for simultaneous vector genome titration by testing the yields of 13 different serotypes of ssAAV and scAAV. Thus, this procedure can be applied to screen large panels of novel serotypes/variants for vector production. Importantly, we show across independent experiments that titer quantification has high inter-experimental reproducibility among multiple batches of samples. In summary, our described method fulfills unmet needs for quantifying vector genomes in crude lysates from both large- and small-scale AAV preparations in a high-throughput, sensitive, accurate, and reproducible manner. This will significantly improve in-process quality control, batch/lot monitoring in large-scale preparations, and good manufacturing practices (GMP) for AAV production - all key for vector manufacturing, as AAV continues to garner use and impact in both basic research and clinical applications.

291. CD26 Inhibition Enhances Chimerism of Lentivirally Transduced Hematopoietic Stem Cells in a Non-Myeloablative Setting

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Despite the progress of gene therapy for monogenic diseases over the last years, the engrafting capacity of gene-modified cells, as well as constant and high-level gene expression *in vivo,* require further optimization, especially when a reduced intensity conditioning is mostly preferred. Culture conditions applied for effective gene transfer lead to significant loss of long-term repopulating cells and ultimately, impaired engraftment. The *ex vivo* inhibition of CD26 peptidase activity with Diprotin A was shown to improve homing and engraftment of unmanipulated hematopoietic stem cells (HSCs). We here, sought to assess the homing and engrafting capacity of lentivirally GFP-transduced murine bone marrow (BM) cells under competitive niche settings generated by a non-myeloablative conditioning. Following CD26 inhibition with 5mM Diprotin A of GFP-transduced murine HSCs, we assessed migration towards SDF-1 in transwell systems and the engraftment capacity after partial myeloablation (Busulfan 100mg/kg, equivalent to 8mg/kg in humans) in a syngeneic mouse model, allowing for donor chimera detection (C57Bl6-CD45.2$^+$ donors/PepBoy-CD45.1$^+$ recipients). We also investigated the possible effects of Diprotin A on gene transfer efficiency and transgene expression in bulk and clonogenic cultures of HSCs. *In vitro*, Diprotin A significantly increased the expression of the CXCR4 receptor (25.6±0.75 vs 16.4±1.02, P=0.02), as well as the %migration of gene-modified HSCs towards SDF-1 over untreated transduced cells (71.78±3.79% vs 55.71±5.34%, P=0.03), implying a potentially enhanced engrafting dynamic in the BM. Indeed, *in vivo,* the Diprotin A-treated transduced HSCs exhibited faster hematologic reconstitution by, at least, one week (P=0.03) and both superior long-term engraftment and GFP expression in all hematopoietic tissues (peripheral blood, BM, spleen) of the recipients, over non-Diprotin A-treated transduced cells (blood 4th month post-transplant, %CD45.2$^+$: 77.26±5.26 vs 13.11±11.69, P=0.0002; %GFP$^+$: 30.21±6.86 vs 3.9±2.33, P=0.03). The increased GFP expression observed *in vivo* in the Diprotin A cohort reflected the enhanced engraftment rates, since Diprotin A *per se* did not affect gene transfer efficiency or transgene expression prior to transplantation (%transduction with Diprotin A: 93±3.05 vs without Diprotin A: 89.9±7.47, P=0.73; %GFP in pools of colonies with Diprotin A: 74.8±7.14 vs without Diprotin A: 72.1±8.45, P=0.81). Upon sacrifice, the Diprotin A animals displayed sustained gene marking with ~1 vector copy in all hematopoietic tissues, as opposed to almost undetectable vector copy number (VCN) in the non-Diprotin A cohort. Likewise, individual colonies from the chimeric BM, demonstrated significantly higher VCN in the Diprotin A mice (2.8±0.48 vs 0.37±0.37, P=0.01). Overall, the *ex vivo* treatment with Diprotin A seems to abrogate the negative impact of culture conditions, allowing for enhanced donor chimerism under partial myeloablation and consequently, increased gene marking *in vivo*. This *ex vivo*, easily applicable approach may serve to overcome major constraints for the clinical implementation of gene therapy should the data be confirmed with human CD34$^+$ cells.

292. Towards Large-Scale Manufacturing of Adeno-Associated Virus by Transient Transfection of HEK293 Suspension Cells in a Stirred Tank Bioreactor Using Serum-Free Medium

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Adeno-Associated Virus (AAV) vectors showing safety profile in phase I clinical trials and its ability to transduce gene expression in various tissues have made it a vector of choice for gene delivery. There are different modes of AAV vector production and each has advantages and disadvantages. Here we demonstrated that the production of AAV by transient transfection in a serum-free medium using NRC's patented cGMP compliant human embryonic kidney HEK293 cell line (clone HEK293SF-3F6) adapted for growth in suspension can be readily scaled-up in stirred tank bioreactors. We employed triple-plasmid / polyethylenimine (PEI) based transient transfection technique. As a proof of concept, we demonstrated that nine serotypes of AAV (AAV-1 to AAV-9) encoding GFP can be produced by our cell line HEK293SF with yields of about 1E+13 genome-containing particles per liter (Vg/L). Depending on the serotypes 4-30% of AAV is present in the supernatant of the cell culture at 48hpt. The presence of plasmids and plasmid polyplexes that were not taken up by the cells or were not brought into the cell nucleus were removed by Iodixanol-ultracentrifugation method and Benzonase treatment before analyzing by real-time PCR. About 25% loss in genome containing viral particle counts were observed by Iodixanol purification method based on infectivity assay. Productions of AAV2 and AAV6 encoding GFP were demonstrated in 3L stirred tank bioreactors. Purification scheme was based on column chromatography - a scalable process. Different chromatography media, such as cation exchanger, anion exchanger and hydrophobic interaction chromatography, were tested with each AAV serotypes for their ability to adsorb and elute efficiently. The purification scheme was then adopted by integrating best chromatography medium and sequence dependent upon the AAV serotype in use. We demonstrated the purification scheme for AAV2 based on ion-exchange and hydrophobic interaction chromatography steps. The SDS-PAGE showed the purity of the final product and the presence of three capsid proteins VP1, VP2 and VP3 on Western blot corresponding to the only three bands present in the final product on SDS-PAGE. To extend the storage life of AAV we explored lyophilization technique to study the stability of AAV2 and AAV6 under lyophilized conditions. The AAV2 and AAV6 were stable for over 40 weeks based on infectivity assay. We demonstrated the scalability of the process up to 45L. Productions tested in 20 and 500 mL cultures in shake flasks were scaled up in 2 and 45L cultures (in 3- and 60-L stirred tank bioreactors, respectively). The volumetric yields and purification recoveries were comparable

at all of these production scale levels demonstrating scalability of transient transfection at even larger scale is possible to generate material necessary for dosages required for gene therapy application.

AAV Vectors II

293. Discovery of an Essential Receptor for Adeno-Associated Virus Infection

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Cellular entry of adeno-associated viruses (AAV) is poorly understood, despite the prominent use of AAV vectors in gene therapy for several monogenic diseases. Using an unbiased, haploid genetic screen, we identified critical players in AAV serotype 2 (AAV2) entry including members of distinct protein complexes involved in retrograde trafficking as well as genes involved in the biosynthesis of the AAV2 attachment factor, heparan sulphate. We focused on the single most significantly enriched gene of the screen, an uncharacterized type-I transmembrane protein. Based on the evidence below we renamed this gene AAV receptor (AAVR). We discovered AAVR as capable of rapidly endocytosing from the plasma membrane and trafficking to the trans-Golgi network, taking a similar path as AAV particles utilize. Genetic ablation of *AAVR* using CRISPR/Cas9 technology demonstrated a robust resistance to AAV2 infection in a wide range of mammalian cell types, which could be reversed upon *AAVR* complementation. This confirmed the essentiality of AAVR in AAV2 infection. Further investigation revealed that AAVR was also required for the infections of all tested human and simian-derived AAV serotypes including AAV1, 3B, 5, 6, 8 and 9. Deeper characterization of AAVR showed it to contain Ig-like domains, which are commonly found in many virus receptors including those for poliovirus and measles. We observed that these domains are capable of binding to AAV2 particles, and anti-AAVR antibodies efficiently block AAV2 infection. Moreover, the importance of AAVR for AAV infection *in vivo* was demonstrated by the strong resistance of AAVR$^{-/-}$ mice to AAV9 infection, recapitulating what we showed *in vitro*. Collectively, the data indicates that AAVR is a universal receptor involved in AAV infection. This has significant implications for the improvement of future AAV vector design.

294. Characterizing AAV CaptureSelect Affinity Ligand Interactions

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Adeno-associated virus (AAV) is one of the most widely studied viral vector systems for therapeutic gene delivery. This application has already experienced success in several human clinical trials, including the treatment of hemophilia B with an rAAV8 vector expressing therapeutic levels of Factor IX protein and for the treatment of Pompe disease, utilizing AAV1 vectors. Recently, an rAAV1 vector packaging a gene for the treatment of lipoprotein lipase deficiency was approved as the first gene therapy drug, validating the utility of this system. However, the effective production and purification of enough viral vector for use in research, preclinical studies, and human treatment is essential for success. Towards this goal, camelid single domain antibody fragments directed against AAV were used to make affinity resin products suitable for fast, one-step purification of AAV vectors of several serotypes. This affinity based purification method can replace current standards of density purification, which are time consuming and require large volumes of reagents, such as sucrose or iodixanol. At present, the collection of affinity resins suitable for AAV purification, i.e. AVB-Sepharose™, POROS™ CaptureSelect™ AAV8 and -AAV9, show varied affinity for different AAV serotypes, resulting in varied efficacies of virus purification. To better understand these differences and improve broad range utility, it is important to understand how these affinity ligands bind to the surface of the AAV capsids. We have thus mapped their binding footprints on several AAV serotypes, including AAV1, AAV5, and AAV9, using cryo-electron microscopy and image reconstruction combined with pseudo-atomic modeling. The sites for different affinity ligands are clustered on regions of the capsid that are common among most serotypes, but display minor variations which likely account for the varied affinities. This information will assist in the synergistic development of affinity ligands with broader serotype coverage and AAV vectors with improved purification outcomes.

295. Intrathecal Delivery of rAAV9-ABCD1 by Osmotic Pump in a Mouse Model of Adrenomyeloneuropathy

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Adrenomyeloneuropathy (AMN) is a debilitating neurological disorder caused by mutations in the ABCD1 gene, which encodes a peroxisomal ATP-binding cassette transporter (ABCD1) responsible for transport of CoA-activated very long-chain fatty acids (VLCFA) into the peroxisome for degradation. The Abcd1-/- mouse develops a phenotype similar to AMN, manifesting spinal cord axon degeneration as well as peripheral neuropathy due to affected dorsal root ganglion neurons (DRGs). We previously reported successful transduction of central nervous system cells in vitro and in vivo using recombinant adeno-associated virus serotype 9 (rAAV9) vector for delivery of the human ABCD1 gene. Unfortunately, intravenous delivery in young mice was associated with cardiac toxicity due to transgene overexpression. We therefore set out to optimize delivery to the spinal cord while minimizing systemic leakage using an intrathecal osmotic pump. Self complementary AAV9 GFP(scAAV9GFP) and rAAV9 encoding ABCD1 (rAAV9-ABCD1) were delivered to Abcd1-/- mice intrathecally (IT) either by bolus over a 2min duration or by osmotic pump over 24h duration with PBS injection as sham control. Two weeks after injection, mice were sacrificed and perfused with 4% PFA. Tissues were then collected, sectioned and stained for immunofluorescence analysis. scAAV9-GFP delivered IT by osmotic pump led to widespread expression across CNS-relevant cell types and DRGs in a dose-dependent manner. Spinal cord and DRG had higher expression compared with brain, but GFP expression was also detected in peripheral organs (liver, heart and adrenal gland), with highest expression seen at $3X10^{11}$GC. A similar distribution pattern of ABCD1 protein was detected after rAAV9-ABCD1 intrathecal pump delivery. In general, higher doses ($2X10^{11}$GC and $1X10^{11}$GC) led to more expression in CNS and peripheral organs compared with a lower dose ($0.5X10^{11}$GC). However, widespread expression of ABCD1 across CNS was even detected after direct intrathecal bolus injection of $0.5X10^{11}$GC. Importantly, the same dose delivered by pump led to higher expression in brain and spinal cord far from the injection