Enhancing AAV6 Vector Production for Cell Therapies: Harnessing the Potential of Viralgen's Pro10™ Platform for Scalable Manufacturing

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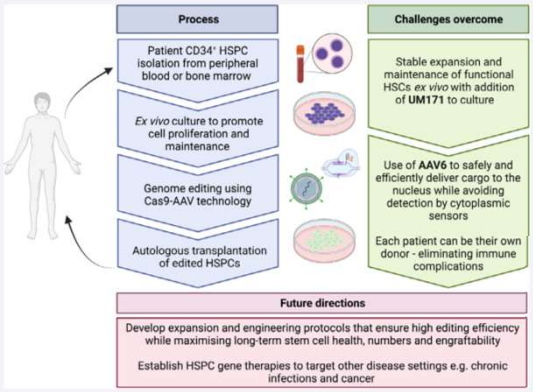
Abstract

The field of cell and gene therapy has shown remarkable promise in treating a wide range of diseases and disorders, ranging from genetic disorders to cancer. In order to fully harness the potential of cell and gene therapies, efficient and targeted delivery of therapeutic genes or factors into cells is essential. Typically, the delivery vehicle used in both cell and gene therapies is a viral vector. For cell therapies in particular, lentivirus and retrovirus have been the vectors of choice, and for gene therapies, Adeno-associated virus (AAV) vectors have emerged as the predominantly used tool to deliver genetic material. More recently, however, due to their ability to transduce a variety of cell types, AAV vectors can offer immense potential for advancing the field of cell therapies. More specifically, the serotype AAV6 presents a broad tropism, demonstrated high transduction efficiency in many cell types, enhanced muscle targeting, low pre-existing immunity, and a favorable safety profile, making it an advantageous choice for various cell therapy approaches. For the robust manufacturing of this serotype, Viralgen's Pro10™ production platform has proven reliability and scalability, making it suitable for meeting the demands of large-scale manufacturing for cell therapies. The Pro10™ platform includes a well-established process that promotes the consistent production of high-quality AAV6 vectors, enabling a robust supply to relevant cGMP scales. Viralgen's experience with AAV6 includes the successful manufacturing of 9 batches for toxicological studies and 15 cGMP (250/500 L cell culture) batches, in addition to over one hundred 2L batches, showcasing a commitment to excellence and continuous advancement in the field. Run data collected show consistency and robustness across different batches produced, despite variations in the Gene of Interest (GOI) within the AAV6 constructs tested, confirming the efficacy of using a manufacturing platform for reliable production of AAVs to advance cell therapies.

State of the Art: the Role of AAV6 in cell Therapies and our Proprietary Pro10™ AAV Platform

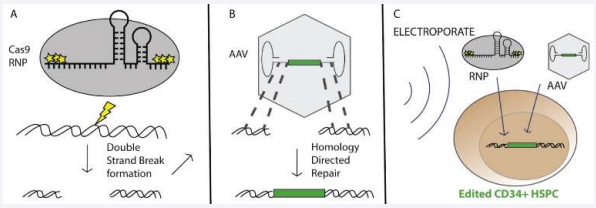
Gene editing system that combines CRISPR-Cas9 technology with the use of recombinant adeno-associated viruses (AAVs)[1]



- Recent progress in HSC gene editing and ex-vivo expansion have opened exciting new opportunities to cure a range of hematological diseases.
- Novel protocols and technology have allowed the field to overcome technical challenges and improve HSC gene editing and expansion efficiency.

Cas9-AAV=Cas9-adenoassociated virus / HSC= Hematopoietic stem cell / HSPC = hematopoietic stem and progenitor cell / UM171 = Novel and Potent Agonist of Human Hematopoietic Stem Cell Renewal

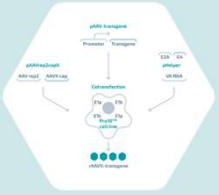
Overview of AAV-based hematopoietic stem and progenitor cell gene editing [2]



Cas9 protein complexed with guide RNA ribo-nucleoprotein complex (RNP) induces a double strand break at a specific locus **(A);** site flanking regions of homology within the recombinant AAV genome serve as templates for homology directed repair and incorporation of the corrective sequence (green) after DNA delivery by the AAV vector **(B)**. The genome editing components are delivered simultaneously ex-vivo (C).

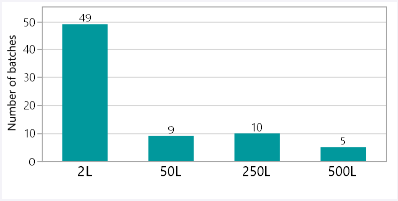
AAV Pro10™ Manufacturing Platform

- Viralgen uses a proprietary cell line the Pro10™, derived from HEK293 cells that is maintained and cultured in animal-derived component-free suspension-adapted cell culture.
- Pro10™ is a unique high yield universal system that can produce all serotypes and chimeric forms of rAAV.
- Viralgen's platform process consists of a plasmid-based triple transfection, from 2L to 2000L scale, that uses single-use stirred-tank bioreactors.



Results[3)]

AAV6 Manufacturing – Substancial Number of Batches

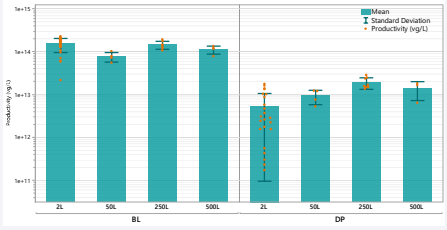


IND authorized AAV6 products

3

- Our AAV6 track record includes the successful manufacturing of 9 batches for toxicological studies and 15 cGMP (250/500 L cell culture) batches, in addition to almost 50 full trained 2L batches.
- At 2L additionally, more than 50 AAV6 batches have been manufactured but were not fully purified until DP.

High Productivity – Reliable AAV Titers – Scalable Platform

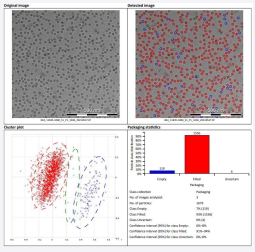


- Productivity in vg/L cell culture for Bulk Lysate (BL) and Drug Product (DP).
- Manufacturing scales: 2L (Non-GMP), 50L (Non-GMP), 250L (GMP) and 500L (GMP).
- Titer assay: ITRddPCR
- Sampling for QC withdrawn

- Our productivities from 2L, 50 L and 250L are in the range of 1e13VG/L for Drug Product
- Our Pro10™ production platform presents reliability and scalability, making it suitable for meeting the demands of large-scale manufacturing for cell therapies.

High Quality Product – Enriched full *vs* Empty Capsid Ratio

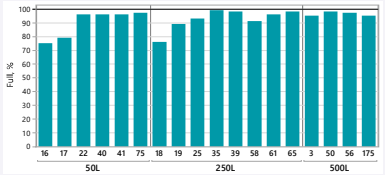
High % of full particles in AAV6 batches at Bulk Drug Substance (BDS) measured by CryoTEM across different scales



- Transmission Electron Microscopy (Cryo-TEM) original image vs detected image; AAV particles over layered subjected to internal density analysis.

  Red "Full": inner density with no distinct boundary between the shell and the core
  Blue "Empty": distinct outer shell and a minute internal density
  Green "Uncertain": discrepancy is found between the analyst assessment and the semi-automated classification

- Principal Component Analysis (PCA) of each AAV particle radial density profile: dashed rings 99% of confidence interval

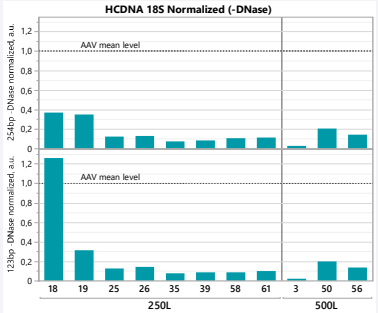


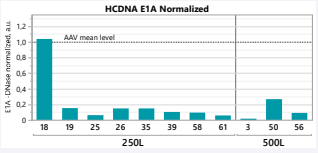
Batches out of the analysis: 50L: (21,163,53); 250L: (26,143) and 500L: (2)

- % Full > 70% and for several batches very close to 100%
- Consistent results scaling up from 50L to 250L and 500L

High Quality Process – Reduced Residual Host Cell DNA (HCDNA)

Low Levels of HCDNA 18S and E1A at GMP grade related to the average value of HCDNA in AAV





- Levels of HCDNA 18S and E1A for AAV6 are generally significantly lower than for AAV
- Robust clearance of HCDNA along the downstream process at 250L and 500L

Batches out of the analysis: 250L: (65,143) and 500L: (2,175)

References

1) Hematopoietic stem cell gene editing and expansion: State-of-the-art technologies and recent applications - Myriam L.R. Haltalli, Adam C. Wilkinson, Alejo Rodriguez-Fraticelli and Matthew Porteus

2) *Answered and Unanswered Questions in Early-Stage Viral Vector Transduction Biology and Innate Primary Cell Toxicity for Ex-Vivo Gene Editing* - Amanda Mary Dudek and Matthew Porteus

3) Data generated at Viralgen

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