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product quality profile across manufacturing-friendly operating ranges. The new upstream process has demonstrated comparable titers and consistent product quality across multiple batches at 2L, 50L, and 500L scale for different constructs and AAV capsid serotypes. Successful scale-up of this process is enabled by critical control of our high-performing transient transfection.

The benefits of a highly productive upstream process may not be realized through to drug substance without a similar effort and focus on the downstream side. At OXB Solutions, we have combined our new 1E15 vg/L upstream process with a comparably intensified downstream process that allows us to manufacture over 1E17 vector genomes of drug substance per 500L bioreactor.

P038

Dyno-86m and Dyno-gvk: cell-type resolved validation of AAV capsids optimized for intravitreal delivery to the non-human primate retina

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1: Dyno Therapeutics

Safer and more effective intravitreally delivered ocular gene therapies may be enabled by engineering AAV capsids for highly efficient transduction of the retina at low doses, thereby reaching a sufficient number of cells without eliciting an inflammatory response. Here we characterize Dyno-86m, a capsid with field-leading potential for intravitreal delivery. The Dyno-86m capsid was designed with generative AI leveraging in vivo data from multiplexed measurements of capsid libraries. In pooled experiments using NGS to measure bulk retina transduction in Cynomolgus monkeys, Dyno-86m was 80-fold improved vs AAV2.

Understanding delivery efficiency in specific cell types is key to successful ocular gene therapy. Towards this goal we further characterized the performance of Dyno-86m through single-nuclei RNA sequencing and histology, comparing to both wild-type AAV2 and an external engineered capsid.

We compared Dyno-86m to the external engineered capsid in a 2-capsid Cynomolgus monkey study, co-injecting each at a dose of ~1e11vg per eye. Transduction was measured through cell-segmentation and quantification of immunofluorescent histological images. Dyno-86m consistently outperformed the external engineered capsid, with 2.6-fold higher transduction across the entire retina, and between 2.2 and 3.2-fold higher transduction across ganglion cells, inner nuclear layer cells including bipolar cells, and photoreceptors in the central retina.

In a separate Cynomolgus monkey study, single-nuclei RNA sequencing confirmed Dyno-86m delivery to all major NHP retinal cell types, including ganglion cells, rods and cones. Dyno-86m showed 2-3 fold higher transduction across these cell types compared to the external engineered capsid, closely matching histology results from the 2-capsid study. Similar results were observed when quantifying transduction of the macula by snRNA-seq, where percent cells transduced was greatest.

These results demonstrate the potential of Dyno-86m as the field-leading capsid for intravitreally delivered gene therapies, opening new opportunities for gene therapies to treat a wider range of ocular diseases.

P040

Airway-specific gene expression and biodistribution in ferrets and nonhuman primates utilizing recombinant Human Bocavirus Type 1

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1: Carbon Biosciences

The lung presents several obstacles to gene delivery that provides selective and durable gene expression, prevents achieving meaningful clinical outcomes for severe pulmonary diseases, including cystic fibrosis (CF). Current viral-based gene delivery approaches are limited by genetic capacity and nonspecific tropism for lung epithelial cells. Human Bocavirus Type 1 (HBoV1) is a nonpathogenic parvovirus of genus *Bocaparvovirus* that readily infects human airway epithelial cells repeatedly and offers 20% extra genomic capacity beyond adeno-associated virus 2 (AAV2). CBN-1000 is a hybrid viral vector featuring a HBoV1 capsid and engineered with conventional AAV2-vector genome elements. The hybrid vector displays exquisite lung tropism while simultaneously leveraging the much of the safety, regulatory and CMC profile of AAV vectors. In the present study, we evaluated the cell type transduction and durability of CBN-1000 in primary human lung epithelial cells from normal donors as well as those from CF patients cultured in air-liquid interphase and differentiated into mature human airway epithelial cells that resemble airway epithelium *in situ*. Results indicate sustained transduction of both progenitor basal cells and terminally differentiated specialized cells of the airway epithelium.

Studies in ferrets and nonhuman primates were conducted to characterize the biodistribution and expression of a GFP transgene delivered by CBN-1000. In addition, an ^{124}I-labeled HBoV1 capsid was administered intratracheally and evaluated for tissue tropism in wild-type (WT) and CF transgenic ferrets. Results indicate similar biodistribution, tissue penetration and clearance in both WT and CF ferrets, despite a moderate-to-severe lung disease phenotype in the transgenic animals. In addition, selective biodistribution and gene expression in the lung was demonstrated in both ferrets and nonhuman primates. Administration of CBN-1000 was well tolerated in both species at clinically relevant dose levels. Overall, these data suggest CBN-1000 is a novel and selective viral vector for lung delivery with the potential for delivering transgenes up to 5.5kb in healthy or diseased lungs resulting in durably expression. CBN-1000 solves several of the most daunting challenges of current pulmonary gene delivery and exemplifies the potential of capsids derived from non-AAV, autonomous parvoviruses to expand the scope of gene therapy for diverse applications.

P041

Characterization of AAV integrations and rearrangements from long and short reads with RAAVioli

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Gene therapy (GT) applications based on recombinant Adeno Associated Viral (rAAV) vectors were proven successful in

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treating several diseases, thanks to their high transduction efficiency and specificity. Even though rAAV primarily remains episomal in the nucleus of transduced cells, a growing number of studies published over the past years have demonstrated the integration of fragmented or full-length AAV DNA within the transduced cell genome where double-strand DNA breaks (DSBs) or nicks have occurred. Yet, the occurrence of hepatocellular carcinoma and clonal expansion events consequent to rAAV insertions have posed safety concerns for their clinical use.

However, bioinformatics tools able to identify AAV integration sites (IS) and characterize vector rearrangements are still missing. Here, we studied the DNA collected from a humanized liver mouse model, where human primary hepatocytes have been transduced ex-vivo or in-vivo with a tomato expressing AAV. AAV IS were retrieved using SLiM-PCR and short-paired end Illumina technology as well as by long reads PacBio sequencing after probe-based capture. Sequencing reads were analysed using RAAVioli (Recombinant Adeno-Associated Viral IntegratiOn analysis) to characterize vector rearrangements and IS. Python and R scripts parse the alignments to identify IS and reconstruct rearrangements using CIGAR strings.

Overall, 811 and 370 unique IS were identified from short paired-end Illumina reads and long PacBio reads respectively, confirming the higher efficiency of PCR-based approach. The IS were distributed all along the human genome showing the typical preference of targeting CpG islands and transcriptional start sites. Moreover, 32 IS were found in both datasets demonstrating the consistency of the results obtained independently from the platform adopted. Both IS datasets showed a similar percentage ($\sim$25%) of fragments containing AAV rearrangements, although more than 2 and up to 6 AAV rearrangements were observed only when long PacBio reads were adopted.

Precision and accuracy of RAAVioli pipeline were assessed through simulated datasets obtaining scores >0.95 in IS identification and rearrangement characterization. These results indicate that RAAVioli is a versatile and comprehensive bioinformatic tool that can efficiently map AAV IS using both long and short paired ends sequencing reads. These approaches are fundamental to characterize AAV integration and recombination events in gene therapy and gene editing applications, allowing and improving the assessment of safety in AAV studies.

P044

AAV vector-based transgene expression in primary human NK cells

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Natural killer (NK) cells are specialized innate immune effector cells. Their innate ability to recognize and kill tumorigenic cells makes them an interesting candidate for cellular immunotherapy, especially in combination with chimeric antigen receptors (CARs). Indeed, CAR-NK cells are considered as an alternative approach to CAR T cells with the advantages of a better safety profile with no or minimal neurotoxicity or cytokine release syndrome and the potential for an allogenic ''off-the-shelf'' manufacturing. However, the safe and efficient modification of NK cells using viral or non-viral vector gene transfer is still a challenge.

Here, we report on establishing adeno-associated virus (AAV) vectors as a novel and promising gene transfer tool for NK cells. We systematically tested a broad range of AAV vectors for uptake and transgene expression in human peripheral blood mononuclear cell (PBMC)-derived NK cells. We determined the impact of in vitro culture conditions, cultivation times, as well as different transduction protocols on transgene expression.

The level of transgene expression did not seem to be linked to cell entry but rather to the NK activation state and to cellular metabolic processes. In general, the basal level of transgene expression was highly donor dependent, ranging from below 10 % up to more than 50 %. By optimizing transduction conditions, we now reach a donor-independent increase of the eGFP transgene expression levels to around 80%.

In conclusion, we report here for the first time efficient AAV vector-mediated transgene expression in primary human NK cells. Our optimized conditions for ex vivo NK cell transduction, are now used to equip NK cells with anti-CD19 and anti-CD4 CAR constructs.

P045

Tetracycline Enabled Self-Silencing Adenovirus (TESSA^{TM}) – a versatile and high-yielding scalable platform for rAAV manufacture

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Recombinant adeno-associated virus (rAAV) is the vector of choice for *in vivo* gene therapy, however, efficient manufacture of rAAV to meet pre-clinical and clinical demands remain challenging. To solve this critical manufacturing challenge in generating high yield and high quality rAAV vectors, we developed a novel self-silencing helper adenoviral vector system entitled 'Tetracycline-Enabled Self-Silencing Adenovirus' (TESSA^{TM}) to deliver the AAV genes and adenoviral 'helper' functions, into HEK293 cells, for efficient rAAV manufacture without adenovirus contaminations. TESSA^{TM} is a robust and versatile platform to produce rAAV, either by using two TESSA^{TM} vectors to deliver AAV rep/cap and the rAAV transfer genome, or wherein the rAAV transfer genome is delivered via stable integration in the cell's chromosome, plasmid transfection, or co-infection with existing rAAVs for vector propagation. This multitude of approaches to rAAV manufacture using TESSA^{TM} enables researcher to produce cost-effective rAAV vectors, both rapidly and scalable. The TESSA^{TM} platform yields >30-fold more rAAV vectors compared to the triple plasmid transfection process across a wide range of AAV serotypes including AAV1-9 & rh10, and generating rAAV vectors at productivities of >1E+6 vector genome copies (GC) per cell. We successfully demonstrated that up-scale production of rAAV6 and rAAV2 in 50 L and 200 L bioreactors runs using TESSA^{TM} is capable of yielding >7E+11 GC per mL of cell culture and >1E+17 GC of Drug Substances. Importantly, TESSA^{TM} enables significant improvement in the efficiency of rAAV genome packaging with AUC analysis showing >60% of full capsids after affinity-capture and >95% of full capsids after