

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

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**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 1 $\alpha$  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

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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 insoluble 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

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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