534. Preparation for a First-in-Man Lentivirus Trial in Cystic Fibrosis Patients

Uta Griesenbach[1], Eric W. F. W. Alton[1], Jeffery M. Beekman[2], Christopher A. Boyd[3], Jane C. Davies[1], Lee A. Davies[4], Johanna F. Dekkers[2], Deborah R. Gill[4], Mamoru Hasegawa[5], Tracy E. Higgins[1], Takashi Hironaka[5], Makoto Inoue[5], Stephen C. Hyde[4], Alastair J. Innes[3], Ian A. Pringle[4], Kamila M. Pytel[1], Stephanie G. Sumner-Jones[4], Shu Tsugumine[5], Marguerite W. Wasowicz[1]
[1]Department of Gene Therapy, National Heart and Lung Institute, Imperial College London, London, United Kingdom, [2]Department of Pediatric Pulmonology, University Medical Centre, Utrecht, Netherlands, [3]Medical Genetics, Centre for Genomic and Experimental Medicine, University of Edinburgh, Edinburgh, United Kingdom, [4]Gene Medicine Group, NDCLS, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom, [5]ID Pharme Co. Ltd. (DNAVEC Center), Tsukuba, Japan

Background: We have recently shown that non-viral gene therapy can stabilise the decline of lung function in cystic fibrosis (CF) patients. However, the effect was modest, and it is important to develop more potent gene transfer agents in parallel. F/HN-pseudotyped lentiviral vectors are more efficient for lung gene transfer than non-viral vectors in pre-clinical models. In preparation for a first-in-man CF trial using the lentiviral vector we have undertaken key translational pre-clinical studies. **Methods:** Regulatory-compliant vectors carrying a range of promoter/enhancer elements were assessed in mice and human air liquid interface cultures to select the lead candidate; CFTR expression and function were assessed in CF models (knockout mice and human intestinal organoids) using this lead candidate vector. Toxicity was assessed and "benchmarked" against the leading non-viral formulation recently used in a Phase IIb clinical trial. Integration site profiles were mapped and transduction efficiency determined to inform clinical trial dose-ranging. The impact of pre-existing and acquired immunity against the vector and vector stability in several clinically relevant delivery devices was assessed. **Results:** A hybrid promoter consisting of the elongation factor 1α promoter and the CMV enhancer was most efficacious in both murine lungs and human air liquid interface cultures. The efficacy, toxicity and integration site profile supports further progression towards clinical trial and pre-existing and acquired immune responses do not interfere with vector efficacy. The lead rSIV.F/HN candidate expresses functional CFTR and the vector is stable in clinically relevant delivery devices. **Conclusions:** The data support progression of the F/HN pseudotyped lentiviral vector into a first-in-man CF trial due to start in Q2 2017. Regulatory-compliant toxicology studies are currently being performed.

536. The Viral Transduction Enhancer Vectofusin-1 Is a Nanofibrillar Peptide Capable of Increasing the Contact between Viral Vectors and Target Cells

Saliha Majdoul[1], Louic Vermeer[2], Loic Hamon[3], Justine Wolf[2], Ababacar K. Seye[4], Nathalie Holic[1], David Pastre[3], Daniel Stockholm[5], Burkhard Bechinger[2], Anne Galy[1], David Fenard[1]
[1]Genethon/INSERM UMR_S951, University of Evry, Evry, France, [2]Institut de Chimie, CNRS UMR_7177/ University of Strasbourg, Strasbourg, France, [3]University of Evry/ INSERM UMR_S1204, Evry, France, [4]Genethon/INSERM UMR_S951, Evry, France, [5]Genethon/INSERM UMR_S951, Ecole Pratique des Hautes Etudes, Evry, France

Gene transfer into hCD34+ hematopoietic stem/progenitor cells (HSPCs) using HIV-1-based lentiviral vectors (LVs) has several therapeutic applications ranging from monogenic diseases, infectious diseases and cancer. In such therapeutic context, the gene therapy could be improved by enhancing transduction levels of target cells and by reducing the amount of LVs used on the cells for greater safety and reduced costs. We recently identified a new cationic amphipathic peptide, Vectofusin-1, with viral transduction enhancing capacity, enabling higher transduction levels with low amounts of LV. Vectofusin-1 promotes the entry of several retroviral pseudotypes into target cells when added to the culture medium and is not toxic to HSPCs. Here, we present the first insights into the mechanism of action of this new transduction enhancer. First, a viral pull down assay showed that viral particles were easily pelleted by low speed centrifugation in presence of Vectofusin-1, suggesting that this latter may form unsoluble nanofibrils, trapping lentiviral particles. Atomic force (AFM) and electron microscopy (EM) of Vectofusin-1 confirmed that this peptide is rapidly forming annular aggregates and nanofibres in culture medium. Furthermore, these fibres were shown to be auto-fluorescent in medium with high-protein content (X-Vivo20), allowing the observation of a nanofibrillar network of Vectofusin-1 using confocal microscopy. Next, Vectofusin-1 was shown to strongly interact with Congo Red, especially in presence of lentiviral particles, but labeling with Thioflavin T was inefficient, suggesting that Vectofusin-1 fibres are not amyloid-type fibrils. Structural studies by circular dichroism confirmed this result. The capacity to form nanofibrils appears to be essential for the mechanism of action of Vectofusin-1 since a defective mutant called LAH2-A4, unable to promote lentiviral transduction (Majdoul S. *et al* (2016) J. Biol. Chem.), was also unable to form nanofibrils. In conclusion, biophysical, nanoscopic and microscopic observations have helped us to define Vectofusin-1 as a new nanofibrillar peptide capable of enhancing lentiviral transduction of target cells in conditions well-adapted to cGMP and scalable gene therapy protocols.

537. New Graph-Based Algorithm for Comprehensive Identification and Tracking Retroviral Integration Sites

Andrea Calabria[1], Stefano Beretta[2], Ivan Merelli[3], Giulio Spinozzi[1], Stefano Brasca[1], Fabrizio Benedicenti[1], Erika Tenderini[1], Alessandra Biffi[4], Eugenio Montini[1]
[1]San Raffaele Telethon Institute for Gene Therapy, Milan, Italy, [2]University of Milano-Bicocca, Milan, Italy, [3]National Research Council, Milan, Italy, [4]Children Hospital, Boston, MA

Vector integration sites (IS) in hematopoietic stem cell (HSC) gene therapy (GT) applications are stable genetic marks, distinctive for each independent cell clone and its progeny. The characterization of IS allows to identify each cell clone and individually track its fate in different tissues or cell lineages and during time, and is required for assessing the safety and efficacy of the treatment. Bioinformatics pipelines for IS detection used in GT identify the sequence reads mapping in the same genomic position of the reference genome as a single IS but discard those ambiguously mapped in multiple genomic regions. The loss of such significant portion of patients' IS may hide potential malignant events thus reducing the reliability of IS studies. We developed a novel tool that is able to accurately identify IS in any genomic region even if composed by repetitive genomic sequences. Our approach exploits an initial genome free analysis of sequencing reads by creating an undirected graph in which nodes are the input sequences and edges represent valid alignments (over a specific identity threshold) between pairs of nodes. Through the analysis and decomposition of the graph, the method identifies indivisible subgraphs of sequences (clusters), each of them corresponding to an IS. Once extracted the consensus sequence of the clusters and aligned on the reference genome, we collect the alignment results and the annotation labels from RepeatMasker. By combining the set of genomic coordinates and the annotation labels, the method retraces the initial sequence graph, statistically validates the clusters through permutation test and produces the final list of IS. We tested

the reliability of our tool on 3 IS datasets generated from simulated sequencing reads with incremental rate of nucleotide variations (0%, 0.25% and 0.5%) and real data from a cell line with known IS and we compared out tool to VISPA and UClust, used for GT studies. In the simulated datasets our tool demonstrated precision and recall ranging 0.85-0.97 and 0.88-0.99 respectively, producing the aggregate F-score ranging 0.86-0.98 which resulted higher than VISPA and UClust. In the experimental case of sequences from LAM-PCR products, our tool and VISPA were able to identify all the 6 known ISs for >98% of the reads produced, while UClust identified only 5 out 6 ISs. We then used our tool to reanalyze the sequencing reads of our GT clinical trial for Metachromatic Leukodystrophy (MLD) completing the hidden portion of IS. The overall number of ISs, sequencing reads and estimated actively re-populating HSCs was increased by an average fold ~1.5 with respect the previously published data obtained through VISPA whereas the diversity index of the population did not change and no aberrant clones in repeats occurred. Our tool addresses and solves important open issues in retroviral IS identification and clonal tracking, allowing the generation of a comprehensive repertoire of IS.

538. Transient Non-Viral RNA Delivery Mediated by a Lentiviral Particle

Pascale Bouillé[1], Régis Gayon[1], Alexandra Iché[1], Christine Duthoit[1], Lucille Lamouroux[1], Jean-Christophe Pagès[2]
[1]Research & Development, Vectalys, Toulouse, France, [2]Faculté de Médecine, Inserm U966, Tours, France

Safe and efficient gene therapies including gene-targeting technologies are very challenging but very promising approaches nowadays. The scientific and clinical communities have been working for a long time together to encounter substantial clinical advances they have made possible thanks to numerous improvements in cell culture and gene transfer methods. Opportunities to improve gene transfer into primary or stem cells involve a better design of the vectors used. Such improvements must lead to an increase of the transduction efficiency including the percentage of positive cells, as well as a better level and duration of expression, cell phenotype preservation and the number of genes delivered··· Lentiviral vectors have seen their use largely increased in clinical protocols over the past few years but safety concerns have been highlighted. First, the permanent genetic modification remains a focus of significant regulatory oversight and even integrase- or reverse transcriptase-deficient lentiviral vectors leads to residual integration events. Moreover, all the gene-editing technologies entail a "hit-and-run" mechanism that requires only a transient expression of the nuclease complex. In parallel, mRNA delivery is a versatile, flexible, and safe mean for protein therapies but chemical or electroporation-based transfection protocols are known to induce cell toxicity and phenotype modifications of the target cells. Here, we describe a new chimeric lentiviral platform that allows mRNA delivery into the target cells without any genomic signature. The respective properties of the MS2 bacteriophage and the lentiviral vectors have been combined to build a non-integrative packaging system in which the wild type HIV packaging sequence is replaced by the MS2 stem-loop repeats and the MS2 Coat sequence is inserted into the NucleoCapsid sequence. The resulting lentiviral particle is able to deliver a non-viral RNA into the cytoplasm of target cells, directly available for protein translation. Transduction of immortalized cells but also of T cells and HSC with these RNA lentiviral particles (RLP) shows an efficient, fast and transient expression of both reporters and functional proteins such as genome editing enzymes. Particles structure and functionality, cell transduction and characterization of such engineered cells have been compared with those obtained with an integrative lentiviral vector. Particularly by recruiting the RNA independently of dimerization with more than four molecules per particles, RLPs allow the cotransfer of different species of RNA into target cells. This new delivery system is a great candidate handle the safe and clinically suitable delivery of the editing machinery, which can transiently act without inducing cellular toxicity or immunogenicity.

AAV Vectors III

539. Screening for Recombinant Adeno-Associated Viral Vectors That Selectively Transduce Hepatitis B Virus Infected Cells

Gustavo de Alencastro, Katja Pekrun, Mark Kay
Genetics and Pediatrics, Stanford University, Stanford, CA

Hepatitis B is a disease caused by infection with the hepatitis B virus (HBV), a small enveloped DNA virus which belongs to the *Hepadnaviridae* family. Even though a safe and effective vaccine is available, HBV represents the ninth most common cause of death worldwide with an estimated 350 million chronically infected individuals. HBV infection can cause cirrhosis and hepatocellular carcinoma and currently there are 7 approved drugs for the treatment of HBV infection in the USA. These drugs decrease the risk of liver damage from HBV by slowing but not eliminating viral replication in most patients, possibly related to the persistence of integrated HBV genomes. Gene therapy using recombinant adeno-associated viral (rAAV) vectors constitute a promising tool to combat HBV infection as rAAV vectors have the ability to transduce and establish long-term and stable transgene expression in liver cells. Preclinical studies have successfully utilized rAAV vectors to combat viral infections after delivering short-hairpin RNA (shRNA) expression cassettes for knocking down viral coding or host genes required for viral replication and spread. Because promiscuous targeting of host cellular genes in the majority of non-infected cells may be detrimental, specific targeting of HBV-infected cells adds a new layer of potential therapeutic targets and safety. Thus we set out to identify new AAV capsids that selectively transduce HBV-infected cells. To do this, we created new AAV shuffled capsid libraries and used these for multiple rounds of infection in HBV-infected hepatoma cells. Each library is co-infected with wild-type Adenovirus-5 (helper virus) allowing the rAAV genomes to replicate in the HBV-infected cells. Isolation of replicating rAAV over successive passages in cultured cells allows for a more stringent selection as all steps in AAV transduction must occur. We have varied the screens using various multiplicity of infections (MOIs) of the library and/or utilized a pre-clearing step using a HBV negative hepatoma cell line. Several selected capsids derived from multiple parental capsids were vectorized. One capsid (AAV-GK4) was found to transduce the infected cells approximately 6 times more robustly than uninfected cells (using an MOI of 100 and 1,000). Interestingly, the same result was observed with AAV-DJ, a variant previously selected on human hepatoma cells. Additional screens and candidates are pending further evaluation. The possibility of finding new rAAV capsids with an increased transduction efficiency and selective tropism for HBV-infected cells will provide a reagent or therapeutic that can deliver a transgene that will allow one to selectively modify or potentially kill infected but not the uninfected cells contained within the liver.