the compliance of standard AAV NAB assays with the percentage law, formulated by Andrewes and Elford in 1933, a standard for virological NAB assays, which seeks stoichiometric conditions of NAB and viral load that enables measurement of NAB titers independent of viral input in the assay. Our data shows that at the conditions in common use in the field the percentage law is not upheld, and NAB titers are highly dependent on input viral load of the assay, directly impacting sensitivity and specificity, and leading to the occurrence of false negatives. Indeed, quantitative assessment of seroprevalence in primate populations with assays that reduce the amount of AAV to within percentage law range affects assay outcome qualitatively and quantitatively. We also independently assessed other assay parameters to improve sensitivity, robustness and reproducibility, and to study whether they may also have qualitative effects on titer outcomes. Our studies highlight the importance of pre-existing immunity in AAV gene therapy and limitations of the current methodologies to measure it in a robust and predictive manner. Data indicates the potential for false positive and negative readout for commonly used assay protocols. An optimized protocol was developed for further validation and evaluation. These studies may impact translational and clinical AAV gene therapy studies.

468. GLP-Compliant Non-Clinical Safety and Biodistribution of a Recombinant AAV2/8 Vector Administered Intravenously for Treatment of Mucopolysaccharidosis Type VI

Rita Ferla[1], Jean-Brice Marteau[2], Severine Pouillot[2], Karen Kozarsky[3], James Brown[3], Stefania Galimberti[4], Maria Grazia Valsecchi[4], Vincent Zuliani[2], Alberto Auricchio[1]
[1]Telethon Institute of Genetics and Medicine, Pozzuoli (NA), Italy, [2]Genosafe, Evry Cedex, France, [3]REGENXBIO Inc., Rockville, MD, [4]Center of Biostatistics for Clinical Epidemiology, Department of Health Sciences, University of Milano-Bicocca, Monza (Mi), Italy

Mucopolysaccharidosis VI (MPS VI) is a lysosomal storage disorder caused by deficiency of the enzyme arylsulfatase B (ARSB), which results in widespread accumulation and excretion of toxic glycosaminoglycans. We recently developed a successful gene therapy approach based on a single systemic administration of AAV2/8 that targets liver of MPS VI animal models. In view of a gene therapy clinical trial for MPS VI, we performed GLP-compliant non-clinical studies to assess the safety and biodistribution of AAV2/8.TBG.*hARSB*, a recombinant AAV2/8 vector encoding human ARSB (hARSB) under the control of the thyroxine-binding globulin promoter (TBG). We used transgenic C57/BL6-Tg*ARSBC91S* mice that overexpress an inactive *hARSB C91S* mutant and are thus immune tolerant to hARSB. Mice were treated with either AAV2/8.TBG.hARSB or the vehicle alone, as control. Toxicity was evaluated on day 15 (D15) and 180 (D180) after systemic injection of $2x10^{13}$ gc/kg, which is 10X the highest dose proposed for the clinical study [20males(M)+20females(F)/treatment/timepoint]. No mortality, abnormal clinical signs and alteration in body weight, body temperature and food intake were observed through the study. Similarly, no clinically relevant changes in blood chemistry and hematology were found in treated mice compared to controls. Histopathology revealed thyroid epithelial hypertrophy in AAV-treated mice. AAV2/8.TBG.*hARSB* biodistribution and expression was evaluated on D15 and D180 at the dose of $2x10^{12}$ gc/kg, which is 1X the highest dose proposed for the clinical study (5M+5F/treatment/timepoint). Although vector DNA was present in all organs on D15, it was sequestered mainly in liver at levels at least 3 logs higher than those found in other organs. Vector DNA declined on D180, but remained high in liver. Accordingly, hARSB was mainly expressed stably in liver, supporting TBG tissue specificity. Vector DNA was found in gonads of both sexes at 3 logs lower than in liver. A robust reduction of vector DNA was observed on D180. A supportive study conducted in male rabbits showed that vector shedding in semen was only transient, which suggests that the risk of inadvertent germline transmission of AAV2/8.TBG.*hARSB* is minimal at least in male animals. An *in situ* hybridization study is ongoing in ovaries to elucidate AAV localization. Finally, AAV DNA was only transiently present in plasma, urine and stools of mice (up to D37, D2 and D14, respectively), which minimizes the potential risk associated with transmission to third parties and/or the environment. In conclusion, these studies show a safe profile of intravenous administrations of AAV2/8.TBG.*hARSB* and pave the way for the phase I/II clinical trial.

469. Improved Protocol and Use of Mitochondrial DNA as Reference for qPCR Quantification of Integrase-Defective Lentiviral Vectors (IDLVs)

Bala Sai Sundarasetty, Laura Gerasch, Renata Stripecke
Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Medical School Hannover, Hannover, Germany

Integrase defective lentiviral vectors (IDLVs) are attractive tools for genetic manipulation and are increasingly being tested as recombinant viral vaccines against infectious pathogens and cancer. IDLVs remain mostly as extrachromosomal/episomal DNA circles in the infected cells, hence with reduced risk of genotoxicity mediated by insertional mutagenesis. We have demonstrated in our previous work the production and characterization of a tricistronic IDLV (packaged with the D64V mutation in the integrase and co-expressing GM-CSF, IFN-alpha and the CMV pp65 antigen) and IDLV-mediated monocyte transduction achieved under Good Manufacturing Process (GMP) compliant conditions (Sundarasetty et al., JTM 2015). One of the main criteria for batch release of the cell vaccine is to confirm and quantify IDLV copies in the thawed cell product. Currently used DNA extraction methods are not efficient in isolating small molecular weight episomal DNA from cells. In addition, genomic DNA loci used as reference controls are not informative regarding quantification of episomal DNAs. Mitochondrial DNA (mtDNA) is an episomal small molecular weight circular DNA (16.5 kb) that can serve as a reference for IDLV quantification. In order to maximize recovery of episomal DNAs, we explored the total DNA (tDNA) extraction described for mtDNA isolation (Badralmaa et al., J Vir. Meth. 2013), based on dehydration and precipitation of proteins and subsequent tDNA precipitation from the supernatants by isopropanol. As a reference for the qPCR quantification, we constructed and validated a plasmid containing a sequence homologous to a regulatory region of transfer vector (wPRE), a sequence for a genomic house-keeping gene (PTBP2) and a sequence for a mitochondrial house-keeping gene (Cytochrome B). The amplification of three target regions was validated by generating a standard curve with the reference plasmid ranging from 5 x10 to $5x10^5$ copies (n=3). As a reference, we used an in-house generated 293T cell line (B5) containing three LV genomic copies. Total DNA extracted form B5 was serially diluted in non-transduced 293T DNA in order to result into 0.25, 0.5, 1, 2, 3 LV copies and the assay linearity was assessed in three independent runs. The reliable detection limit of the qPCR assay was 0.5 LV copies/cell and 0.9 copies/ ng of genomic DNA. Having assessed the linearity, we assessed if this method could be used to quantify IDLV in transduced monocytes. The tricistronic IDLV was used to transduce monocytes of different donors in triplicates at increasing multiplicities of infection (MOI: 1, 2.5, 5 and 10). After thawing each cryopreserved batch, tDNA was extracted and analyzed. Our results showed a direct correlation between the IDLV copies per cell and ng of DNA used for the assay and MOI, whereas detection of the genomic and mtDNA references remained constant. Thus, this simple