or without an IgG1-CH2CH3 spacer and a *piggyBac* transposase plasmid. We compared 2 new culture conditions with our original method for expansion of CD19 CAR-T cells. All cells were cultured in serum-free medium (TexMACS) containing IL-7 and IL-15 in 24-well plates. Electroporated cells were immediately transferred to irradiated autologous activated T-cells (ATCs), either pulsed with 4 viral peptide pools (PepTivator; AdV5 Hexon, CMV pp65, EBV EBNA-1, and BZLF1) (ACE), or unpulsed (non-ACE). The next day, non-ACE cells were transferred to CD3 and CD28 antibody-coated plates for 5 days. In the original method, electroporated cells were transferred to CD3/CD28-coated plates one day after electroporation without ATCs. On day 7, all cells were transferred into G-Rex10 culture flasks with ACE-pulsed or unpulsed irradiated ATCs or no ATCs for ACE, non-ACE and original cells respectively. On day 14, we collected CAR-T cells from all conditions. **Results** We obtained $4.7 \pm 3.0 \times 10^7$ ACE CAR-T cells, $6.7 \pm 2.1 \times 10^7$ non-ACE CAR T cells, and $5.3 \pm 2.3 \times 10^7$ original CAR T cells after 14 days of culture. ACE CAR-T cells showed significantly higher expression of the CAR transgene (33.0 ± 9.7% and >50% for CD19.CAR with and without the CH2CH3 spacer respectively) than non-ACE (10.7 ± 7.8%) or original CAR-T cells (4.6 ± 3.2%). All CAR-T-cells inhibited the growth of CD19+ tumors (95%, 60% and 51% for ACE, non-ACE and original CAR-T at an E:T ratio of 1:50; ACE CAR-T cells contained 29.6 ± 17.3% CD4+ cells and 64.3 ± 16.8% CD8+ cells, with 79.9 ± 5.9% cells co-expressing CD45RA and CCR7 and 62.6 ± 20.9% cells co-expressing CD45RO and CD62L. In an *in vivo* mouse model, ACE CAR-T cells delayed tumor growth by 30 days compared to no T-cells. **Conclusions** We markedly increased CAR expression from *piggyBac*-mediated CD19 CAR-T cells without cell selection, reducing the culture period to 14 days using viral antigen stimulation. Deletion of IgG1-Fc spacer led to further increase of CAR expression. Together with the simple manufacturing process and cost-effectiveness of DNA transposon technology, *piggyBac*-based gene transfer provides an alternative to retro- or lentiviral gene transfer for CAR T-cell therapy.

706. Consistent Viral Vector Manufacturing for Phase III Using iCELLis 500 Fixed-Bed Technology

Minna Karhinen[1], Elisa M. Nurminen[1], Piia Valonen[1], Outi Rautsi[1], Vesa Huusko[1], Kati Heikkilä[1], Janne Weisell[1], Achim Muller[1], Hanna P. Lesch[1], Outi Närvänen[1], Minna M. Hassinen[1], Minna Pollen[1], Steve Parrish[1], Robert Shaw[1], Nigel R. Parker[2], Seppo Ylä-Herttuala[3]

[1]Finvector Vision Therapies, Kuopio, Finland, [2]FKD Therapies, Kuopio, Finland, [3]AIV Institute for Molecular Science, University of Eastern Finland, Kuopio, Finland

Single use disposable technology faces further challenges in producing viral vectors in sufficient large quantities especially where adherent cells are needed. Scaling up the adherent system has proven to be troublesome. The PALL iCELLis® disposable fixed-bed bioreactors offer a novel option for viral vector manufacturing in large quantities in an adherent environment. We have made process development in iCELLis Nano, where the cultivation area varies between 0.53 - 4 m^2, after which we went forward to iCELLis 500, where the cultivation volume can be upgraded to 500 m^2 (66 - 500 m^2). iCELLis 500 has proven to be ideal to satisfy upstream demand and large-scale downstream purification process was developed to supply high quality recombinant adenovirus based gene products in our fully-licensed GMP manufacturing facility for pre-clinical and clinical trials The process is initiated by HEK293 cell mass expansion in suspension mode using Biostat® CultiBag RM (Sartorius Stedim Biotech S.A.) bioreactor. The expanded suspension cell mass is inoculated into iCELLis 500 for further expansion in adherent mode. This is infected by Working Viral Seed Stock and subsequent virus is released from the infected cells by chemical lysis. Downstream process contains Benzonase digestion, clarification, concentration and conditioning by crossflow ultrafiltration, capture and polishing by anion exchange chromatography, and final concentration and formulation is achieved using crossflow filtration. All product contact parts are fully disposable. Several batches have been produced with consistent results. Further validation of the process for commercial manufacturing is currently ongoing. We established a scalable, large-scale manufacturing process to supply high quality recombinant adenovirus based gene products in our fully-licensed GMP manufacturing facility for clinical trials. Alongside this we have validated assays which are providing a relevant quantitative measure of biologic function of the vector and demonstrating the quality and comparability of drug product batches in Phase 3 and commercial use.

707. Attenuated HIV Does Not Amplify in Primary Human T-Lymphocytes During a Model *Ex Vivo* Gene Therapy Procedure

Pratima Cherukuri[1], Scott Witting[2], Lilith Reeves[2]

[1]Indiana Univeristy, Indianapolis, IN, [2]Cincinnati Childrens' Hospital Medical Center, Cincinnati, OH

Current regulatory guidelines require RCL testing of lentiviral vector preparations used in human clinical trials. Cells transduced *ex vivo* and cultured four days or more must also be tested before administering to the patient. Attenuated HIV (R8.71) can be considered as a theoretical RCL that could develop from a recombination event with wild-type HIV. Importantly, R8.71 lacks accessory proteins (*vip, vif, vpr and nef*) that are known to be essential for viral replication in T-lymphocytes. Based on this fact, the risk of a recombinant RCL being amplified in T-lymphocytes during an *ex vivo* transduction and expansion protocol is questionable. To test this possibility, we transduced human T-lymphocytes with one of two RCL-negative lentiviral vector preparations (eGFP or a chimeric antigen receptor (CAR)) that were spiked or not with R8.71 or wild-type HIV. C8166 cells served as a positive control for cells permissive to R8.71. The transduction and subsequent expansion lasted 10 days total and the experiment was performed on two independent occasions. Using day 10 supernatants from all groups, a full RCL assay (with amplification and indicator phases) was performed. Product enhanced reverse transcriptase (PERT) and P24 ELISA assays served as readouts for the RCL assay. Transduction rates were determined by flow cytometry and found to be 57-60% and 10-37% for the eGFP and CAR vector, respectively. The RCL assay results showed that all T-lymphocyte groups exposed to R8.71 were negative but those exposed to wild-type HIV were positive. C8166 cells exposed to R8.71 or wild-type HIV were positive in the RCL assay. These results suggest that a recombinant RCL that possess the wild-type HIV envelope would NOT amplify in human T-lymphocytes during a model *ex vivo* transduction procedure. The amplification of an arguably more dangerous VSVG-enveloped recombinant RCL is still possible; however, it could be reliably detected by quicker and less expensive assays such as qPCR to VSVG sequences.

708. Process Development of Lentiviral Vectors for Large Scale Production Using a HEK293 Producer Cell Line

Alexandre Audy[1,2], Marc-André Robert[1,2], Parminder Chahal[2], Rénald Gilbert[2], Bruno Gaillet[1]

[1]Laval University, Québec, QC, Canada, [2]National Research Council, Montréal, QC, Canada

Lentiviral vectors (LVs) are promising vectors for gene therapy. Most often, they are used to deliver a functional copy of a gene to sustainably replace a defective or missing gene. However, processes for LVs must be improved to increase the yield, facilitate the scale

up and satisfy Health regulatory agencies. For these reasons, we have developed and optimized a LVs production process in serum-free medium using an inducible HEK293 producer cell line which possesses the capacity to grow in suspension culture. By adding two inducing molecules, (cumate and doxycycline) this cell line produces LVs pseudotyped with the protein G of the vesicular stomatitis virus without the need of any transfection. Our tested LV carried an expression cassette for GFP to facilitate LV quantification. To optimize the process, a design of experiment (DoE) was prepared which included the study of different culture media, high cell density production using six cell boosts commercially available and the addition of sodium butyrate, caffeine and valproic acid. We found that two cell boosts were outperforming the other cell boosts tested. At the present time, two commercial media (Hycell TransFx-H and SFM4TransFx-293 media) were our best candidates to maximize viral titer by achieving high cell density culture. In parallel, a LV carrying the cDNA for a shorter version of dystrophin (mini-dystrophin) was constructed. The truncated version of the dystrophin was produced by transient transfection in 293A cells and its presence was confirmed by western blot. We are planning to evaluate if the optimal conditions for the production of LV-GFP will be also applicable to LV-mini-dystrophin, a LV encoding a much longer transgene than GFP (0.7 kb vs 5.8 kb). This LV could be first evaluated for cell therapy in animal models and later, in patients suffering from Duchenne muscular dystrophy, where the dystrophin gene is defective and the protein is absent.

709. Characterization of Nanoparticles in Lentiviral Vector Preparations

Erik Hansen, Kristen Lee, Suzanna Melotti, Jesse Milling, Maya Miatkowski, James Warren, Mercedes Segura
Process Development Vector Analytics, Bluebird Bio, Cambridge, MA

The potential of lentivirus-based 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-to-late stage clinical development requires enhanced characterization of the purified lentivirus vector product. Lentiviral vector preparations are complex in nature and contain a heterogeneous mixture of transduction-competent as well as transduction-deficient virus particles. In addition, lentiviral vector production utilizes host cells that can produce not only the viral particles of interest, but a variety of closely-related impurities that include exosomes and microvesicles. These cell-derived impurities can overlap key biophysical and biochemical attributes of the lentiviral vector, including size, net charge and composition, making them challenging to analyze. We used a variety of analytical tools to further characterize lentiviral vector preparations in terms of size distribution, particle counts and to determine the total particle (viral or non-viral) to infectious particle ratio. These tools included Nanoparticle Tracking Analysis (Nanosight), Field-Flow Fractionation coupled to Multi-Angle Light Scattering (FFF-MALS), and p24-ELISA. Particle protein composition was evaluated by several standard orthogonal protein quantitation assays. Characterization of nanoparticle subpopulations is important as it can support the refinement of manufacturing processes. The impact of variation of these parameters on lentivirus vector performance still remains largely unknown, so interpretations of the results must be carefully assessed and further study is warranted.

710. Surface Adsorptive Loss of rAAV on Materials Used in cGMP Manufacturing

Monica Jiang, Colleen M. Dupuis, Amy A. Medeiros, Samuel C. Wadsworth, K. Reed Clark, Christopher J. Morrison
Downstream Process Development, Dimension Therapeutics, Cambridge, MA

Continued interest in using rAAV vectors as therapies for a range of indications with varying prevalence often translates to increased manufacturing scales. A significant challenge that is encountered is the use of different manufacturing materials during the transition between early research and development and the clinical manufacturing scale. For example, manufacturing components, ranging from bioprocessing bags and hard plastics to bioprocess tubing and filters can be constructed of different materials and are not often engineered for use with rAAV. In this work we investigate the loss of rAAV to non-specific surface adsorption on various materials of construction. In addition, the effect of different background matrices was also investigated. These data demonstrate that surface adsorptive loss of rAAV can be significant, and that losses can vary significantly even for materials of similar construction. To mitigate adsorptive loss, several surfactants and other components were examined for their ability to reduce surface adsorption of rAAV. A number of these agents were identified as viable options for cGMP manufacturing. These results provide insight for the development of scaleable, more robust and higher yielding manufacturing processes to meet the continued demand for rAAV therapeutics.

711. Development of a Clinically-Acceptable Lentiviral Vector for Cystic Fibrosis Airway Gene Therapy

Alexandra McCarron[1], Chantelle McIntyre[2], Martin Donnelley[2], Patricia Cmielewski[3], David Parsons[3]
[1]School of Medicine, University of Adelaide, Adelaide, Australia, [2]Robinson Research Institute, University of Adelaide, Adelaide, Australia, [3]Respiratory and Sleep Medicine, Women's and Children's Hospital, North Adelaide, Australia

Background: Lentiviral (LV) vectors are a promising gene delivery vehicle for cystic fibrosis airway gene therapy. However, the development of a clinically-acceptable method suited to large-scale lentivirus production remains a barrier to the translation of gene therapy to the clinic. Current virus production methods have limited-scalability and often result in contamination of the vector preparation with potentially immunogenic components such as bacterially-derived plasmid DNA, and animal sera products. Accordingly, a scalable process for the production of clinical-grade vector is required.

Methods: LV vector preparations were treated with Deoxyribonuclease I (DNase I) to remove residual plasmid DNA, with the effectiveness measured by PCR using primers to detect the presence of the cytomegalovirus promoter sequence. The potential for scalable LV vector production in serum-free conditions was investigated by performing transient transfection of HEK 293FT cells adapted to grow in serum-free, suspension culture. To determine the efficiency of the virus production system RNA titering was performed using a commercial qPCR kit, and reporter gene expression of GFP and Luciferase were quantified using FACS and IVIS bioluminescence imaging.

Results: Plasmid DNA was detected in the vector preparations following DNase treatment, indicating that DNase I was ineffective at eliminating the residual plasmid DNA. Lentivirus was successfully produced using transient transfection of serum-free, suspension growing HEK 293FT cells, and was scaled-up to a one-litre culture capacity in shake flasks. Titres of up to 10^8 TU/mL were achieved in the suspension cell culture system, which is comparable to the yields obtained in the standard adherent culture system.