Poster 1147

Manufacturability and product quality of novel AAV capsids optimized for specific targeting using capsid library selection in non-human primates

Hari Acharya, David Chu, Adam Cockrell, Jesse Granados, Carol Liu, Alex Mitchell, Heidy Morales, Carrie Pedeferri, Jenna Rodden, Troy Sandberg, Claire Simington, Emily Springfield, Brandon Wheeler, Jordyn Wheeler, Nick Goeden, Laura A. Adamson

Capsida Biotherapeutics inc., Thousand Oaks, California, USA

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Introduction

Use of recombinant adeno-associated viruses (rAAVs) as a delivery tool for gene therapy has significantly increased in the last 10 years. Despite broad application, challenges to the field include the ability to efficiently cross the blood–brain barrier, cell-specific targeting and productivity, and process recoveries sufficient to meet the clinical material demands.

Capsida Biotherapeutics has developed a library screening platform to improve rAAV targeting through the insertion of small peptide sequences into the capsid's surface. A library of insertions can be screened *in vivo* for organ-specific targeting and improved transduction.

Objectives

The objectives of this study include:

- Evaluating multiple capsid insertions during single variant production that demonstrate improved productivity in a pooled library
- Comparing productivity across multiple production platforms
- Demonstrating scalability of these novel capsids containing a therapeutic gene in Capsida's suspension-based manufacturing platform
- Determining product quality of therapeutic transgenes packaged in novel capsids

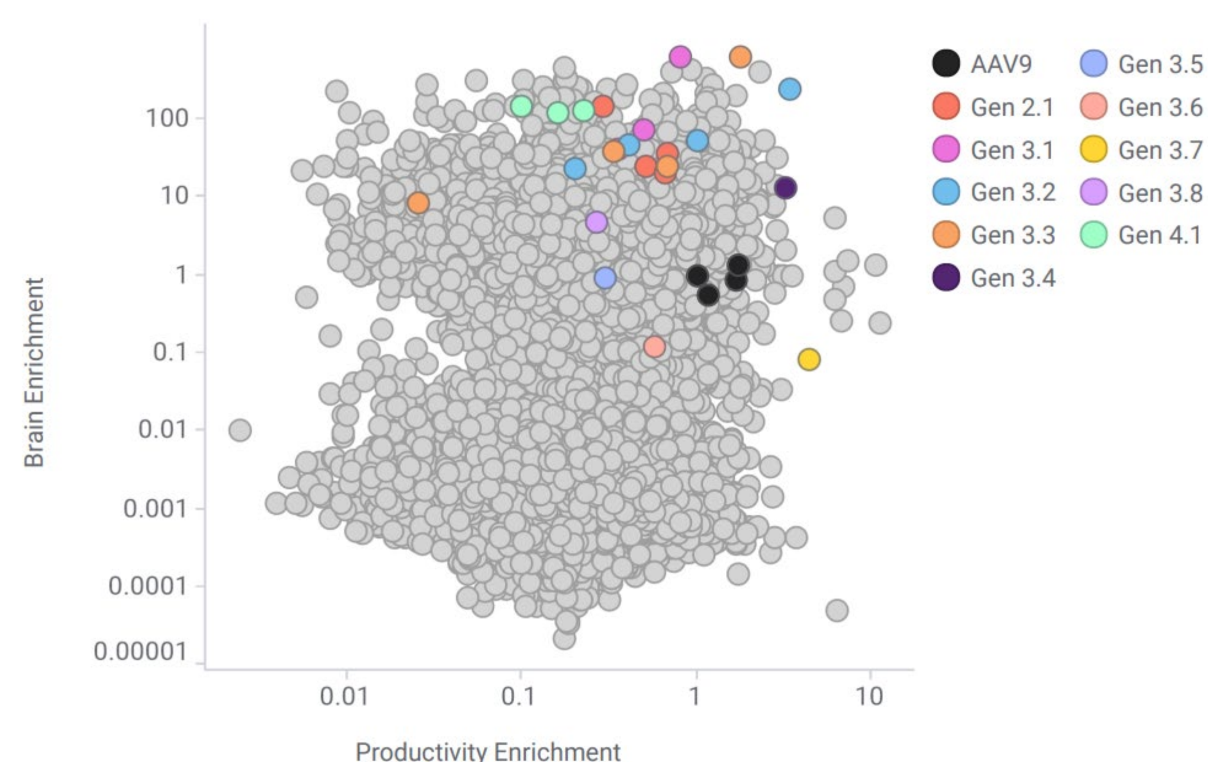
Methods

Capsid selection: For this study, capsid libraries are created by inserting random peptide sequences in the surface of rAAV9 capsids. Following intravenous administration of these libraries in non-human primates, capsid sequences were selected for both productivity and tissue enrichment by screening for high sequence prevalence both in the target tissue and injected virus pools.

Productivity screening: Multiple capsids demonstrating enhanced brain enrichment were selected from this platform and evaluated for productivity during single variant production by packaging a therapeutic construct in each selected capsid. All capsids tested had similar vector genome titers and ability to bind commercially available purification resins when compared with AAV9, demonstrating that variants with high prevalence in viral libraries maintain high productivity as a single variant.

Manufacturing scale-up: Production of one of Capsida's lead capsids and therapeutic construct was further optimized in a serum-free, chemically defined, transfection-based production platform and demonstrated consistent volumetric productivity up to 200 L. High product quality of purified novel variants was demonstrated in both development and scaled-up productions through evaluation of overall product purity, aggregation, infectivity and other process and product-related impurities.

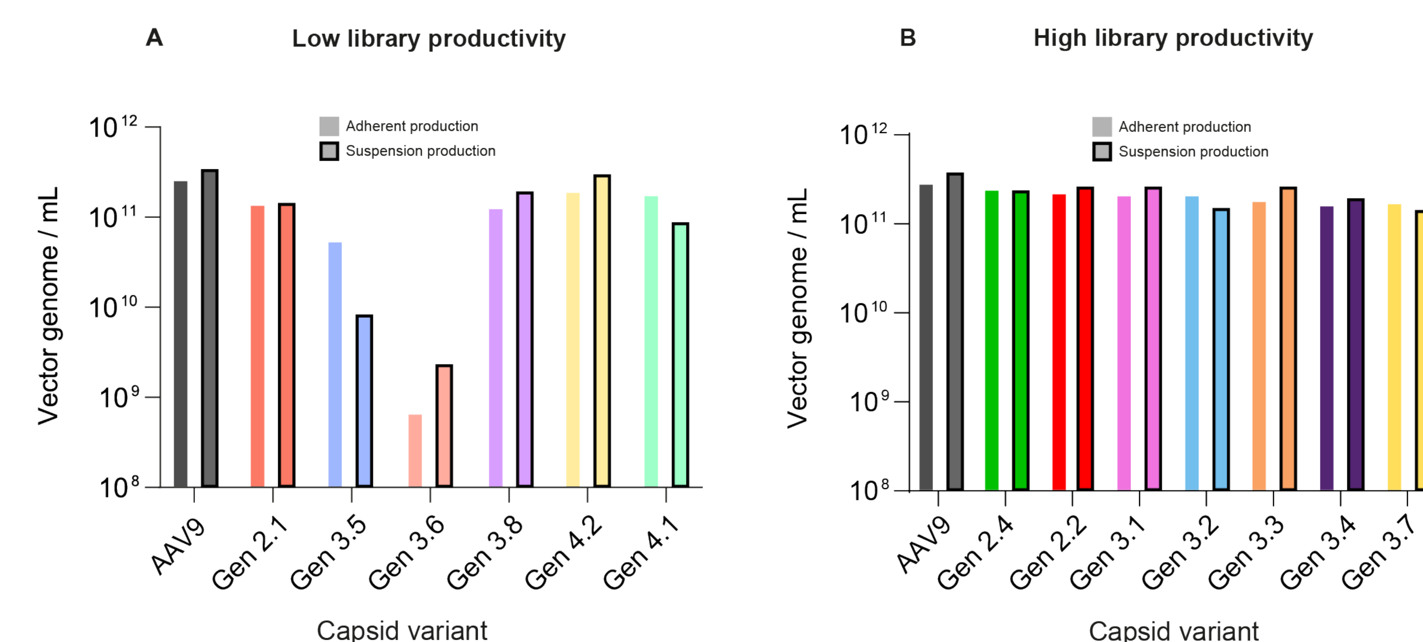
Figure 1. Next generation sequencing analysis of capsid variants in a pooled library compares to AAV9



Multiple vector designs show productivity within 50% of AAV9 spiked control in a pooled library

- Each generation (Gen 2, 3, or 4) denotes a different library design strategy of insertions into the AAV9 base serotype (Figure 1)
- AAV9 was spiked in each library at a fixed concentration to normalize productivity and tissue targeting
- Productivity of each vector variant was calculated by determining the amount of each variant in the plasmid DNA and in the purified virus pool in correlation with the AAV9 spiked control
- These values were compared for enrichment of each vector in the brain compared with AAV9 to determine vectors enriched for both productivity and targeting

Figure 2: Comparison of capsids selected from libraries in single-variant productions



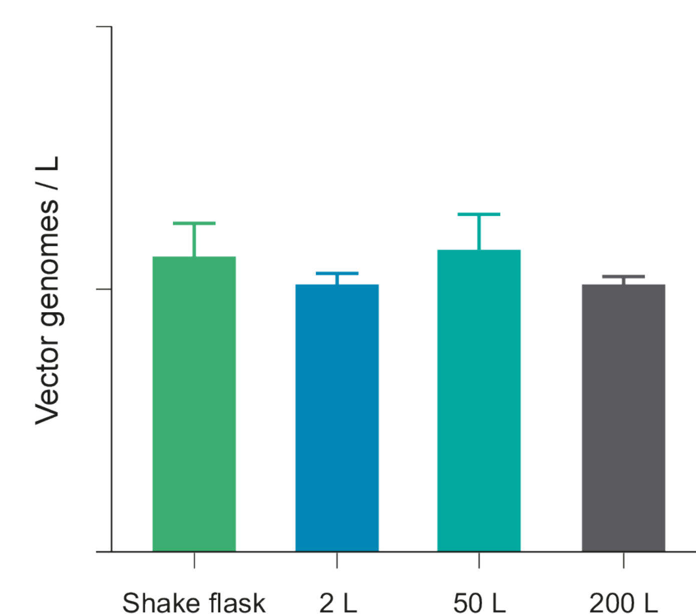
Capsid variants demonstrating higher productivity in a library maintain comparable productivity to AAV9 when produced as single variants

- 13 capsid variants from multiple libraries were produced in adherent and suspension cultures, packaged with a green fluorescent protein cargo and productivity was then quantified in crude lysates by droplet digital PCR (ddPCR)
- Variants were grouped based on their productivity in the library pool; 'low library productivity' if the value in the library was less than 50% of the AAV9 titer or 'high library productivity' if the value in the library was within 50% of the AAV9 titer
- Most variants grouped as 'low library productivity' showed a 1.5–5x reduction in titer compared with AAV9, except for the Gen 3.6 capsid, which had a titer approximately 2 logs lower than AAV9 (Figure 2A)
- All variants in the 'high library productivity' group had titers within 50% of AAV9 when packaging the same cargo in a single-variant production (Figure 2B)

Productivity of novel variant CAP-001 shows linear scalability up to 200 L

- Capsida's suspension-based transfection production process is in HEK293 cells, in a serum-free, chemically defined media
- Transfection conditions were optimized for the production of CAP-001 by evaluating polyethylenimine (PEI) to DNA and plasmid ratios, transfection reagents, cell density and media
- Optimized parameters were confirmed in the shake flask and 2 L scale, demonstrating similar productivity
- This process was scaled to the 50 L and 200 L bioreactor and demonstrated consistent linear productivity throughout scale-up (Figure 3)

Figure 3. Linear scalability of CAP-001 up to 200 L



Multiple novel variants demonstrate similar recovery across commercially available purification resins

- A primary affinity capture purification step was developed for AAV9 and tested with multiple novel capsid variants
- Crude lysates from the suspension production process were purified using the affinity chromatography protocol developed for AAV9, and recovery of each capsid was measured by ddPCR
- Each capsid showed similar recovery to AAV9 using this platform process (Figure 4). These data demonstrate that the insertion of novel peptides using Capsida's current strategy does not disrupt binding to commercially available purification resins

Figure 4: Affinity chromatography recovery of three novel variants compared with AAV9

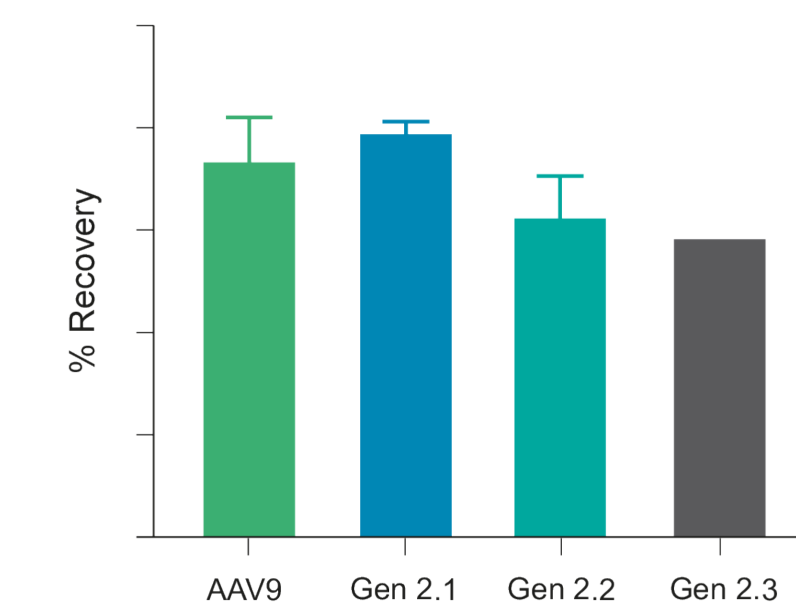
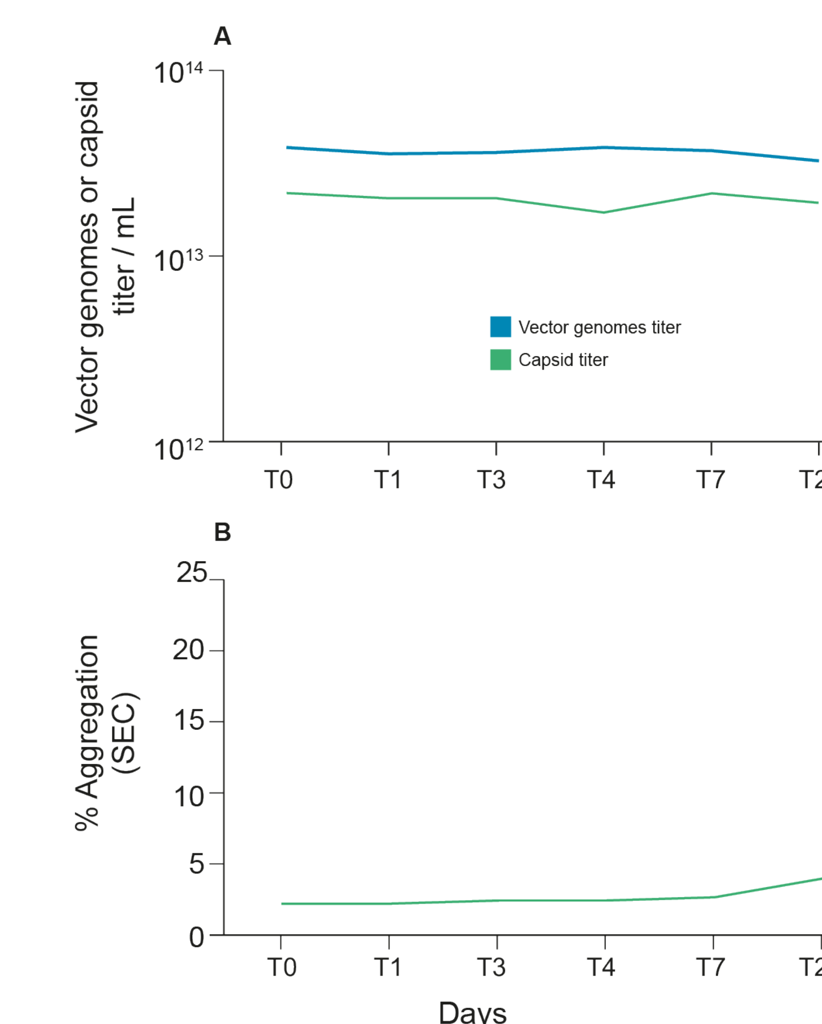


Figure 5: Minimal product loss (A) or aggregation (B) observed for vector incubated for 28 days



Novel capsid variants demonstrate a high level of stability under accelerated stress conditions

- Formulation buffer for CAP-001 was optimized using freeze-thaw, +4°C and –80°C long-term storage and 37°C accelerated stress testing to determine the optimal conditions for long-term storage and maintenance of critical product quality attributes
- CAP-001 in optimized formulation buffer was stored at 37°C for up to 28 days and maintained consistent vector genome and capsid titer (Figure 5A) and minimal aggregation (Figure 5B)
- Additional data has demonstrated minimal change to genome or capsid titer, purity and aggregation following multiple freeze-thaw cycles (data not shown)

High product quality is observed in CAP-001 produced at 200 L scale

- Analytical testing of CAP-001 vector has further demonstrated:
  - >95% purity by capillary electrophoresis (CE-SDS)
  - <5% aggregation by size exclusion chromatography (SEC)
  - <5% empty capsids by analytical ultracentrifugation
  - Consistent infectivity from 50 L to 200 L scale by median tissue culture infectious dose (TCID50)

Conclusions

Capsid variants selected from Capsida's library screening platform, not only show improved brain enrichment but are also capable of generating high titer and high-quality products in a suspension-based scalable production platform.

Acknowledgements

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