achieved.

INV07

Novel AAV vectors for classical and genome-editing based gene therapy

M A Kay [1]

1: Departments of Pediatrics and Genetics Stanford University Stanford, CA USA

Recombinant AAV vectors (rAAV) have provided some successes in early clinical trials. However, when moving from animals to humans a number of unanticipated responses have occurred that resulted in lower than expected efficacy. Based on our published (Lisowski et al., Nature 2014) and more recent unpublished studies, we propose that murine-human xenotransplant models for muscle and liver represent a robust animal model that may more closely predict clinical trial outcomes. Moreover, such models in combination with multi-species DNAse-shuffled AAV capsid libraries have allowed us to select for AAV vectors that show at least 10 times enhanced transduction of human muscle and liver. Current screens for rAAV vectors that show selective transduction of Hepatitis B virus infected cells and increased DNA carrying capacity are ongoing. The episomal nature of the vector genomes in transduced cells restrict classical rAAV-mediated gene transfer to quiescent tissues. Moreover, even with the low rate of AAV integration, high rates of hepatocellular carcinoma resulting from promoter

activation of oncogenic loci in young mice, and loss of episomal AAV genomes (and hence transgene expression) with normal growth and development has raised concerns about treating infants. To circumvent these concerns and provide a viable approach to treating infants, we have developed an AAV promoterless gene targeting approach without the use of nucleases (Barzel et al Nature 2015) and use this tactic to successfully treat mice with hemophilia B. We are now expanding this approach for treating a variety of diseases as well as developing novel methods for increasing gene targeted-mediated transgene expression. The discovery and characterization of novel AAV vectors as well as their use in both classical gene transfer and genome-editing approaches broadens their application in both biological discovery and therapeutic applications.

INV08

Prosthetic Gene Networks for Biomedical Applications

M Fussenegger [1]

1: ETH Zurich, Department of Biosystems Science and Engineering, Basel

Since Paracelsus' (1493-1541) definition that the dose makes the drug, the basic treatment strategies have largely remained unchanged. Following diagnosis of a disease the doctor prescribes specific doses of small-molecule drugs or protein pharmaceuticals which interfere with disease-associated molecular targets. However, this treatment concept lacks any diagnostic feedback, prophylactic impact and dynamic dosage regimen. We have pioneered the concept of metabolic prostheses which, akin to mechanical prosthesis replacing defective body parts, interface with host metabolism to detect and correct metabolic disorders. Metabolic prostheses consist of designer cells containing synthetic sensor-effector gene networks which detect critical levels of disease metabolites, processes pathological input with Boolean logic and fine-tune in-situ production and release of protein therapeutics in a seamless, self-sufficient and closed-loop manner. When implanted inside insulated, immunoprotective and autovascularizing microcontainers the metabolic prostheses connect to the bloodstream, constantly monitor the levels of disease-associated metabolites and trigger an immediate therapeutic response to prevent, attenuate or correct the disease. With their unique characteristic to dynamically link diagnosis to dose-specific in-situ production and delivery of protein pharmaceuticals, metabolic protheses will enable new treatment strategies in the future. We will present our latest generation of remote-controlled gene switches, biosensor circuits and metabolic prostheses and highlight the impact of synthetic biology on future biomedical applications.

INV09

Towards Clinical Translation of Hematopoietic Stem Cell Gene Targeting for the Correction of Inherited Mutations

P Genovese [1]

1: HSR TIGET, San Raffaele Telethon Institute for Gene Therapy

Targeted genome editing by artificial nucleases has brought the goal of site-specific transgene integration and gene correction within the reach of gene therapy. However, its application to long-term repopulating Hematopoietic Stem/Progenitor Cells (HSPCs) has remained for long time only elusive. By tailoring delivery platforms and culture conditions we overcame the barriers that specifically constrain homology directed DNA repair in the primitive HSPC subset and provide stringent evidence of targeted integration in human HSCs by long-term multilineage repopulation of transplanted mice. We demonstrate the therapeutic potential of our strategy by targeting a corrective cDNA into the IL2RG gene of HSCs from healthy donors and a subject with X-linked Severe Combined Immunodeficiency (SCID-X1). Gene edited HSCs sustained normal hematopoiesis and gave rise to functional lymphoid cells that possess a selective growth advantage over those carrying disruptive IL2RG mutations. Based on these encouraging results, we refined and scaled-up the gene targeting protocols to develop a robust, scalable and clinically ready process for ex vivo editing of HSPC. This process will possibly enable the first clinical testing of an HSPC gene correction approach for the treatment of SCID-X1, chosen as paradigmatic disease because it provides a favorable risk-benefit ratio.

INV10

Genome editing with CRISPR-Cas9 : tools and strategies

J B Renaud [1] M Haeussler [2] M Charpentier [1]
C Boix [1] L Perrouault [1] A De Cian [1] C Giovannangeli [1]
J P Concordet [1]

1: Inserm U1154 2: University of California Santa Cruz

The success of genome editing with CRISPR-Cas9 depends on the choice of the guide RNA sequence, which is facilitated by various websites. We have evaluated on-target and off-target scoring algorithms and developed online tools for selection of guide RNAs in more than a 100 species (crispor.org). Unlike other tools, we find that bioinformatic predictions are very reliable. For on-target activity, the correlation between scores and guide activity varied considerably with the dataset. With novel data, we show how one can significantly reduce the time spent on guide RNA screening when using the most appropriate efficiency prediction model. In collaboration, we have contributed to developing genome editing in the rat and zebrafish as well as in less common model organisms, a moth and a jellyfish. For example, we have successfully targeted the rat DMD gene and generated a very promising model of Duchenne muscular dystrophy, notably for testing effects on disease progression and cardiac anomalies that were difficult to assess in the

current DMD animal models. We now show that using phosphorothioate-modified oligonucleotides strongly enhances efficiency of single-stranded oligonucleotide (ssODN) donors in genome editing and provides better design flexibility, allowing insertions more than 100 bp long. In particular, high rates of homozygous loxP site insertion and of point mutations were achieved at the mouse ROSA and rat CFTR loci, respectively. Our results demonstrate that phosphorothioate-modified oligonucleotides will significantly facilitate the generation of models of human disease in cultured cells and in mouse and rat.

INV11

Current progress of gene editing to provide a therapy for Duchenne muscular dystrophy

L J Popplewell [1] M Moore [1] H Kymalainen [1] D Vallese [1] J G Dickson [1]

1: Royal Holloway, University of London

Duchenne Muscular Dystrophy (DMD) is a hereditary, X-linked neuromuscular disease, resulting from mutations across the DMD gene. The subsequent absence of dystrophin protein prevents the correct formation of the dystrophin-associated protein complex (DAPC), a structural link between the intracellular actin and extracellular matrix. This compromises muscle stability and contractility, giving rise to progressive muscle wasting. A number of different gene therapies are currently in clinical trial and are reporting varying degrees of therapeutic benefit. These include: AAV microdystrophin delivery, premature termination codon read-through using Ataluren, exon-skipping and utrophin upregulation. These gene therapies would require repeat administration, and/or carry an adverse immunological risk, and/or are restricted by mutation specificity, which may limit their clinical relevance. Such problems may be circumvented through the use of gene editing. With the development of prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 as a gene editing platform, work is progressing at an incredible rate in this area. Recent studies performed at Royal Holloway University of London will be described including; - Use of various endonucleases to target mutation hotspots in the DMD gene - Correction of the mutated genetic reading frame through InDel disruption of splice acceptor sites - Homology-directed repair of the mutated DMD gene using ssODN and cDNA templates - Optimisation of ex vivo application and description of in vivo studies in preclinical models The work presented will demonstrate the potential of gene editing to provide a permanent corrective gene therapy that, with the correct design, could hold high DMD patient applicability.

INV12

Engineering and phenotyping human stem cell cardiomyocytes to understand genetic heart disease

C Denning [1]

1: Dept of Stem Cell Biology, Centre for Biomolecular Sciences, University of Nottingham, University Park, NG7 2RD, United Kingdom

Over the last 15 years, human pluripotent stem cell (hPSC) technologies have progressed from academic curiosities into tools with the promise to underpin commerce, leading to real progress in understanding of disease, improving drug safety and providing novel routes to clinical translation. With an emphasis on the heart, this presentation will discuss our progress in producing models of genetic disease by reprogramming somatic cells into human induced pluripotent stem cell (hiPSC). This includes various conditions such as long QT syndrome, Duchenne muscular dystrophy and CPVT, which affect the function and / or structure of cardiomyocytes. We will show how the Cas9/CRISPR system is being used to produce defined sets of polymorphisms in the ADRB2R and GRK5 loci, which encode proteins that underpin b2-adrenoceptor signaling. These polymorphisms reflect the genotypes in the patient population and we will present early data on how these changes may influence receptor density, internalization and both receptor and heart function. Since these panels of hiPSC and engineered lines can now be created with relative ease, bottlenecks of scaled culture, differentiation and phenotyping are becoming a considerable issue. Thus, we have developed an automation suite that includes a bespoke robotic platform to culture and differentiate hPSCs at scale into cardiomyocytes. Into this suite, we have incorporated high content platforms that allow assessment of structure (confocal plate reader imaging) and function (mitochondrial activity, contractility and electrophysiology). Despite these advances, numerous challenges remain, such as incomplete epigenetic reprogramming of hiPSC relative to hESCs, which impedes development of phenotypes such as hypertrophy and our current progress will be discussed.

INV13

Opportunities for modeling neurodegenerative diseases using iPS cell technology

A Raya [1]

1: ICREA Research Professor and Director, Center for Regenerative Medicine in Barcelona (CMRB), Barcelona

The reprogramming of somatic cells to induced pluripotent stem (iPS) cells using delivery of defined combinations of transcription factors is a groundbreaking discovery that opens great opportunities for modeling human diseases, including Parkinson's disease (PD). iPS cells can be generated from patients and differentiated into disease-relevant cell types, which would capture the patients' genetic complexity. Furthermore, human iPS-derived neuronal models offer unprecedented access to early stages of the disease, allowing the investigation of the events that initiate the pathologic process in PD.

Recently, human iPS-derived neurons from patients with familial and sporadic PD have been generated and importantly they recapitulate some PD-related cell phenotypes, including abnormal α-synuclein accumulation in vitro, and alterations in the autophagy machinery. I will introduce our efforts to generate PD iPS-based models and discus the potential future research directions of this field.

INV14

Differentiation of human induced pluripotent stem cell-derived otic placode progenitors into inner ear hair cell lineage

H Lahlou [1] A Lopez [1] A Fontbonne [1] F Feron [2] E Nivet [2] A Zine [1 3]

1: Laboratory of Integrative and Adaptive Neurosciences, UMR 7260, AMU, Marseille 2: Neurobiology of Cellular Interactions and Neurophysiopathology, UMR 7259, AMU, Marseille 3: Neurosensory Biophysics, Faculty of Pharmacy, Montpellier University, Montpellier

Human induced pluripotent stem cells (hiPSCs) technology holds great expectations for drug discovery and cell therapy applications. Recent progresses have been made in applying hiPSC technology to a variety of organ systems such as the retina, as well as the peripheral and central nervous systems. Noteworthy, the inner ear represents another system of particular interest for translational studies relying on the use of hiPSCs.
In this study, our main objective is to develop a hiPSC-based protocol allowing efficient derivation of otic progenitors and HC-like cells.
We first sought to induce the generation of otic progenitor cells prior to proceed to their further differentiation into HC-like cells. To this end, hiPSCs were cultured onto a laminin using a medium supplemented with FGF3/FGF10 during 12 days. Then, newly generated otic progenitors were maintained in a medium containing either retinoic acid and EGF or Notch pathway modulators for 2-4 weeks. At the end of each of the two steps, qPCR and immunocytochemical analyses were performed to assess the expression of otic- and HC-associated markers, respectively.
We observed the generation of cells upregulating comprehensive otic/placode markers at the end of the induction phase. Interestingly, a fraction of these cells were found to express HC markers upon further differentiation.
Our data indicate that interference with Notch pathway as a potent mean to derive human HC-like cells from hiPSC-derived otic progenitors. This work could contribute to better understanding of the mechanisms controlling human HC differentiation and could benefit for a cell-based therapy for inner ear disorders.

INV15

An armed oncolytic adenovirus platform for delivering checkpoint inhibitors and other therapeutic antibodies directly and selectively to human tumours

K Fisher [1]

1: Oxford University

Enadenotucirev is a chimeric Ad11p/Ad3 oncolytic adenovirus with potent and selective activity against a range of epithelial cancer cells in vitro and in vivo and has blood stability enabling systemic dosing. Data from ongoing clinical trials have shown that i.v. dosed EnAd infects and replicates in tumour cells, producing significant amounts of viral protein (hexon), and that this is associated with CD8+ cell infiltration in tumour cell nests, consistent with immune stimulation within the tumour.
To develop 'armed' Enadenotucirev variants for delivery of therapeutic agents targeting the tumour microenvironment for enhanced efficacy, we have exploited the deletion mutations in EnAd to develop a novel cloning system for rapid generation of modified viruses that can produce antibodies and other immunomodulatory products. Using this platform, we have successfully produced viruses encoding full-length (NG-135) and ScFv (NG-76) forms of anti-human VEGF antibodies which retain the virus activity profiles of EnAd in vitro and in vivo (virus replication, gene expression and oncolytic action), but also produce the respective anti-VEGF antibody forms. Antibody expression by these viruses could be detected in tumour tissue as both mRNA and functional antibodies early, within 3 days of treatment, and expression was sustained over several weeks. The NG-135 virus was also efficacious at reducing tumour burden and prolonging survival in an A549 lung orthotopic tumour model. Viruses expressing anti-PDL1 and anti-CTLA4 antibodies have now been generated and shown to retain activity characteristics of EnAd and to produce antibody "payloads" with the correct target binding and immunomodulatory functional properties. Studies evaluating the impact of these different viruses in vivo, individually and in combination, on the growth and microenvironment of tumours as well as immune responses are in progress.
Studies are in progress to evaluate the impact of these different viruses, individually and in combination, on in vivo tumour growth, the tumour microenvironment and anti-tumour immune responses.

INV16

Exploiting antiviral immune mechanisms for cancer immunotherapy

U Sahin [1]

1: TRON- University Medical Center of the Johannes Gutenberg University Mainz

Abstract not available.

INV17

Chimeric Antigen Receptor Therapy for Cancer

S Guedan [1]

1: IDIBELL-ICO, Barcelona

Adoptive immunotherapy using chimeric antigen receptor (CAR)-transduced T cells is a promising cancer therapy. CARs are recombinant receptors that combine the targeting specificity of monoclonal antibodies with the delivery of defined T-cell activating signals. Recently, some dramatic tumor regressions in patients with B-cell neoplasms, like chronic and acute lymphoblastic leukemia and non-Hodgkin lymphoma, using CARs targeting CD19 have sparked great interest. Despite these promising results, significant challenges remain with regard to understanding the factors that impact effective T cell persistence and function, particularly in the context of solid tumors. Here we will present recent and ongoing successes in developing CART cell therapies. We will focus on the design of CARs and the requirements for optimal costimulation, as well as the selection of the right cell type for engineering.

INV18

Fueling T cell immunotherapy: Metabolic Challenges

N Taylor [1]

1: Université de Montpellier

Abstract not available.

INV19

Complementarity and redundancy of innate lymphoid cells

E Vivier [1]

1: Centre d'Immunologie de Marseille-Luminy (CIML) Aix Marseille Université - INSERM - CNRS

Innate Lymphoid Cells represent an emerging population of lymphocytes. ILCs include Natural Killer (NK) cells and three main subsets, ILC1, ILC2 and ILC3. In contrast to T and B cells, ILCs do not express antigen-specific receptors derived from gene rearrangements. Besides this major difference in their recognition repertoire, ILC and T cell subsets share striking similarities as ILC1, ILC2 and ILC3 are driven by T-bet, GATA-3 and RORg?t transcription factors, and produce IFN-?, IL-5/IL-13 and IL-17/IL-22 respectively. In addition, NK cells are driven by Eomes and T-bet, can be cytolytic and produce IFN-g?, inasmuch as CD8+ T cells. These common features led to suggest that ILCs might correspond to innate counterparts of T cells. Over the course of evolution, two highly parallel systems have thus emerged in which ILCs mimic the effector profile of T cell subsets. However, it is still unclear how the innate and adaptive immune systems integrate these two arms. We will review the emerging set of data showing that ILCs and T cells can exert redundant functions in natura in humans and in models of experimental disease in mice, and discuss how the overlapping functions of ILCs and T cells contribute to the robustness of immunity and hence to the fitness of the hosts.

INV20

Humoral immune responses to AAV vectors

F Mingozzi [1]

1: Genethon

Adeno-associated virus (AAV) vector mediated gene therapy has shown promising results in preclinical and clinical studies. However antibody responses directed against the AAV capsid are an important limitation to the use of AAV vectors as therapeutic tools, as even low-titer anti-capsid neutralizing antibodies (NAb) can lead to vector clearance and lack of efficacy. Anti-AAV antibodies are commonly found in adult individuals, as they result form the exposure to the wild-type virus in life. This forces to exclude up to 60% of subjects from enrollment in AAV gene therapy trials. Additionally, anti-AAV NAbs develop at high titers following vector administration and persist for several years after AAV vector administration, making vector re-administration hard if not impossible. In this presentation, an overview of strategies to overcome the limitation of anti-AAV antibody responses will be given, will particular emphasis on the translation of these strategies to the clinic.

INV21

From a cancer causing virus to a potentially safer strategy for gene therapeutics

J D Suerth [1] V Labenski [1] E Verhoeyen [2 3] A Schambach [1 4]

1: Institute of Experimental Hematology, Hannover Medical School, 30625 Hannover, Germany 2: CIRI, International Center for Infectiology Research, EVIR team, INSERM U1111, CNRS, UMR5308, Université de Lyon-1, ENS de Lyon, Lyon, France 3: INSERM, U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), équipe 'contrôle métabolique des morts cellulaires', Nice 06204, France 4: Division of Hematology/Oncology, Boston Children's Hospital, Harvard Medical School, Boston MA 02115, USA

Gene therapy enabled by integrating retroviral vectors has proven its general effectiveness for applications in basic science and clinical treatment. However, vector integration-associated adverse events related to insertional mutagenesis were documented in clinical trials, fueling the efforts to develop safer vector systems. Of note, alpharetroviruses, originally identified as a cancer-causing agent, have a more random and potentially safer integration pattern, when compared to gammaretro- and lentiviruses. This special integration pattern has likely evolved, as alpharetroviruses transfer their incorporated proto-oncogenes and are thus not dependent on insertional mutagenesis for transformation. Moreover, the special biology of alpharetroviruses is reflected by their inability to replicate in mammalian cells, as they are only replication-competent in bird cells.

Here, we outline how alpharetroviruses can be converted into state-of-the-art vectors with improved safety features, capable of efficiently transducing human cells and thus harboring a perspective for clinical applications. Based on a split-packaging technology with high titer production in human cells, we effectively transduced human and murine hematopoietic stem cells, as demonstrated in a bone marrow transplantation model. Moreover, we obtained proof-of-principle for transduction of human T cells, a major target population for anti-cancer therapies. In this presentation, we will explain and discuss clinically relevant examples, in which alpharetroviral SIN vectors could be used for translation into clinical application. In summary, together with a potential production perspective from stable producer clones and the associated upscaling potential, alpharetroviral SIN vectors represent promising tools for clinical application in gene therapy of inherited diseases and anti-cancer immunotherapy.

INV22

Nucleic acid delivery systems for RNA therapy and gene editing

D G Anderson [1 2 3 4]

1: Massachusetts Institute of Technology 2: Institute for Medical Engineering and Science 3: David H. Koch Institute for Integrative Cancer Research 4: Harvard-MIT Division of Health Sciences & Technology

High throughput, combinatorial approaches have revolutionized small molecule drug discovery. Here we describe our work on high throughput methods for developing and characterizing RNA delivery and gene editing systems. Libraries of degradable polymers and lipid-like materials have been synthesized, formulated and screened for their ability to delivery RNA, both in vitro and in vivo. A number of delivery formulations have been developed with in vivo efficacy, and show potential therapeutic application for the treatment of genetic disease, viral infection, and cancer.

INV23

Bioinspired delivery systems for RNA medicine and DNA vaccines

B Pitard [1]

1: inCellArt

Protein expression and RNA interference require efficient delivery of DNA, mRNA or small double stranded RNA into cells. Although cationic lipids are the most commonly used synthetic delivery vectors, a clear need still exists for better delivery of any types of nucleic acids molecules to improve their biological activity and especially after local in vivo delivery. To optimize the transfection efficiency, a molecular approach consisting in optimizing each part of the different domains of a given cationic lipid (cationic polar headgroup, linker and hydrophobic moiety) is usually performed. Although this approach has allowed synthesizing new generations of in vitro efficient and bio-inspired cationic lipids, no particular lipid has really emerged as universal delivery vectors for all nucleic acids in vivo. We therefore hypothesized that modulating the supramolecular assembly of the nucleic acids complexes, rather than modifying the cationic lipid molecule itself, may have a greater impact on the in vivo transfection efficiency. To validate this supramolecular approach, we synthesized novel classes of amphiphilic molecules made of polymers rather than lipids. Combinations of amphiphilic block copolymers with nucleic acids led to the formation of particles where nucleic acids are not entrapped inside particles characterized by a lamellar structure sandwiching nucleic acids molecules between lipids bilayers, like observed with cationic lipids. Results show that amphiphilic block copolymers led to the dramatic improvement of DNA and messenger RNA transfection efficient after delivery in the muscle allowing efficient DNA vaccination and protein replacement therapy, respectively.

INV24

Recombinant protein therapy for achondroplasia

E Gouze [1]

1: Centre Méditerranéen de Médecine Moléculaire

Achondroplasia is a rare genetic disease characterized by abnormal bone development resulting in short stature. It is caused by a single point mutation in the gene coding for fibroblast growth factor receptor 3 (FGFR3), which leads to prolonged activation upon ligand-binding. To prevent excessive intracellular signaling and rescue the symptoms of achondroplasia, we have developed a recombinant protein therapeutic approach using a soluble form of human FGFR3 (sFGFR3), which acts as a decoy receptor and prevents FGF from binding to mutant FGFR3. sFGFR3 was injected subcutaneously to newborn Fgfr3ach/+ mice—the mouse model of achondroplasia—twice per week throughout the growth period during three weeks. Effective maturation of growth plate chondrocytes was restored in bones of treated mice, with a dose-dependent enhancement of skeletal growth in Fgfr3ach/+ mice. This resulted in normal stature and a significant decrease in mortality and associated complications, without any evidence of toxicity. Proof-of-concept has thus been clearly established describing a novel approach for restoring bone growth and suggest that sFGFR3 could be a potential therapy for children with achondroplasia and related disorders.

INV25

Vector Safety 2.0 in Gene Therapy

M Schmidt [1]

1: NCT DKFZ Heidelberg

Viral vectors have shown their efficiency in clinical studies of rare diseases. However, insertional mutagenesis led to side effects and even malignant transformation in few patients. These observations prompted new vector designs, such as self-inactivating (SIN) LTR configurations and tissue specific promoters that promise safe and efficient gene-correction in vivo. With the advent of next

generation sequencing (NGS) technologies, large-scale identification of vector integration profiles and vector persistence studies over time became feasible, leading to profound new insights into vector trafficking and the biology of the affected cells, tissues and organs. Thus, clonality and vector safety analyses may represent an essential part of pharmacokinetics studies for a gene therapeutic drug. Here, the current state-of-the-art and future perspectives in vector safety studies will be presented. This includes our current (linear amplification-mediated (LAM) PCR assay coupled to NGS and also new technologies like capture based quantitative sequencing of viral vector sequences (and integration sites) as well as associated bioinformatical data mining tool suites.

INV26

Tricyclo-DNA: highly promising antisense oligonucleotides for splice switching therapeutic approaches

G Griffith [1] S Relizani [1] L Echevarria-Zamora [1] V Robin [1] B Dugovic [2] C Vaillend [3] C Leumann [2] L Garcia [1] A Goyenvalle [1]

1: Université de Versailles Saint Quentin 2: University of Bern 3: Université Paris sud

Antisense oligonucleotides (AON) hold promise for therapeutic splice-switching correction in many genetic diseases; however, despite advances in chemistry and design, systemic use of AONs is still limited due to poor tissue/cellular uptake. This talk will describe a novel class of AONs made of tricyclo-DNA (tcDNA), which displays unique pharmacological properties and unprecedented uptake in many tissues after systemic administration. These outstanding properties have been demonstrated in different mouse models of genetic diseases such as Duchenne muscular dystrophy (DMD) and Spinal muscular atrophy (SMA). DMD is a neurogenetic disease typically caused by frame-shifting deletions or nonsense mutations in the gene encoding dystrophin and characterized by progressive muscle weakness, cardiomyopathy, respiratory failure and neurocognitive impairment. While current naked AONs do not significantly enter the heart or cross the blood brain barrier, systemic delivery of tcDNA-AONs allow high levels of dystrophin rescue in skeletal muscles as well as in heart and to a lower extent in the brain. Our results demonstrate for the first time physiological improvement of the cardio-respiratory functions and correction of behavioural features linked to the emotional/cognitive deficiency associated with the lack of dystrophin. These properties, together with the safe toxicology profile of tcDNA make this chemistry particularly attractive for future therapies in DMD patients as well as in other neuromuscular disorders or diseases eligible for splice-switching approaches requiring whole-body treatment.

INV27

Manufacturing of AAV vectors: what are the technical challenges before treating patients with muscular diseases?

M Hebben [1]

1: Généthon, Evry

While AAV-mediated gene therapy has shown promising clinical efficacy, manufacturing of large amounts of AAV vectors to address product commercialization remains a challenge. Particularly for muscular diseases like Duchenne Muscular Dystrophy, the predicted dose requirement per patient is so high that producing enough product even for phase I trials is difficult to achieve.
Whereas several AAV production methods are available at moderate scales, the transition to industrial levels may raise difficulties that are often underestimated in early development: quality of the final product, productivity and cost of the manufacturing process.
To gain knowledge on these aspects, we have evaluated two production platforms to produce recombinant AAV8 - a serotype commonly used for its tropism for the muscles - in stirred tank bioreactors: on one hand, the standard triple transfection process in suspension HEK293 cells; on the other hand, the dual Baculovirus expression system in Sf9 cells. Both processes displayed comparable productivity and generated similar ratios of full/empty capsids. However, the results revealed that the HEK293 transfection process was the most efficient to produce AAV8 vector with the foreseen target profile, particularly with regards to genome integrity, capsid proteins content and infectivity in vitro and in vivo.
Despite the highest quality of AAV8 vectors obtained by transfection, the productivity and the cost of such a process remain a critical issue to envisage commercialization of AAV-based gene therapy products. A comparative analysis of the costs and options for cost reduction will be presented.

INV28

Clinical Manufacturing of Lentiviral Vectors by Transient Transfection in Single Use Systems

J Warren [1]

1: bluebird bio, Cambridge, MA, USA

The potential of lentiviral gene therapy vectors for the treatment of severe genetic diseases using genetically modified CD34+ cells and hematological malignancies using chimeric antigen receptor T-cells (CAR-T) is supported by recent positive data in clinical trials showing promising therapeutic benefits and safety. The progress from early clinical development stages to late stage development requires a robust clinical manufacturing process for the vector product, and the establishment of a characterized connection between process unit operations and product quality attributes. Substantial clinical manufacturing experience has enabled the retrospective analysis of in-process and product release data as a method of identifying potential sources of process and product variability. An extensive risk-based approach to process characterization has been applied to

the vector production process, beginning with an assessment of vector product attributes to define those expected to impact drug product quality, and proceeding to an analysis of the overall impact of vector production process parameters. Establishment of a next-generation manufacturing process requires consideration of processing platforms, with the guiding principles of preserving the comparability of the lentiviral product profile while achieving benefits in scalability and cost of goods. The production of lentiviral vectors involves unique challenges relative to those seen with alternative vectors or traditional biologics, which will be discussed.

INV29

Gene Therapy of Hemoglobinopathies

M Cavazzana [1]

1: Hôpital Necker – Enfants Malades, Paris

Abstract not available.

INV30

Generation of diverse organ buds from stem cells towards therapy

T Takebe [1 2 3]

1: Department of Regenerative Medicine, Yokohama City University Graduate School of Medicine, Japan.
2: Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, USA
3: PRESTO, Japan Science and Technology Agency, Japan

In vitro organogenesis is now becoming a realistic goal of stem cell biology; however, one practical challenge is to develop a four-dimensional (4-D) stem cell culture system whereby multiple progenitors communicate in a spatiotemporal manner. During early hepatogenesis, the multicellular communication that occurs among mesenchymal stem cells, undifferentiated vascular endothelial cells and anterior visceral endodermal cells are required to initiate the budding of the rudimentary liver in the foregut. To recapitulate early organogenesis, we recently showed that specified hepatic cells self-organized into 3-D iPSC-derived liver buds when co-cultivated on solidified Matrigel with multiple stromal cell populations. By transplanting in vitro grown organ bud, we have demonstrated the vascularized and functional liver tissues in an immunodeficient animal with therapeutic potential (Nature, 2013 & Nature Protocols, 2014). Furthermore, we also demonstrated the applicability of this approach to other systems by delineating the mechanisms guiding organ bud formation. Specifically, mesenchymal progenitors initiated organ bud formation within these heterotypic cell mixtures, which was dependent upon substrate matrix stiffness. Defining optimal mechanical properties of the substrate promoted formation of 3D, transplantable organ buds from tissues including kidney, pancreas and cartilage (J Clin Invest, 2014 & Cell Stem Cell, 2015). In this talk, I will summarize the state-of-art of these organ bud based approaches, and discuss their future potential applications.

Oral Presentations

OR01

Gene editing of PKLR gene in human hematopoietic progenitors

S Fañanas Baquero [1 2] I Orman [1 2] A Gouble [3]
R Galetto [3] L Poirot [3] J A Bueren [1 2]
O Quintana-Bustamante [1 2] J C Segovia [1 2]

1: Division of Hematopoietic Innovative Therapies, Centro de Investigaciones Energéticas Medioambientales y Tecnológicas/Centro de Investigación Biomédica en Red de Enfermedades Raras (CIEMAT/CIBERER). Madrid. Spain. 2: Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD, UAM). Madrid. Spain 3: CELLECTIS Therapeutics. Paris. France.

Pyruvate Kinase Deficiency (PKD) is a rare erythroid metabolic disease caused by mutations in the PKLR gene which encodes the erythroid specific Pyruvate Kinase (RPK) enzyme. The defective enzyme fails to produce normal ATP levels consequently, erythrocytes from PKD patients show an energetic imbalance. Site-specific hematopoietic stem cell gene therapy using gene editing would be the safest approach to treat PKD patients. We have developed a gene editing approach that addresses the treatment of most PKD patients through a Knock-In strategy using PKLR specific TALEN, which has been shown effective to revert PKD phenotype in PKDiPSC lines. However, it is direct application in hematopoietic progenitor cells (HPCs) is challenging. So that, a matrix carrying an exon 3-11 partial codon optimized version of RPK cDNA and a puromycin selection cassette was designed to be inserted in the second intron of the PKLR gene assisted by TALEN nucleases. Thus, therapeutic matrix and PKLR TALEN were electroporated in purified CD34+ from healthy cord blood. Cells were then expanded and puromycin selected to enrich the population for gene edited ones. Although a high toxicity and low efficiency were observed, up to 96% colony forming units showed the specific integration. Experiments to improve efficacy and reduce toxicity have been conducted and a high percentage of gene edited HPCs were detected after shortening cell expansion and puromycin selection. Importantly, gene edited HPCs were detected after engraftment in immunodeficient (NSG) mice. Overall, we showed that gene editing in engraftable HPCs is feasible, but more improvements are needed.

OR02

Modeling coenzyme Q10 deficiency using iPS cells

D Romero-Moya [1] C Santos-Ocaña [2]
J A Rodriguez-Gómez [3] C Bueno [1] J Castaño [1]
A Giorgetti [1] I Velasco [1] M F Fraga [4] A Fernández [4]
J López-Barneo [3] P Navas [2] P Menéndez [1 5]

1: Josep Carreras Leukemia Research Institute 2: CABD-UPO 3: IBIS 4: IUOPA 5: ICREA

Primary Coenzyme Q10 (CoQ10) deficiency is a rare metabolic syndrome, which originates from mutations in genes responsible for CoQ10 biosynthesis. It is clinically/genetically heterogeneous and begins during childhood leading to a disorder associated to encephalopathies, myopathies and ataxias; all clinical features affecting tissues with high-energy demand such as brain and skeletal muscle (SKM). Mutations in COQ4 cause a heterogeneous pathological phenotype associated to CoQ10 deficiency. We diagnosed a 4-y.o. patient with CoQ10 deficiency carrying a novel heterozygous c.483G>C (E161D) mutation in COQ4 gene. The patient presented weakness, mild psychomotor retardation, clumsiness and myoglobinuria due to massively elevated levels of creatine kinase in serum with eventual lethal rhabdomyolysis. Metabolically, patient's fibroblasts showed a reduction in [CoQ10] (75%), CoQ10 biosynthesis (87%) and mitochondrial respiratory chain defects in complexes I+III (25%) and II+III (54%). Multiple bona fide iPSC clones harboring the mutation were generated and characterized. Interestingly, metabolic analyses revealed that CQ4-iPSCs retained CoQ10deficiency but resumed the MRC function, likely reflecting the glycolytic nature of iPSCs. To elucidate the developmental impact of COQ4 mutation, iPSCs were differentiated into disease-affected tissues: motor neurons and dopaminergic neurons. No differences were observed between CQ4- and Ctrl-iPSC in their specification into neural lineages. Although functional experiments are underway, this iPSC model does not seem to reproduce the patient`s mild neurologic phenotype. We successfully repaired the single point mutation using the CRISPR/Cas9-based editing system. iPSCs are currently being differentiated into SKM which is the most affected tissue and studying the effect of the mutation using the iPSC-edited clone.

OR03

Benefits of human cardiac progenitor cell seeded-collagen patches applied on failing right ventricle: progenitor cells differentiation/migration may impact the RV function.

V Lambert [3] C Rucker-Martin [3] J Boissardier [2] A Hodzic [3] M Ly [3] E Le Bret [3] M Puceat [1 2]

1: Aix-Marseille University 2: Inserm 3: Centre Medical Marie Lanelongue

Cell-based myocardial repair is an innovative approach to treat right ventricle (RV) failure. The aim was to regenerate the RV myocardium in a repaired Tetralogy of Fallot pig model with RV dysfunction. After 4 months of a combined overloaded RV, cell therapy was surgically administrated in one pig using human NKX2.5+ cardiac progenitor cell-seeded collagen pressured-patches (3 patches, 107 cells within each patch) applied on the epicardium. One pig also received patches of Nkx2.5+ cells added with endothelial CD31+/KDR+cells (5x 106 each). Myocardial function was measured 1 month after transplantation by standard and strain echocardiography. The fate of progenitors was studied using antibodies directed against an anti-human mitochondrial marker, troponin T (TnT), Nkx2.5, CD31, Ki67, actinin. Fully differentiated human cells were found in the patch and migrated within the myocardium close to the endocardium. These cells were still immature but some featured TnT sarcomeric units. Addition of endothelial cells to the patches improved migration of the pluripotent stem cell cardiac derivatives but less fully differentiated human cardiomyocytes was observed. Regarding RV functional data, cell therapy slowed down (pig receiving only cardiac cells) or reversed (pig receiving both cardiac and endothelial cells) the RV dilation and increased the RV anterior free wall thickness. Global RV functional parameters were improved in the pig receiving only cardiac cells. Segmental parameters increased in both. Endothelial cells may have speed up migration of cells out of the epicardial patch. Human cardiomyocytes migrated and differentiated, contributing to maintain RV adaptation to overload.

OR04

Generation and characterization of induced Pluripotent Stem Cells from an infertile man carrying a complex chromosomal rearrangement

A Mouka [3 4] V Izard [2 3] S Brisset [2 3 4] F Yates [1 5] A Mayeur [2 3] L Drévillon [2 3] R Jarray [1 5] G Tachdjian [2 3 4] L Maouche-Chrétien [1 4] L Tosca [2 3 4]

1: CEA 2: AP-HP 3: Hôpitaux Universitaires Paris-Sud 4: Université Paris-Saclay 5: Sup'Biotech

Infertility is a major health concern affecting about 15% of couples. Despite progress in human reproductive biology, the cause of male infertility remains often undefined. The use of iPSCs derived from infertile and normal individuals will allow to produce primordial germ cells. Reconstituting the first steps of spermatogenesis in a dish will allow to understand the biology of male gonad development and to decipher certain infertilities. We generated iPSCs from an infertile man carrying a complex chromosomal rearrangement (CCR) associated with azoospermia. The CCR was characterized by karyotype, FISH and array-CGH assays. Analyses showed a two-step CCR: An insertion of a portion of chromosome 12 into the short arm of chromosome 7 and a pericentric inversion of the derivative chromosome 12. Successive analysis of chromosomes allowed to estimate the location of breakpoints and determine the gene cartography in these regions. Array-CGH analysis did not detect DNA copy number variation in the breakpoints or elsewhere. Pluripotency of iPSCs were assessed by RT-PCR which confirmed the expression of the pluripotency markers Oct4, Nanog, Sox2 and Rex-1. Immunofluorescence staining highlighted the expression of pluripotency markers SSEA4, TRA-1-60 and OCT3/4. Intra-muscular injection of infertile-iPSC into immuno-deficient mice induced teratoma formation. Generating patient-specific iPSCs provides a priceless in vitro model for studying meiosis and germ cell development and opens new therapeutic perspectives for infertility treatment. Indeed, in contrast to somatic cells, iPSCs can be readily propagated before inducing their differentiation into suitable tissues making their use favorable for therapeutic applications as drug screening.

OR05

Lessons learned from first-in-man gene therapy clinical trial: identification of new vulnerabilities for pancreatic cancer treatment

M Gayral [1] E Saland [1] H Lulka [1] A Vignole-Vidoni [1] N Hanoun [1] A Lemarié [1] L Ligat [1] N Saint-Laurent [1] F Lopez [1] L Buscail [1] J E Sarry [1] P Cordelier [1]

1: INSERM U1037 Toulouse France

Pancreatic cancer (PDAC) remains a deadly disease with no cure. We demonstrated that non-viral gene therapy to sensitize cancer cells to chemotherapy was safe and feasible, and offers therapeutic benefit in patients. However, alternative molecular pathways must be targeted to relieve resistance to treatment for best therapeutic benefit. We identified cytidine deaminase (CDA) that catalyzes the hydrolytic deamination of cytidine and deoxycytidine to uridine and deoxyuridine, as overexpressed (i) in cohorts of patients resisting to gemcitabine, (ii) in PDAC as compared to normal parenchyma, and (iii) in patients receiving gene therapy. Targeting CDA using genetic tools sensitizes cancer cells to chemotherapy both in vitro and in vivo, with very high efficacy. In the absence of chemotherapy, loss of CDA unexpectedly alters cell proliferation and tumor progression. Both pyrimidine and purine levels are decreased and TCA cycle is altered, strongly suggesting mitochondrial dysfunction, as we further demonstrate decreased mitochondrial ATP and down expression of key proteins of the mitochondrial OXPHOS complexes and □-oxidation. Consequently, mitochondrial ROS are elevated with major changes in the redox balance. Remarkably, tumor cells retaliate using compensatory mechanisms resembling Pasteur effect with elevated lactate levels. This study illustrates that genetic depletion of CDA reverses the chemoresistance of PDAC cells to standard-

of-care therapy, and, for the first time, that CDA depletion induces a severe energetic stress. This study paves the way for new synthetic lethality strategies, based on the genetic targeting of CDA, alone or in combination, for the management of patients with PDAC.

OR06

T lymphocyte immunotherapy: Utilising cell surface Glut1 levels to select T cells with distinct effector functions

G Cretenet [1] M Matias [1] I Clerc [1] C Mongellaz [1] N Taylor [1] V Dardalhon [1]

1: Institut de Génétique Moléculaire de Montpellier, Centre National de la Recherche Scientifique UMR5535, Université de Montpellier

There have been many advances in cancer treatments but metastatic cancer remains extremely difficult to treat. As a result, new strategies are being developed with one focus based on adoptive T cell immunotherapy. While this type of approach has shown much promise, numerous challenges remain. The potential for adoptively transferred T cells to mediate an efficient anti-tumor response requires their in vivo persistence and effector function. We and others have shown that these parameters are dependent on the T cell's metabolic fitness, strongly suggesting that it will be critical to identify lymphocytes whose metabolic characteristics promote optimal anti-tumor immunity. As the energetic requirements of T lymphocytes following antigen stimulation are met by an increased glycolysis, due, at least in part, to induction of the Glut1 glucose transporter, we assessed whether surface Glut1 levels could be used to distinguish T lymphocytes with distinct features. Notably, antigen-stimulated human CD4 as well as CD8 T lymphocytes, sorted on the basis of Glut1-Hi and Glut1-Lo profiles, maintained distinct characteristics. Furthermore, the distinct features were detected in both atmospheric oxygen conditions as well as in the hypoxic oxygen conditions that often characterize tumors. Glut1-Hi lymphocytes exhibited increased effector phenotype acquisition, a skewing towards a cytotoxic fate, and an augmented proliferation that resulted in an inverted CD4/CD8 ratio. Consistent with these data, Glut1-Hi T lymphocytes displayed an enhanced ability to secrete IFN-γ as well as IL-17. Thus, separating T lymphocytes on the basis of Glut1 levels may allow the development of new T cell immunotherapy strategies.

OR07

A lentiviral vector allowing physiologically-regulated membrane-anchored and secreted antibody expression depending on B cell maturation status

F Fusil [1 2 3 4 5 6] S Calattini [1 2 3 4 5 6] F Amirache [1 2 3 4 5 6] J Mancip [1 2 3 4 5 6] C Costa [1 2 3 4 5 6] J B Robbins [7] F Douam [1 2 3 4 5 6] D Lavillette [1 2 3 4 5 6] M Law [7] T Defrance [1 2 3 4 5 6] E Verhoeyen [1 2 3 4 5 6 8] F L Cosset [1 2 3 4 5 6]

1: Centre International de Recherche en Infectiologie 2: INSERM U1111 3: Ecole Normale Supérieure de Lyon 4: Université Lyon 1 5: CNRS UMR5308 6: LabEx Ecofect 7: Department of Immunology and Microbial Science, The Scripps Research Institute 8: INSERM U1065

The development of lentiviral vectors for expression of a specific antibody can be achieved through the transduction of mature B cells. This approach would provide a versatile tool for active immunotherapy strategies for infectious diseases or cancer, as well as for protein engineering. Here, we created a lentiviral expression system mimicking the natural production of these two distinct immunoglobulin isoforms. We designed a lentiviral vector (FAM2-LV) expressing an anti-HCV-E2 surface glycoprotein antibody (AR3A) as a membrane-anchored Ig form or a soluble Ig form, depending on the B cell maturation status. FAM2-LV induced high-level and functional membrane expression of the transgenic antibody in a non-secretory B cell line. In contrast, a plasma cell (PC) line transduced with FAM2-LV preferentially produced the secreted transgenic antibody. Similar results were obtained with primary B cells transduced ex vivo. Most importantly, FAM2-LV transduced primary B cells efficiently differentiated into PCs, which secreted the neutralizing anti-HCV E2 antibody upon adoptive transfer into immunodeficient NSG (NOD/SCIDγc-/-) recipient mice. Altogether, these results demonstrate that the conditional FAM2-LV allows preferential expression of the membrane-anchored form of an anti-viral neutralizing antibody in B cells and permits secretion of a soluble antibody following B cell maturation into PCs in vivo.

OR08

Lipoaminoglycosides: Vectors and adjuvants for vaccination

T Colombani [1] T Haudebourg [1] D Habrant [2] B Pitard [1 2]

1: Inserm UMR 1087; CNRS UMR 6291- Institut du thorax 2: In-Cell-Art

Lipoaminoglycosides, synthetic molecules belonging to the cationic lipids family, are commonly used as efficient DNA carrier for transfection. This family has the capacity to stimulate the innate immune response as well as the humoral and cellular immune response, making them potential adjuvants for vaccination. However, a better understanding of the structure-function relationship in this stimulation is needed in order to develop molecules tailored for specific applications. By in-vitro experimental

approach, we investigated the influence of the composition of our lipoaminoglycosides by treated different cell types (C2C12, MEFs, JAWSII) with these molecules, then followed the expression of innate immune cytokines (Interferon-β1, Interleukine-6), chemokine (CXCL10) or sensors (Z-DNA Binding Protein 1, Interferton gama-inductible Protein 16/204). We demonstrated that some criteria are crucial to design lipoaminoglycosides with adjuvant properties: (i) They should have a dio-oleyl hydrophobic segment (C18:1); (ii) They must be composed of a succinyl spacer (2C); and (iii) the polar head group must include a Kanamycin or Tobramycin aminoglycosides. In addition, we observed a lack of correlation between positive charges possessed by cationic lipids and adjuvant effect as well as a deleterious effect of cholesterol hydrophobic segment. This study allowed the optimization of 2 lipoaminoglycosides: DOST and DOSK, simple to synthetize and characterize. Capable to efficiently transfect DNA and possessing immune-stimulations properties, they appear as promising nanocarrier and adjuvant for DNA vaccination. Other studies are ongoing in order to determine receptors involved in cells stimulations as well as in-vivo validation.

OR09

Gene transfer of both APP and PS1 induces hippocampal impairments close to human early phases of Alzheimer's disease

M Audrain [1 3] R Fol [1 3] P Dutar [5] B Potier [5] J M Billard [5] J Flament [1] S Alves [1] M A Burlot [1 3] G Dufayet-Chaffaud [2] A Bemelmans [1] J Valette [1] N Deglon [1] P Hantraye [1 5] N Cartier [2 4] J Braudeau [1]

1: CEA 2: Inserm 3: Paris Descartes University 4: Paris Sud University 5: CNRS

Alzheimer's disease (AD) is the most frequent form of dementia and no treatment is yet available. We describe here the development of an alternative AAV-based mouse model with two major objectives. First, to create a relevant model closer to human physiopathology and second, to mimic early stages of AD. This model was obtained by co-injection in the hippocampus of wild-type mice of two AAV vectors coding the human Amyloid Protein Precursor (APPsl) and the human Presinilin 1 (PS1 M146L). Our strategy allows a stable expression of transgenes without significant overexpression. This leads to a production of human APP and its cleavage products as soon as one month post-injection. These productions are stable during at least 12 months, without classical late symptoms appearance such as senile plaque, inflammation or atrophy, and trigger a hyperphosphorylation of the murine Tau protein. We performed a comparative analysis with human samples and showed similar amounts of APP following by a very close Ab42/Ab40 ratio between our model and AD cases hippocampi. Finally, behavioral impairments appeared from 3 months post-injection in association with synaptic defects such as PSD-95 decrease, extrasynaptic NMDARs activity impairments and a reduced neuromodulator production. This strategy overcame some major pitfalls of transgenic models such as continuous expression of transgenes from in utero and APP over-production. Moreover, results highlighted here are strong evidences that a human-like APP processing leads to various mechanisms occurring independently of plaques genesis and reinforce the importance to focus research on early events of AD.

OR10

BaEV pseudotyped LVs mediate high-level gene transfer in human B cells allowing secretion of Factor IX at therapeutic levels in NSG mice

C Levy [1 2] F Fusil [1 2] F Amirache [1 2] C Costa [1 2] A Girard [1 2] D Nègre [1 2] O Bernadin [1 2] G Garaulet [3] A Rodriguez [3] N Nair [4] T Vandendriessche [4] M K Chuah [4] F L Cosset [1 2] E Verhoeyen [1 2 5]

1: CIRI; Inserm U1111 2: Université Lyon 1 3: Universidad Autonoma de Madrid 4: Free University of Brussels 5: Centre de Médecine Moléculaire, Nice

B-cells are attractive targets for gene therapy of diseases associated with B-cell dysfunction and particularly interesting for immunotherapy. Moreover, B-cells are potent protein secreting cells and tolerance inducing cells. We tested here for the first time our newly developed baboon envelope pseudotyped lentiviral vectors (BaEV-LVs) for human (h) B-cell transduction. Upon B-cell receptor stimulation, BaEV-LVs transduced up to 80% of hB-cells, while VSV-G-LV transduction did no exceed 5%. Remarkably, BaEV-LVs permitted efficient transduction of 20% of resting naive and up to 40% of resting memory B cells. Importantly, BaEV-LVs reached up to 100% transduction of human plasmocytes ex vivo. Adoptive transfer of BaEV-LV-transduced mature B-cells into NOD/SCID/-/- mice allowed normal differentiation into plasmablasts and plasma B-cells confirming a sustained high-level gene marking in vivo. Since B-cells can induce tolerance, we tested BaEV-LV for transfer of hFactor IX (hFIX) into B-cells for treatment of hemophilia. BaEV-LVs encoding FIX efficiently transduced hB-cells and their transfer into NSG mice demonstrated for the first time secretion of functional hFIX from hB-cells at therapeutic levels in vivo. Concluding, the BaEV-LVs might represent a valuable tool for therapeutic protein secretion from autologous B-cells in vivo in the treatment of hemophilia and other acquired or inherited diseases.

OR11

IRES-based vectors for a combined gene therapy of heart ischemia

E Renaud-Gabardos [1] B Lebas [1 2] P Sicard [1 4] D Calise [3] F Hantelys [1] O Kunduzova [1] F Pujol [1] J Roncalli [1 2] X Chaufour [1 2] B Garmy-Susini [1] A Parini [1] A C Prats [1]

1: Inserm UMR1048 2: CHU Rangueil 3: US006 Service de microchirurgie 4: UMR CNRS 9214-Inserm 1046

Despite of considerable advances in the treatment of cardiovascular diseases, heart failure remains an important problem of public health. In such a context, gene therapy appears as an attractive approach. Several

studies demonstrate the interest of combining therapeutic genes in treatment efficiency. Our laboratory develops combined gene therapy of cardiovascular diseases, based on translational activators, IRESs (internal ribosome entry sites), to generate combined gene transfer vectors. We have validated the concept of IRES-based vector for therapeutic angiogenesis of hindlimb ischemia, using a vector co-expressing the angiogenic factors FGF2 and Cyr61. This association creates a synergistical effect of the two molecules that are more efficient at low doses. We have designed IRES-based vectors for gene therapy of myocardial ischemia. The aim is to express combinations of molecules stimulating angiogenesis, myocardium contractile function and cardiomyocyte survival. A bicistronic lentivector expressing two luciferase genes separated by the FGF1 IRES has been successfully assessed by direct intracardiac injection in a mouse model of infarcted myocardium, showing that the IRES drives increased transgene expression when heart is ischemic. Lentivectors have been designed to express combinations of therapeutic genes including Apelin, FGF2 or Serca2a. They have been assessed in vivo, and the combinations of Apelin-FGF2 and Apelin-FGF2-Serca2a improve cardiac function and limit dilated features. The two multicistronic lentivectors exhibit stronger effects on angiogenesis, while the tricistronic vector Apelin-FGF2-Serca2a generates the best therapeutic benefits. These data validate the use of IRES-based lentivectors for combined gene therapy of heart ischemia and reveals the benefits of the Apelin-FGF2-Serca2a triplet.

OR12

Exhaustive evaluation of DNA species packed in adeno-associated vectors produced in insect cells by High-Throughput Sequencing technology

M Penaud-Budloo [1] E Lecomte [1] B Tournaire [1] B Cogné [1] V Blouin [1] A Donnart [2] P Moullier [1 3] A Léger [1] E Ayuso [1]

1: Atlantic Gene Therapies, INSERM 1089, Nantes, France 2: Plateforme Genomique, Nantes, France 3: Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, Florida, USA

Recombinant adeno-associated vectors (rAAV) are viral vectors of choice for gene therapy of many inherited diseases. Medicinal products based on rAAV are predominantly manufactured by transient transfection of mammalian cells or baculovirus expressing vectors (BEV) infection of insect cells, being the latter method more suitable for large-scale production. With the success of clinical trials and advancements towards commercialization, the regulatory agencies ask for a more exhaustive characterization of the rAAV stocks. Indeed, viral capsids produced in mammalian cells are known to internalize not only the therapeutic gene but also illegitimate DNA such as plasmid DNA which can be transferred in vivo. Here, we adapted the single-strand virus sequencing protocol developed in our laboratory to assess the contaminating DNA in rAAV stocks generated in insect cells using the Illumina high-throughput sequencing technology. Preliminary data obtained from rAAV2/rh10 stocks purified by CsCl ultracentrifugation or immunoaffinity chromatography revealed that baculoviral and cellular DNA correspond to ≤1.5% and ≤0.02% of the total reads, respectively. The sequencing coverage showed that the proximity to the ITRs increases progressively the probability for baculoviral DNA to be encapsidated. Nonetheless, these baculovirus-derived reads are found at a frequency of 3 logs lower than the rAAV genome reads. Compared to the real-time PCR, our technique allows a non-biased identification and quantification of DNA species packed in rAAV batches. The development of accurate quality control methods is not only critical for fulfilling regulatory requirements, but will also provide novel insights into the biological mechanism of rAAV assembly in insect cells.

Poster Presentations

P01

Initial characterization of integrase-defective lentiviral vectors for pancreatic cancer gene therapy

N Hanoun [1] M Gayral [1] A Pointreau [1] L Buscail [1] P Cordelier [1]

1: Inserm U1037 Toulouse France

The vast majority (85%) of pancreatic adenocarcinoma (PDAC) are discovered at too late stage to allow curative surgery. In addition, PDAC is highly resistant to conventional methods of chemotherapy and radiotherapy that only offer a marginal clinical benefit. Consequently, the prognosis of this cancer is devastating, with a 5-year survival rate of less than 5%. In this dismal context, we recently demonstrated that PDAC gene therapy using non-viral vectors is safe, feasible, with early signs of efficacy in selected patients. Our next step is to transfer to the clinic HIV-1 based lentiviral vectors that outshine other therapeutic vectors to treat experimental models of PDAC. However, a primary safety issue presented by lentiviral vectors that may delay their use in patients is the risk of oncogenesis following vector integration in the host's cell DNA. Thus, we developed a novel anti-cancerous approach based on integrase-defective lentiviral vectors (IDLVs) and demonstrated that IDLVs can be successfully engineered to transiently deliver therapeutic genes to inhibit pancreatic cancer cells proliferation. This work stems for the use of therapeutic IDLVs for the management of PDAC, in forthcoming early-phase gene therapy clinical trial for this disease with no cure.

P02

Kinetic live cell imaging enables direct, long-term measurement of immune cells functions

M Lucas [1] J B Pénigault [1] N J Bevan [1] C Szybut [1] T O'Callaghan [1] K Patel [1] T Dale [1]

1: Essen BioScience

The role of the immune system in cancer research has been investigated through different approaches such as chemotaxis, migration, cytotoxicity, and phagocytosis. Research in these areas has traditionally been performed using various end point assays. With end point approaches, the contribution and behavior of different cell types to the final readout is often unclear and there is limited information concerning the temporal nature of events. Using a co-culture model of tumor and effector cells in the IncuCyte ZOOM®, we have successfully followed tumor cell death, post immune cell activation in real time. Using a caspase 3/7 reagent, dying cells are identified as fluorescent green objects that can be quantified by the IncuCyte ZOOM®. Dying and labelled effector cells can be removed from this identified population with the use of size and shape exclusion filters. Chemotaxis is an essential component during the immune response. Chemotactic properties of immune cells and tumor cells are often monitored with Boyden chambers. We have developed a new technique for use on the IncuCyte ZOOM®. Our strategy allows visualization and quantitation of directed cell migration and chemotaxis over time using 10-fold fewer cells, without cell labelling, all through real-time image processing and quantitation. Overall IncuCyte ZOOM® enables the generation of high definition phase contrast images, data analysis of images and time lapse videos to demonstrate events or cell functions in real time which can greatly enhance the understanding of the biology involved.

P03

Modelisation of a laminopathy in a dish uncovers a potential therapeutic approach

I Jebeniani [1] A C Guénantin [2] N Vignier [3] G Bonne [3] M Pucéat [1]

1: INSERM UMR S910, Marseille 2: INSERM UMRS938, Paris 3: INSERM UMRS974, Paris

H222P lamin A/C mutation in patients is responsible for a dilated cardiomyopathy. The molecular mechanisms underlying development of the pathology is still unknown. Herein, we used mouse and human pluripotent stem cells harboring a H222P LaminA/C mutation to investigate potential therapeutic approaches. Echocardiography of H222P Lmna mice in utero revealed dilatation of heart as early as as E13.5. Accordingly, ventricular ejection fraction was decreased, pointing to a developmental origin of the disease. Cardiac differentiation of both mouse and human pluripotent stem cells was impaired as early as the mesodermal stage. Expression of Mesp1, a mesodermal cardiogenic gene involved in epithelial-to-mesenchymal transition (EMT) of epiblast cells as well as snai1 and twist was decreased in mutated cells when compared to wild type in the course of differentiation. In turn, cardiomyocyte differentiation was impaired. Lamin Associated Domain (LAD) define nuclear regions where lamin interacts with chromatin and points to a role of lmna in epigenetic regulation of gene transcription. Chromatin immunoprecipitation assays of different epigenetic marks including H3K4me1 in differentiating cells revealed a specific decrease of this histone mark on regulatory regions of MesP1, and of other EMT genes such as twist. The demethylase LSD1 specifically demethylates H3K4me3 and H3K4me2. LSD1 siRNA-mediated down-regulation rescued the epigenetic landscape in mutated cells. Furthermore, preliminary experiments revealed that downregulation of LSD1 rescued contraction in cardiomyocytes differentiated from H222P lamin A/C pluripotent stem cells. Hence, our data point t LSD1 inhibitors used in clinical trials in cancerology as potential therapeutic molecules for a laminopathy.

P04

Chromatin architecture and Cardiac homeostasis

T Moore-Morris [1] M Puceat [1]

1: Inserm

Congenital heart diseases represent a huge burden for society and involve multiple structural and functional defects. The growing population of adult CHD patients lack therapeutic solutions and are prone to heart failure. Cardiac development and homeostasis rely on a network of transcription factors that interact with transcriptional enhancers and promoters. Recently, cohesin and its loading partner Nipped B-like (NIPBL) have been identified as key players in the regulation of chromatin conformation. Accordingly, NIPBL haploinsufficient Cornelia de Lange Syndrome (CdLS) patients present developmental defects, including CHDs. Using conditional deletion of NIPBL in mouse, we have established that specific cardiac lineages are sensitive to NIPBL haploinsufficiency. Our work will help identify specific regulatory elements within the cardiac program responsible for signaling underlying CHDs in CdLS patients.

P05

Targeted integration of hFANCA in the mouse Mbs85-AAVS1 human orthologous safe harbor locus in Fanca-/- fibroblasts and hematopoietic progenitor cells.

M J Pino-Barrio [1 2] R Chinchon [1 2] Y Gimenez [1 2] P Rio [1 2] R Sánchez-Dominguez [1 2] S Rodriguez-Perales [3] R Pujol [4 5] C Mussolino [6 7] T Cathomen [6 7] J A Bueren [1 2] S Navarro [1 2]

1: Division of Hematopoietic Innovative Therapies, CIEMAT/CIBERER, 28040 Madrid, Spain 2: Advanced Therapies Unit, IIS-Fundación Jimenez Diaz (IIS-FJD, UAM), 28040 Madrid, Spain 3: Molecular Cytogenetics Group, Human Cancer Genetics Program, Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernandez Almagro, 3, 28029 Madrid, Spain. 4: Genome Instability and DNA Repair Group, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain. 5: Center for Biomedical Network Research on Rare Diseases, Instituto de Salud Carlos III, Bellaterra, 08193 Barcelona, Spain. 6: Institute for Cell and Gene Therapy, Medical Center - University of Freiburg, 79106 Freiburg, Germany. 7: Center for Chronic Immunodeficiency, Medical Center - University of Freiburg, 79106 Freiburg, Germany

Fanconi anemia (FA) is an inherited syndrome mainly associated with bone marrow failure and cancer predisposition, caused by mutations in any of the 19 FANC genes so far discovered. Here we aimed to develop a gene therapy approach in a mouse model of FA-A, the most frequent complementation group in FA patients, by the insertion of the hFANCA gene in the murine Mbs85 locus; the orthologous of the human AAVS1 'safe harbor locus'. Using a TALEN pair targeting the mouse AAVS1 locus, we achieved 44% and 25% cleavage efficiencies in murine embryonic fibroblasts (MEFs) and hematopoietic progenitors, respectively. Thereafter, lineage negative (Lin-) bone marrow cells from Fanca-/- mice were nucleofected with plasmids encoding the TALEN and a donor PGK-hFANCA construct flanked by Mbs85 homology arms. Nucleofected cells were grown in methylcellulose cultures in presence of 10 nM mitomycin-C (MMC) for positive selection. MMC-resistant colonies were characterized for site-specific integration of the therapeutic cassette via PCR. Based on CFU-GMs survival rates after MMC-selection, the efficacy of targeted integration of hFANCA in Lin- cells was about 1-2%. When similar experiments were performed in Fanca-/- MEFs, the efficacy of targeted integration increased to 2-5%, and was confirmed by Sanger sequencing of the targeted Mbs85-locus. As happened in the hematopoietic progenitors, evidence of phenotypic correction was deduced in edited fibroblast because of their sensitivity to MMC. Our data demonstrate for the first time the feasibility of performing targeted gene therapy in fibroblasts and hematopoietic progenitor cells in a mouse model of FA

P06

Intrathymic correction of a genetic immunodeficiency by AAV gene transfer

M Pouzolles [1] S Gailhac [1] O Adjali [2] P Moulier [2] N Taylor [1] V S Zimmermann [1]

1: IGMM, 1919 route de Mende, 34293 Montpellier 2: UMR1089, 8 quai de Moncousu, BP70721, 44007 Nantes

Patients with combined immunodeficiency can be cured by allogeneic hematopoietic stem cell transplantation but complications often occur if the donor is incompatible. To circumvent these problems, significant efforts have been invested in the development of gene therapy strategies. Nevertheless, adverse events indicate the necessity of exploring other avenues. Our group hypothesized that in situ gene correction of T lymphoid progenitors in the thymus itself may overcome some of the drawbacks of ex vivo gene correction.

As lentiviral gene transfer in the thymus is minimal, we assessed the potential efficacy of adeno-associated virus (AAV) serotypes for thymocyte transduction. Intrathymic administration of scAAV2/8, 2/9 and 2/10 vectors resulted in a >10-fold higher transduction of thymocytes (3-5%) as compared to lentiviral vectors with rapid transgene expression, detected by three days post injection. scAAV2/8 vector promoted the highest level of transduction and strikingly, transduced cells represented up to 1% of peripheral T lymphocytes following scAAV2/8 gene transfer, even in immunocompetent mice. In the context of ZAP-70-/- immunodeficient mice, we found that intrathymic injection of an AAV2/8-ZAP-70 vector resulted in a rapid and efficient T cell reconstitution. Furthermore, stable expression of a functional ZAP-70 transgene (> 4 months) allowed functional T cell responses. Thus, AAV vectors, even though they persist mainly as vector episomes, are maintained long-term and can be used for stable in vivo transgene expression in the thymus, promoting T cell function.

P07

An antibiotic-free strategy for miniplasmid production

R Shankar [1] M Telaar [2] K Friehs [2] M Schleef [1] E Flaschel [2]

1: PlasmidFactory GmbH & Co. KG 2: Bielefeld University

The segregational stability of plasmids in a recombinant bioprocess is of extreme significance. Although this is commonly achieved by the selection pressure from antibiotics, their application for the production of therapeutic DNA for gene therapy or for DNA vaccines would be undesirable. Similarly, the presence of antibiotic-resistance genes in the final product would have to be avoided. In addition to the minicircle approach, this type of miniplasmid is able to fulfil the regulatory requirements. The gene tpiA is responsible for the connection of the glycerol metabolic pathway with the essential glycolytic pathway in Escherichia coli. The knockout of genomic tpiA rendered cells completely auxotrophic in minimal medium with glycerol as sole carbon source while allowing growth at a reduced rate with glucose or in complex medium. This was advantageous for optimizing antibiotic-free cloning and selection of recombinant plasmid. Complementation of the auxotrophy by plasmid-borne tpiA led to high segregational and structural plasmid stability, resulting in stable production of a model recombinant enzyme under antibiotic-free conditions in a continuous cultivation. Thus, the complementation of tpiA represents a significant alternative to antibiotics as a selection principle and is therefore of interest for the recombinant production of biotherapeutics in the form of miniplasmids.

P08

Autologous cell transplantation for OPMD patients

S Périé [1 2] P Klein [1] A Malerba [3] M Graham [4] S Suhy [4] G Dickson [3] V Mouly [1] J Lacau St Guily [1 2] C Trollet [1]

1: Sorbonne Universités UPMC Univ Paris 06, Inserm, CNRS, Centre de Recherche en Myologie 2: Service d'Oto-Rhino-Laryngologie et de Chirurgie Cervico-Faciale, Assistance publique-Hôpitaux de Paris (APHP) 3: School of Biological Sciences, Royal Holloway-University of London 4: Benitec Biopharma, Balmain

Oculopharyngeal muscular dystrophy (OPMD) is a late-onset autosomal dominant genetic disease mainly characterized by ptosis and dysphagia and caused by GCN expansion in PABPN1 gene. We conducted a phase I/IIa clinical study using autologous myoblast transplantation following myotomy in adult OPMD patients. This study included 12 patients with clinical diagnosis of OPMD, indication for cricopharyngeal myotomy, and confirmed genetic diagnosis. The feasibility and safety end points of both autologous myoblast transplantation and the surgical procedure were assessed by videoendoscopy in addition to physical examinations. Potential therapeutic benefit was also assessed through several tools (videoendoscopy, videofluoroscopy of swallowing, quality of life score, and a drink test). Short and long-term (2 years) safety and tolerability were observed in all the patients, with no adverse effects. There was an improvement in the quality of life score for all 12 patients, and no functional degradation in swallowing was observed for 10 patients. A cell dose-dependant improvement in swallowing was even observed in this study. However the current protocol involves the autologous transplantation of unmodified cells from spared muscles still carrying the genetic mutation then we are now developing a new gene therapy strategy to genetically engineer patient's cells prior to transplantation in order to restore a normal PABPN1 allele. Using RNA interference we are able to efficiently knock down both expended- and wt-PABPN1 and bring into the cell a functional PABPN1 version untargeted by the shRNAs - thanks to the genetic code redundancy - preventing the cell lethality observed by silencing PABPN1 in myoblast.

P09

NF kappa B motifs on plasmid DNA enhance transfection of skeletal muscles following hydrodynamic limb vein injection in healthy mice

T Le Gall [1] L Pigeon [2] Y Sibiril [1] S Quemener [1] P Guégan [3] P Lehn [1] C Pichon [2] T Montier [1] P Midoux [2] P Midoux [2]

1: INSERM UMR 1078; Faculté de Médecine, Université de Bretagne Occidentale; Université Européenne de Bretagne, 22 avenue Camille Desmoulins, 29238 Brest Cedex 3, France. 2: Centre de Biophysique Moléculaire, CNRS UPR4301 and Université d'Orléans, Orléans, France 3: CNRS, UMR 8232, IPCM, Chimie des Polymères, F-75005, Paris, France

Hydrodynamic injection via the limb saphenous vein (HLV) is an in vivo loco-regional administration procedure useful to deliver plasmid DNA (pDNA) into skeletal muscles. This method has been successfully used in mice, dogs, non-human primates and it was recently shown to be safe in humans. Despite it allows delivering pDNA at the same time into all the muscles of a limb, only few copies actually reach the nucleus of each muscle cell. Besides, it has been shown that a sequence comprising 3 κB motifs (3NF) can greatly enhance the nuclear import of a pDNA. In the present study, we examined the effect of 3NF contained into a luciferase-encoding pDNA (p3NF-CMVLuc-3NF) on the transfection efficiency of skeletal muscles following HLV injection in normal mice. Compared to pDNA lacking 3NF, injection of p3NF-CMVLuc-3NF resulted in 20-30 times more intense in vivo bioluminescence in the whole injected leg. This advantage was lasted at least one month. When considering each muscle separately, we found that p3NF-CMVLuc-3NF mediated up to 200 times higher luciferase activity, especially in muscles of the lower limb, which are more difficult to transfect . After repeated HLV injections of p3NF-CMVLuc-3NF, the luciferase activity was increased for several days, a ~10-fold higher bioluminescence being measured after injection of a 10-fold higher dose. These results indicate that 3NF would be very beneficial to enhance transfection efficiency following HLV injection, especially in the context of Duchenne Muscular Dystrophy. This work was supported

by grants from AFM (Strategic project 2009, n°15628AFM) and VLM.

P10

A novel gene therapy using hematopoietic-stem-cell-derived microglia in animal models of multiple sclerosis

S Tada [1] V Tepavcevic [3 4 5] G Dufayet-Chaffaud [1] M S Aigrot [3 4 5] C Lubetzki [3 4 5 6] N Cartier [1 2]

1: Inserm U1169/MIRcen CEA 2: Universite Paris Sud 3: Pierre and Marie Curie University, University of Paris 06, UM-75, Sorbonne Universities, ICM-GH Pitie-Salpetriere 4: Inserm U1127 5: CNRS Mixed unit of Research 7225 6: Pitie-Salpetriere Hospital, Public Hospital Network of Paris

Multiple sclerosis (MS) is characterized by demyelinating lesions scattered within central nervous system (CNS). Existing medications against MS patients act primarily on suppressing immune system to reduce relapse rates. These treatments are mainly for relapsing-remitting MS patients, where inflammation plays a vital role. However, we need to promote remyelination in order to treat primary progressive or secondary progressive MS patients, in whom failure of remyelination matters more than aberrant inflammation. In fact, there are no drugs available today that act on promoting remyelination. Oligodendrocyte precursor cells (OPCs) differentiate into oligodendrocytes and promote remyelination in demyelinated lesions of CNS. However, oligodendrocytes that are unsuccessful in making contact and sheathing axons undergo programmed cell death, highlighting the importance of targeted OPCs migration. We previously showed that transplanted gene-modified hematopoietic stem cells (HSCs) repopulate the CNS and differentiate into microglia, serving as vehicles to target therapeutic molecules in the lesions of CNS. In addition, we recently confirmed that semaphorin 3F (sema3F) recruit OPCs to the demyelinated lesions and enhance remyelination, making it a promising candidate for the application in treating MS 1,2. In this context, our objective is to deliver sema3F in demyelinated lesions of CNS by using lentivirally gene-modified HSCs as vehicles, and to recruit OPCs to the lesions to promote remyelination. Here we will present our results regarding Sema3F overexpression in HSCs and their transplantation in recipient mice. Reference: 1: Brain 2011: 134; 1156–1167 2: Brain 2007: 130; 2554-2565

P11

Gene therapy using a self-complementary AAV9 (scAAV9) construct expressing a codon-optimized Mecp2 transgene in Mecp2-deficient mice improves survival, behavioral deficits and rescues breathing defects.

V Matagne [1] L Villard [1] J C Roux [1]

1: Aix Marseille Université, INSERM, GMGF UMR_S 910

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder primarily affecting CNS functions. Most RTT cases are due to mutations in the methyl CpG binding protein 2 (MECP2) gene, a global transcriptional modulator. There is currently no cure for the disease and drugs alleviating symptoms are the only available therapies. Recently, two different publications reported that gene therapy using Mecp2-deficient mice partially cured the disease (Gadalla et al 2013; Garg et al 2013). Although both studies showed some rescuing effect, the benefits seemed to be very variable. In order to try and improve transgene expression, we designed a plasmid construct expressing a codon-optimized version of Mecp2 that was used to generate a scAAV9 therapeutic vector. Thirty day-old Mecp2-deficient (KO) male mice were injected with the vector through the tail vein (2X10E11 vg/mouse). The treatment significantly increased survival (median survival 58d vs 99d in Mecp2 KO vs treated) and some behavioral deficits (Open field, Rotarod). Apneas, which are part of the characteristic RTT breathing defects, were also significantly reduced (125±51 vs 6±3, Mecp2 KO vs treated). This improvement could be due to the increased percentage of Mecp2-expressing cells that we found in the posterior brain areas involved in breathing regulation such as the locus coeruleus (23.8±4.7%) compared to more anterior ones (10.9± 1.4% in the cortex). These data indicate that even a low level Mecp2 expression can improve RTT symptoms in Mecp2-deficient mice. Further studies will aim at investigating the therapeutic effect of the same viral construct in female RTT mice.

P12

Neural-competent cells of mouse ventral skin belong to the Schwann lineage

H Iribar [1] A Gutierrez-Rivera [1] V Perez-Lopez [1] A Izeta [1]

1: Biodonostia Institute

In the trunk dermis, dorsal precursors that are traced by Myf5 expression and of supposed dermomyotomal origin are able to generate Schwann cells, a cell type previously believed to be of exclusive neural crest origin. Derivation of neural lineage cells from non-neural tissue is puzzling from a developmental point of view. Since we had previously shown that human ventral dermis-derived precursors originate from Schwann cells, and that their neural competence was mediated by SOX2 expression levels, we wanted to test if this was also the case for mouse ventral dermis-derived Myf5+ precursors. Contrary to expectation, we here describe the existence of a Myf5+ precursor cell in ventral dermis that retains the capacity to generate neural (Schwann) derivatives. Close inspection showed that these precursor cells had an in situ localization consistent with terminal Schwann cells. As expected, SOX2 expression levels where higher in the Myf5+ lineage and in situ localization of SOX2+ cells was partially overlapping that of Myf5+ cells, indicating that neural competence of precursors of the mouse ventral dermis is also regulated by SOX2. These results resolve the conundrum of developmental origin of dermal precursors and highlight the importance of isolating the dermal cells with neural competence prior to their purported use in neural tissue engineering approaches.

P13

mRNA & pDNA based vaccines: Combination of an Imidazolium head group containing lipid and DOPE highly improved dendritic cells transfection

S Akhter [2] M Dubuisson [1] L Pigeon [1] C Gonçalves [1] M Berchel [3] P Jaffrès [3] P Midoux [1] C Pichon [1]

1: Centre de Biophysique Moléculaire 2: Le Studium® Loire Valley Institute for Advanced Studies, Centre-Val de Loire region, France 3: Université de Bretagne Occidentale, UMR CNRS 6521 - UFR Sciences et Techniques, Brest, France

Non-immunogenicity, relatively non-toxic nature, and potential of a design for specific target to dendritic cells (DCs) increasingly making lipidic nanoparticles (called as lipoplexes) the vectors of choice in nucleic acids delivery for DCs transfection in cancer vaccination. While designing lipoplexes it's very critical to optimize the lipids composition for better stability of mRNA and DNA and ultimately better transfection of DCs. In our study, we optimized cationic liposomes composed of lysosomotropic base lipid having imidazolium in head group and DOPE as co-lipid and screened its transfection efficiency as mRNA and DNA-lipoplexes. The nano lipid vesicles prepared having the size varied in between 80nm-150nm with the polydispersity index within 0.2. The zeta potential was ranging from 50mV - 80mV. The lipoplex of these formulation formed with mRNA & pDNA were evaluated for the complexation by gel electrophoresis, circular dichroism and ultimately the transfection efficiency in dendritic cells (DC2.4 cells) studied by flow cytometric analysis. The change in the size and shape/topography of lipoplex were observed by AFM in comparison to cationic liposomes. The transfection study revealed that optimized composition significantly improve the transfection up to 62% and 33% for mRNA & DNA respectively. Particularly, the protein expression in these transfected cells goes remarkably high indicating that our lipid composition help in better intracellular protection of nucleic acids, improved endosomal escape and affluence releasing the payload mRNA and DNAo. At this stage, we able to conclude that the tested lipoplexes could be a good nanomedicine for nucleic acids based cancer vaccination.

P14

Targeting of oncogenic transcription factors by both DNA decoys and interfering RNAs through co-delivery by lipopolyplexes

G Casas [1] M -P Gosselin [1] T Thibault [2] P Baril [1] C Pichon [1] J M Malinge [1] P Midoux [1]

1: CBM-CNRS 2: Universidade Estadual de Campinas

DNA decoys and interfering RNAs (siRNAs) are promising therapeutic oligonucleotides for cancer treatment but several limitations exist towards this goal. A key requirement is to develop oligonucleotide based strategy allowing multitargeting in order to potentiate cancer cell death and to decrease the appearance of drug resistance. Also, DNA and RNA oligonucleotides need efficient delivering system. Herein, we designed a short double-stranded DNA minicircle of less than 250 bp with the capability of trapping several transcription factors. This DNA minicircle was generated by a new method allowing production of minicircles of any base composition with the freedom to choose the position and nature of sequences of interest for the binding of critical transcription factors recognized as targets in cancer. We showed that a 95 base pairs minicircle containing two binding sequences for NF-kB binds efficiently two NF-kB (p50) molecules in vitro and can impaired NF-kB transcription activity in cellulo. We test whether acting at both pre transcriptional level with the DNA decoy and post transcriptional level with siRNA could potentiate transcription factor inhibition in cancer cells. Therefore, we targeted Stat3 by both DNA decoy and siRNA directed against Stat3 which were co-delivered within nanoparticle made of histidinylated lipopolyplexes. We found that the co-delivery induced an increased anti-proliferative activity as compared to additive effect when oligonucleotides were used separately. Works are in progress for in vivo experiments and to define at the molecular level the mechanism of this combinatory effect.

P15

Novel iPSC-derived cellular systems for in vitro disease modeling.

G Salvagiotto [1] C Carlson [1] A Thompson [1] D A Mann [1] S DeLaura [1]

1: Cellular Dynamics International, a Fujifilm Company

A major challenge in disease research and drug discovery is modeling human biology in physiologically relevant and predictive in vitro systems. Human induced pluripotent stem cell (iPSC) technology allows for the generation of virtually any cell type of the human body in unlimited quantities from a donor. This technology also enables access to human disease models which have been shown to recapitulate the native human phenotypes in vitro. Here, we present examples of induced, engineered, and innate disease models generated by the production of iPSC-derived cell types environmentally stimulated to elicit a disease phenotype, genetically modified to introduce a disease mutation, or from patient-derived material, respectively. In particular, we describe the application of iPSC-derived hepatocytes in hepatitis C virus (HCV) infectivity. We present an amyloid-beta dependent toxicity assay developed for a pilot screen to identify compounds protective against Alzheimer's Disease in iPSC-derived cortical neurons. We also provide data from iPSC-derived neurons genetically modified to carry point mutations in the amyloid precursor protein yielding another model of Alzheimer's Disease, or in the potassium channel KCNT1 offering an epilepsy model. Finally, we illustrate a diabetic cardiomyopathy model in iPSC-derived cardiomyocytes. This model was used in a phenotypic screen for rescue from the pathological phenotype during diabetic stress and identified candidate protective molecules for iPSC-derived cardiomyocytes generated from diabetic patient samples. The data presented show how the iPSC technology offers reliable and predictive models not otherwise attainable using

currently available primary and immortalized cells, thus creating new tools and opportunities in drug discovery.

P16

Personalized iPSC-based cell/gene therapy: a Hemophilia B model

E Luce [1 2 3] C Steichen [1 2 3] T Lambert [4] T H Nguyen [5] A Dubart-Kupperschmitt [1 2 3]

1: INSERM U1193 2: Université Paris sud 3: DHU Hepatinov 4: Centre de Référence pour le Traitement des Hémophiles, Hôpital de Bicêtre 5: Inserm U1064

Hemophilia B is an X-linked genetic disorder characterized by a reduced activity of circulating clotting factor IX (FIX). Replacement therapy based on regular intravenous injections of FIX exists but it is lifelong thus cumbersome and costly. The aims of our project are to use induced pluripotent stem cells (iPSCs) i) to generate a new cell model of hemophilia B with iPSC-derived hepatocytes and ii) to study the feasibility of ex vivo gene/cell therapy for this disease. iPSCs have been generated from skin fibroblasts of a patient affected with severe hemophilia B (FIX activity < 1%). These iPSCs were extensively characterized and DNA sequencing confirmed the presence of the patient's mutation. The second step of our strategy was to correct the genetic defect by a genome editing approach. We constructed a cassette including a F9 mini-gene under the control of the APOAII promoter. The encoded FIX included the Padua mutation to enhance its specific activity. We used the CRISP/Cas9 technology to insert this therapeutic cassette into the safe harbor AAVS1 site (adenovirus-associated virus integration site 1). Based on our previously published protocols, we have set up chemically defined conditions to differentiate non-corrected and genetically corrected iPSCs into hepatocyte-like cells (HLCs). The efficacy of the correction approach is currently evaluated by transplanting the two types of iPSC-derived HLCs into a Hemophilia B mouse model, and by comparing FIX expression levels in the two series of transplanted mice.

P17

Risk-based approach: a generic template for Advanced Therapy Medicinal Product

A L Chateau [1 2] J Magalon [1 2] L Giraudo [1] L Blanchet [1] H Mendizabal [1] F Sabatier [1 2 3] J Veran [1]

1: Assistance Publique Hôpitaux de Marseille 2: Aix-Marseille University 3: CIC Biothérapie

Advanced Therapy Medicinal Products (ATMP) are promising new cell or tissue-based treatments. They are regulated in Europe under the status of medicinal product, thereby creating specific challenges for development, commercialization and risk analysis. The Conception University Hospital, through collaboration between its Cell Therapy Unit and Quality and Risk management department developed a generic template for a risk-based approach to determine in what extent data related to pharmaceutical quality and security are complying with European Guidelines. Since the ATMP are very different in nature, manufacturing process, route of administration and intended clinical use, the proposed methodology is the Preliminary Risk Analysis, particularly adapted to innovative system and products under development. In this global ATMP-manufacturing risk analysis, 20 pertinent hazards were identified in 4 groups: means, management, supports, ATMP-specific hazards. From there, 66 dangerous situations were classified into priorities due to their probability of occurrence and severity of their consequences. Although ATMP-specific dangerous situations are the most critical, an acceptable level of risk could be reached through the identification of priority actions and measures. This model provides a decision making tool for risk analysis of any ATMP under development and should be completed by a critical benefits/risks clinical assessment to evaluate the risk profiling of one specific product. The results of each appropriate risk analysis may contribute to decrease patient risks and justify development plan to the health authorities.

P18

Hematopoietic Stem Cell Therapy: Modulating cell fate decisions via manipulation of metabolic pathways

M Romano [1] L Oburoglu [1] S Kinet [1] N Taylor [1]

1: UMR5535 Institut de Génétique Moléculaire de Montpellier

Hematopoietic stem cell (HSC) transplantation allows the hematopoietic system of the transplanted patient to be reconstituted from the newly infused HSCs. In most therapeutic applications, all blood lineages are derived from the infused HSCs but there are also conditions where it is beneficial to bias transplanted progenitors to a specific lineage fate. A large body of previous research has revealed the importance of cytokines, transcription factors and cell-cell contacts in regulating HSC differentiation to a given lineage but recent studies have shown that fuel utilization also conditions progenitor fate. Our group has demonstrated that HSC differentiation towards the erythroid lineage, leading to the production of 2.4 million erythrocytes per second, is not only dependent on erythropoietin but requires glutaminolysis and the shunting of glucose through the pentose phosphate pathway for nucleotide synthesis (Oburoglu et al., Cell Stem Cell, 2014). We now find that manipulating fatty acid oxidation (FAO) potential also alters erythroid differentiation. Blocking FAO inhibited the erythroid differentiation of HSCs, even in the presence of high dose recombinant erythropoietin. In these conditions, erythropoietin-signaled progenitors were diverted to a myelomonocytic fate. Furthermore, we identify long chain fatty acyl-CoA synthetase (ACSL), an enzyme required for FAO, as a key player in this process. Under conditions of mTOR inhibition, ACSL expression is increased, resulting in an enhanced erythroid differentiation. Thus, these data strongly suggest that the erythroid specification of transplanted HSCs can be altered by manipulating therelative levels of glutaminolysis, glycolysis and fatty acid oxidation.

P19

Induction of immune tolerance following AAV-mediated muscle gene transfer using bi-functional fusion proteins combining extracellular domains of CTLA-4 and PD-L1

L Dupaty [1] G Riou [1] L Jean [1] A Salvetti [2] O Boyer [1] S Adriouch [1]

1: U905, INSERM, Normandie University, Institute for Research and Innovation in Biomedicine (IRIB), Rouen, CS 76183, France 2: U1111, CNRS UMR5308, INSERM, Ecole Nationale Supérieure (ENS) de Lyon, Lyon, 69342, France

Recombinant adeno-associated virus (rAAV) has become one of the most promising vectors with successful translation to human diseases. However, immune responses against the transgene (Tg) and/or the vector still represent a major limitation to long-term transgene expression. Thus, strategies to induce immune tolerization against the Tg still represent a major challenge to improve gene therapy efficacy.
Immune tolerization can be achieve by reinforcing the natural immunoregulatory pathways that have evolved to ovoid immune response against self-antigens and that are often subverted in the tumors microenvironment. Among these pathways, CTLA-4 and the PD-L1 molecules have emerged as two complementary immunoregulatory pathways involved in the maintenance of self-tolerance. In a stringent mouse model we show that CTLA-4/Fc or PDL1/Fc chimeric protein significantly improve Tg persistence by different mechanisms. In the next step, we designed new bi-functional Fc-fusion proteins containing the extracellular domains of both CTLA-4 and PD-L1 with the aim to screen for even more potent immunoregulatory chimeric proteins syngergistically acting on these two non-redundant pathways. To rapidly screen the efficacy of our immunoregulatory protein candidates we developed a strategy that we named "vectored immunoregulation" (VIR), based on the use of rAAV vectors encoding for these proteins. The most effective bi-functional protein will then be produced as a recombinant protein and injected in preclinical models with aim to demonstrate its efficiency to induce long-term tolerance to the Tg in the context of rAAVbased muscle gene therapy.

P20

USP44 is highly expressed in pluripotent stem cells under the control of specific regulatory regions

P Tropel [1 2] L Jung [1] C André [1] A Tosch [1] S Viville [1 3]

1: IGBMC 2: Bluestem Engineering SAS 3: CHU de Strasbourg

Developing new therapy based on pluripotent stem cells (PSC) requires an in-depth knowledge of their physiology and gene regulation. To identify new PSCs markers, we focused on ubiquitin specific peptidases (USPs) since they are increasingly described as playing crucial roles in cell physiology, through modulation of protein function and turnover. To get insight into the potential role of USPs in PSCs, we first tested every single gene expression in silico and identified USP44 gene as highly expressed in human embryonic stem cells and induced pluripotent stem cells but dramatically repressed during differentiation in embryoid bodies. Among adult organs, USP44 is substantially expressed in testis while a few others organs displayed much weaker expression levels. Using 5' RACE, we identified 7 different promoter regions driving either tissue-specific or non-specific expression of the gene. Two promoter regions are specifically active in both PSCs and testis, but probably through different regulatory mechanisms. Accurate analysis of initiation actually suggests that transcription may be directly controlled by the core pluripotency factors, Oct4, Nanog and Sox2. Our results drew a far more complex picture of USP44 transcriptional regulation than previously thought and a strong expression in PSCs. This gene was previously described as a key target of the Anaphase Promoting Complex which controls chromosome alignment and metaphase-to-anaphase transition. We hypothesize that variation in expression of the core pluripotency factors may negatively influence USP44 expression and originate major cell dysfunction like aneuploidy. In this hypothesis, tightly checking USP44 expression may help in elaborating safe PSC-based therapy.

P21

Are SOX2 expression levels involved in dermal stem cell aging?

L Yndriago [1] H Iribar [1] A Gutiérrez-Rivera [1] V Perez-Lopez [1] A Izeta [1]

1: Biodonostia Institute

Stem cells constitute a constant repair system for the body and their dysfunctionality or loss leads to a wide range of alterations, including tissue aging. We have previously shown that (i) human dermal stem cells lose functionality with age, becoming senescent; (ii) dermis-resident Schwann cell precursors and pericytes constitute stem cells, their stemness being mediated by SOX2 expression levels. During the dermal aging process the expression levels of SOX2 might also modulate the activity of stem cells. To characterize the aged skin phenotype, we first quantified the number of microvessels and nerve terminals at the subepidermal level in young vs aged C57BL/6 mouse skin. We noticed a reduction in subepidermal microvessels and nerve terminals that correlated with a decrease in the number and functionality of dermal stem cells. Next, we studied haploinsufficient SOX2:EGFP mice, which present half of the normal gene dosage in the SOX2 locus (and thus express lower levels of SOX2). Functional studies in skin thermoreception (hot plate assay) and mechanoreception (von Frey filaments) capacities gave mixed results with regard SOX2 involvement, which was only apparent in thermoreceptors, although in both cases a sharp reduction was detected in aged vs young mice. Finally, lentiviral vector-mediated overexpression of SOX2 in aged dermal stem/precursor cells seemed to rescue the aged phenotype in vitro. These results suggest a role of SOX2 in the maintenance of dermal stem cells in mouse skin. The SOX2:EGFP mouse model might be useful to

understand tissue resident stem cell dysfunction in response to aging.

P22

Measles virus glycoprotein pseudotyped lentiviral vectors transduce cytokine stimulated and resting HSCs and target HSCs in unfractionated bone marrow from Fanconi Anemia patients

C Lévy [1] F Amirache [1] A Girard-Gagnepain [1] C Frecha [3] C Costa [1] D Nègre [1] R Gijsbers [4] J F Román-Rodríguez [5] P Rio [5] J Bueren [5] F L Cosset [1] E Verhoeyen [1]

1: CIRI; Inserm U1111, Lyon France 2: C3M, Inserm U1065, Nice France 3: Faculty of Medicine, University of Buenos Aires, Argentina 4: Faculty of Medicine - KU Leuven, Belgium 5: CIEMAT/CIBERER. Madrid, Spain

A challenge in the field of gene therapy is the transduction of HSCs with high efficiency without compromising their stemness. Here we show that after a single application, MV-LVs transduced 100% of hCD34+-cells prestimulated with a mild cytokine cocktail and transduction of up to 70% of quiescent hCD34+-cells, while VSV-G-LVs reached only 5 % of transduction. Importantly, reconstitution of primary and secondary recipient NSG mice with these MV-LV transduced hCD34+-cells demonstrated that this high transduction levels were maintained in all hematopoietic lineages. Notably, we found increased transduction levels (up to 100%) in all human cells in these secondary recipients for the resting MV-LV transduced hCD34+ cells, emphasizing that these vectors preferentially gene-marked HSCs. Fanconi anemia (FA) is a rare monogenic inherited disease characterized by bone marrow failure, congenital abnormalities and cancer predisposition. HSC-based gene therapy has been proposed for FA treatment but the reduced reservoir of HSCs in FA patients together with the significant loss of HSCs associated with CD34+ cell isolation and cytokine stimulation results in reduced numbers of transduced HSCs for re-infusion in the patient. In this context, MV-LVs showed a marked increase in the transduction efficiency and FA correction of CD34+ cells from unfractionated BM from FA patients in comparison with classical VSVG-LVs. Therefore, these MV-LVs pave the way towards HSC-based gene therapy of multiple diseases including diseases characterized by low numbers of HSCs such as Fanconi Anemia, for which high level HSC correction is needed combined with minimal cell activation to be successful in the clinic.

P23

"RCL-pooling assay": A simplified method for the detection of Replication Competent Lentiviruses in vector batches using sequential pooling.

G Corre [1 2 3] M Dessainte [4] J B Marteau [4] B Dalle [1] D Fénard [1 2 3] A Galy [1 2 3]

1: GENETHON 2: INSERM UMR_S951 3: Université d'evry 4: Genosafe

Non-replicative recombinant HIV-1 derived lentiviral vectors (LV) are increasingly used in gene therapy of various genetic diseases, infectious diseases or cancer. Before they are used in man, preparations of LV must undergo extensive quality control testing. In particular, testing of LV must demonstrate the absence of replication-competent lentiviruses (RCL) with suitable methods, on representative fractions of vector batches. Current methods based on cell culture are challenging because high titers of vector batches translate into high volumes of cell culture be tested in RCL assays. As vector batch size and titers are continuously increasing due to the improvement of production and purification methods, it became necessary for us to modify the current RCL assay based on the detection of p24 in cultures of indicator cells. Here, we propose a practical optimization of this method using a pairwise pooling strategy enabling easier testing of higher vector inoculum volumes. These modifications significantly decrease material handling, operator time, leading to a cost effective method, while maintaining optimal sensibility of the RCL testing. This optimized "RCL-pooling assay" ameliorates the feasibility of the quality control of large-scale batches of clinical-grade LV while maintaining the same sensitivity.

P24

Pseudotyping serotype 5 adenovirus with the fiber from other serotypes uncovers a key role of the fiber protein in adenovirus 5-induced thrombocytopenia

N Raddi [1] F Vigant [1 6] O Wagner-Ballon [2 3] S Giraudier [2 3] J Custers [4] S Hemmi [5] K Benihoud [1]

1: UMR 8203 Vectorologie et Thérapeutiques anticancéreuses, CNRS, Univ. Paris-Sud, Gustave Roussy, Université Paris-Saclay 2: Inserm, U1009, Université Paris-Sud11, Gustave Roussy, Villejuif 3: Assistance Publique des Hôpitaux de Paris, Laboratoire d'Hématologie, Université Paris-Est Créteil, Hôpital Henri Mondor, Créteil 4: Viral Vaccine Discovery and Early Development, Crucell Holland BV, Leiden, The Netherlands 5: Institute of Molecular Life Sciences, University of Zurich, Switzerland 6: Department of Microbiology, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, #1124, New York, New York 10029, USA.

Ad infection in humans is associated with inflammatory responses and thrombocytopenia. While several studies were conducted in mice models to understand molecular and cellular mechanisms of Ad-induced inflammatory responses, only few of them turned their interest towards the mechanisms of Ad-induced thrombocytopenia. Our kinetic studies performed in C57BL/6 mice demonstrated that intravenous Ad5 administration led to a dose-dependent thrombocytopenia with a nadir at 2 days. First, using warfarin and surgery, we ruled out any significant role of vitamin K-dependent factor or the spleen in this phenomenon. Then, we examined the ability of different Ad to trigger thrombocytopenia. Interstingly, pseudotyping Ad5 with capsid fiber protein (or only the shaft) from other serotypes (3 or 35) was shown to dramatically reduce thrombocytopenia. In contrast, a serotype 3 vector was able to trigger platelet reduction as

efficiently as Ad5. These results underline a key role of Ad5 capsid fiber protein, and more precisely of its shaft, in Ad-induced thrombocytopenia. In parallel, we observed that pseudotyping fiber protein strongly reduced Ad's ability to trigger pro-inflammatory cytokines (IL-6, IL-1b) and chemokines (IP-10, KC, CCL2, CCL3 and CXCL2) production in mice. These data draw a link between Ad's ability to produce cytokines/chemokines and its ability to trigger platelet reduction. Taken as a whole, our results suggest that capsid fiber protein (and more precisely its shaft) of Ad serotype 5 triggers the cytokine production that leads to Ad-induced thrombocytopenia.

P25

Enhanced liver transduction and efficient protection from pre-existing neutralizing antibodies with exosome-associated AAV8 vectors

Z Fitzpatrick [1] A Meliani [1] F Boisgerault [1] G Ronzitti [1] F Collaud [1] S Charles [1] C A Maguire [2] F Mingozzi [1]

1: Généthon 2: The Massachusetts General Hospital

Adeno-associated virus (AAV) based vectors are ideal tools for in vivo gene transfer. As a result of exposure to wild-type AAV, however, a proportion of humans harbor pre-existing neutralizing antibodies against the viral capsid, which can prevent successful transduction by AAV vectors, posing an obstacle to the widespread use of AAV vectors in the gene therapy domain. We have previously reported that AAV1, 2 and 9 can associate with exosomes (exo-AAV) in media from 293T producer cells, enabling enhanced transduction and antibody evasion capabilities relative to conventional vectors. Herein, we characterize the in vivo transduction and biodistribution profile of exo-AAV8 encoding human factor IX (F.IX) under the control of a liver-specific promoter. C57BL/6 mice (n=5/group) were passively immunized with intravenous human immunoglobulin (IVIg) intraperitoneally, followed by an intravenous administration of either conventional AAV8 or exo-AAV8 vectors (5x1010 vg/mouse) 24h post immunization. Noteworthy, exo-AAV8 completely shielded the vector capsid from neutralizing antibodies at IVIg doses between 0.5mg and 2mg/mouse (NAb titer ~ 1:3.16), resulting in equivalent levels of F.IX transgene expression to naïve animals treated with conventional AAV8. While we did not detect a significant difference in transgene expression in male mice amongst the two vector cohorts, female mice (in which the efficiency of liver transduction with AAV is extremely low compared with male animals) treated with exo-AAV8 showed a dramatic increase in transgene expression, comparable to that of male mice receiving conventional AAV8 vectors. In conclusion, exo-AAV8 vectors enable an enhanced liver transduction profile compared with standard AAV.

P26

***COL7A1* correction for recessive dystrophic epidermolysis bullosa mediated by CRISPR/Cas9 and Homologous Recombination**

A Izmiryan [1 2] M Bovolenta [3 4] F Mavilio [3] A Hovnanian [1 2 5]

1: INSERM U1163, Institut Imagine, Paris, France. 2: University Paris Descartes Sorbonne Cité, Paris, France. 3: Genethon, 1 bis, rue de l'Internationale, 91002 Evry Cedex, France 4: Department of Life Sciences and Biotechnology, University of Ferrara, 74 Fossato di Mortara, 44121 Ferrara, Italy. 5: Department of Genetics, Necker Hospital, Paris, France.

Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a rare and severe genetic skin disease responsible for blistering of the skin and mucosa. RDEB is caused by a wide variety of mutations in *COL7A1* encoding type VII collagen, the major component of anchoring fibrils which are key attachment structures for dermal-epidermal adhesion. Site-specific CRISPR/Cas9-mediated Homologous Recombination (HR) is emerging as a powerful approach for gene editing to correct disease mutations.

Here we provide preliminary data for *COL7A1* editing using the CRISPR/Cas9 approach without selection. We designed five guide RNAs (gRNAs) in order to correct a RDEB causative null mutation in exon 2 (c.189delG; p.Lys6Trp*40). These gRNAs were cloned into a lentiviral vector and delivered as integration-deficient lentivirus (IDLVs). Two of them showed low toxicity and significant activity in HEK293 cells (up to 35%), in primary keratinocytes and in fibroblasts (up to 15%) when delivered as IDLVs.

To achieve gene editing in RDEB cells, primary keratinocytes and fibroblasts isolated from a RDEB patient homozygous for the c.189delG mutation were co-transduced with IDLVs encoding the site-specific CRISPR/Cas9 and the corresponding Donor. Genetic correction could be detected in bulk-transduced cells when performing droplet digital PCR, allele-specific PCR and direct sequencing. In addition, type VII collagen was detected in up to 10% of these cells as assessed by immunochemistry. Next steps will aim at improving the efficiency of CRISPR/Cas9-mediated HR for the development of transplantable skin models suitable for clinical application.

P27

Antisense mediated exon skipping gene therapy for dystrophic epidermolysis bullosa

M Titeux [1 2 *] S Turczynski [1 2 *] L Tonasso [3]
A Hovnanian [1 2 4]

*1: Inserm, UMR1163 – Institut Imagine, Paris, France 2: Université Paris Descartes – Sorbonne Paris Cité, Paris, France 3: CNRS, UMR5288, Toulouse, France 4: Department of Genetics, Necker Hospital, Paris, France * these authors contributed equally to this work*

Dystrophic Epidermolysis Bullosa (DEB) is a group of genodermatoses inherited in a dominant (DDEB) or recessive (RDEB) manner, characterised by severe skin and mucosae blistering after mild trauma. DEB is caused by mutations in COL7A1 encoding type VII collagen that assembles into anchoring fibrils to form key dermo-epidermal adhesion structures. No specific treatment is available for DEB but the targeted removal of exons carrying recurrent mutations shows therapeutic potential. Exon skipping relies on the capacity of antisense sequences to mask key splicing regulatory elements to modulate splicing of the pre-messenger RNA. Among the 118 exons of COL7A1, exons 73 and 80 are of particular interest since they carry several recurrent mutations and their excision preserves the open reading frame. We have first demonstrated the dispensability of exons 73 and 80 in an in vivo xenograft model and subsequently the efficient in frame exon skipping (up to 90%) of these exons and re-expression of type VII collagen (20 to 30% of normal amount) in cells from 3 RDEB patients transfected with antisense oligoribonucleotides (AONs). Finally, we have demonstrated type VII collagen re-expression and anchoring fibril formation in vivo following subcutaneous injections of AONs in RDEB skin equivalents. About 15% of RDEB patients could benefit from local or systemic injections of AONs targeting exon 73 or 80, and many other COL7A1 exons are theoretically targetable which could expand the number of patients candidate to this non invasive therapy.

P28

Frequency and characterization of recombination events in a gene therapy *COL7A1* retroviral vector during the reverse transcription in human cells

M Titeux [1 2] S Charbonnier [1,2] R Löw [3] K Kuehlcke [3]
A Hovnanian [1 2 4]

1: Inserm, UMR1163 – Institut Imagine, Paris, France 2: Université Paris Descartes – Sorbonne Paris Cité, Paris, France 3: EUFETS Gmbh, Idar-Oberstein, Germany 4: Department of Genetics, Necker Hospital, Paris, France.

Retroviruses, including MLV and HIV-derived viral vectors, package two copies of genomic RNA into viral particles which are subsequently reverse transcribed in a minus-strand DNA in the infected cells. During that step, template switching activity and low processivity of the reverse transcriptases lead to recombination events. We have developed an *ex vivo* gene therapy approach using a SIN retroviral vector encoding the 8.9 kb *COL7A1* cDNA and Southern blot experiments demonstrated that *COL7A1* recombination sometimes occur in the transduced cells (Titeux et al. 2010). To better understand this phenomenon and to quantify the recombination frequency, we have isolated and analyzed a large number of proviruses recombination events at the clonal level. A human keratinocyte cell line was transduced with the SIN γ-retroviral vector expressing *COL7A1* under conditions that favor single provirus integration, and the cells were subsequently cloned and expanded. Three panels of overlapping PCR primers were used to amplify the integrated proviruses sequences, in order to identify and sequence the recombination events. 26% of the proviruses presented with deletions ranging from 0.1kb up to 5.4kb, involving stretches of direct repeats occurring in the highly repeated collagenous domain of *COL7A1*. No mutation hotspot could be identified, which could have been edited to lower the rearrangement frequency. These results have relevant consequences for highly efficient retroviral or lentiviral-based gene transfer, particularly when the expressed cDNA is large and/or contains highly repeated sequences such as TALENs cDNA expressed in Integration-Deficient Lentiviral Vectors (IDLV).