Criticality of Manufacturability Assessment in Supporting Discovery of Novel AAV3B and AAV8 Variants

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Background and Objectives

At REGENXBIO we have been undertaking the effort to engineer adeno-associated virus (AAV) capsids for improved transduction efficiency and minimized off-target effects for certain routes of administration. To achieve this goal, we developed a directed evolution platform NAVIGATE (Novel AAV Vector Intelligent Guided Adaptation Through Evolution) for new capsid discovery, and this is accomplished by generating diverse libraries with randomized 7-mer peptide insertions in the AAV VR-IV and VR-VIII surface loop regions. For one of the projects, we applied NAVIGATE to AAV8 and AAV3B capsid optimization in the context of suprachoroidal space (SCS) delivery to ocular tissues (ASGCT abstract #s 312 & 501). During library screening phase, we used various high-throughput techniques for material generation, such as transfecting with enhanced plasmid ratio or purifying pooled AAV variants using ultracentrifugation technology, which resulted in production of ~300 runs and >1,000,000 peptide variants. The AAV library material produced went through multiple rounds of selection and the biological activity data generated showed differences in folds of improvement for these novel capsids over the AAV8 and AAV3B parental controls. Because manufacturability assessment is crucial in helping to identify the suitability of a therapeutic molecule, to evaluate its fitness to the NAVXpress™ platform process, and to reduce the process development effort due to unexpected results, we incorporated manufacturability assessment after narrowing down to a manageable number of variant candidates.

A panel of variants was selected for production utilizing the NAVXcell®, a proprietary cell line, at 250mL scale for preliminary manufacturability assessment. For this study, we used an in-house developed high throughput chromatography technology that can simultaneously purify 12 runs, at both the capturing and polishing chromatography steps. We were able to quickly observe the process differences between these novel SCS capsids. Then the combination of preliminary bioprocessing information and animal data readouts allowed us to further narrow down these AAV capsid variants to be produced at 10L scale for a more complete manufacturability assessment. More detailed process information was gained to understand how these variants are produced differently. The comprehensive manufacturability information complemented biological activity data in guiding us to select the top capsid candidate that could potentially improve transduction efficiency with minimal off-target effects.

Methods

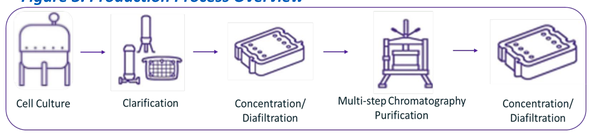
Screening process:

Ultracentrifugation (UC) method (Fig. 1) was used for the first three rounds of screening to generate material (Fig. 4a, b, c). The biological data output from the third round of screening produced some top hits. These top hits along with a few backups were tested for bioprocessing suitability in round 4 (Fig. 4d) via high throughput chromatography screening using the instrument shown in Figure 2. Thus, helping to narrow the capsid pool one more time to the top few for round 5 screening (Fig. 4e) which was produced at 10L production scale for a final large scale production manufacturability assessment (Fig. 3). The lead AAV capsid variant was selected afterwards.

Figure 1. Ultracentrifugation System and Method

Figure 2. High-throughput Purification Instrument

Figure 3. Production Process Overview



Production process:

The 10L production scale was carried out in bioreactors utilizing a triple transfection process. After transfection, cells were lysed, concentrated, and then purified. (Figure 3). The small-scale production was carried out in shake flasks and did not include the concentration/diafiltration steps.

AAV product quality was tested to verify how well the process worked. The analytical assays used to test the AAV vectors are listed in Table 1.

Table 1. Summary of Analytical Assays Used During Manufacturability Assessment

Method	Usage
Digital droplet PCR (ddPCR)	Titer measurement
Optical density	Using UV-Vis spectrophotometer and internally developed formula to measure and calculate percentage (%) of empty and full capsids for high-throughput samples
Bioanalyzer	Measurement for viral protein (VP) ratio of VP1, VP2, and VP3
Differential scanning fluorimetry (DSF)	Measures melting temperature of the vectors for thermal stability testing
Dynamic light scattering (DLS)	When implementing temperature ramp, can be used as an orthogonal way to assess sample thermal stability
TapeStation	Assesses any impacts on genome integrity with the modified capsids or between different serotypes
Visual inspection	Precipitation during downstream process or sample storage stability

Figure 4. Capsid Screening Process Overview

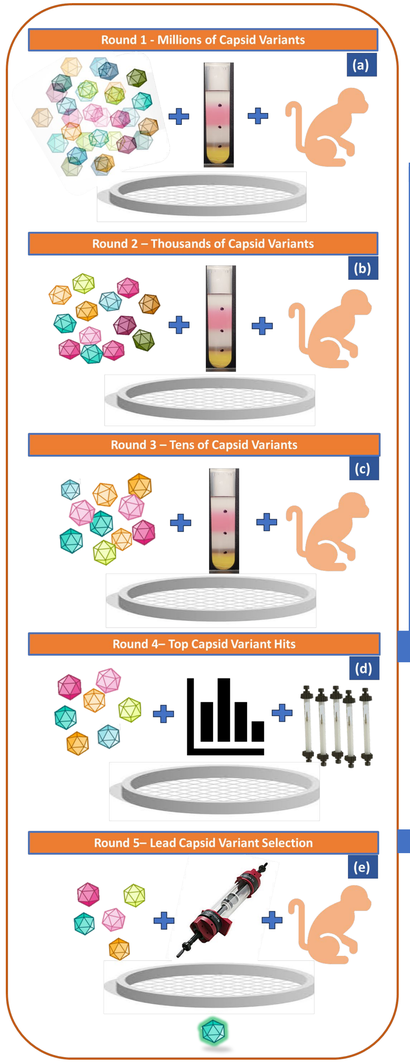
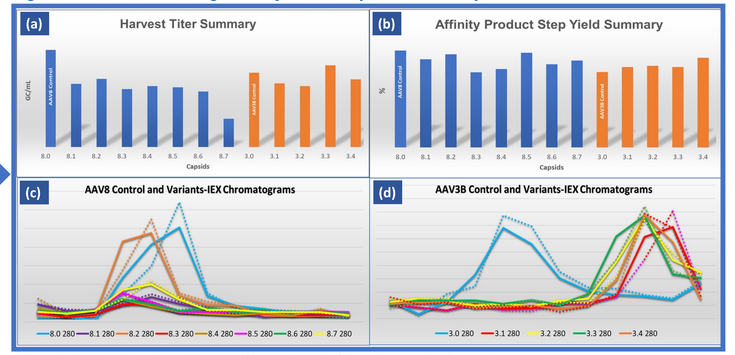


Figure 5. Round 4 Screening - Manufacturability Data Summary



8.0 and 3.0 are AAV8 and AAV3B parental controls, and all the other number designations are AAV variants

Figure 6. Round 5 Screening - Manufacturability Data Summary

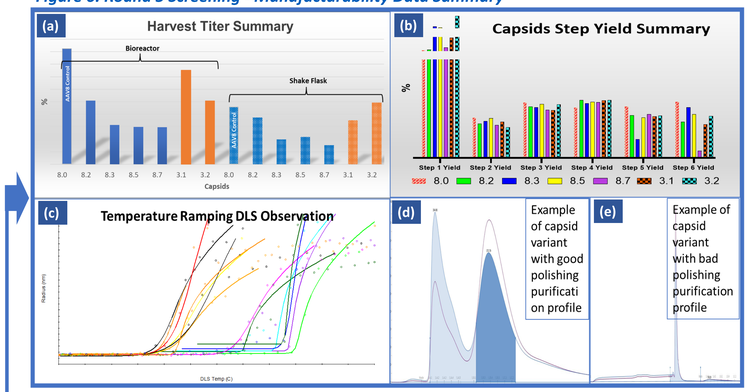


Table 2. Manufacturability Evaluation Matrix

ID	Harvest Titer	%Full	Genome Integrity	VP Ratio	Thermal Stability	Downstream Dev. Effort	Formulation Dev. Effort
8.0							
8.2							
8.3							
8.5							
8.7							
3.1							
3.2							

Relative comparison between the AAV capsid variants and AAV8 parental control. Colors represent ranking between the capsids for a particular assessment parameter (each column).

Best ***Worst***

Results

The small-scale manufacturability assessment for round 4 screening of the top AAV capsid variants showed:

- *All the harvest titers were acceptable, although there is ~3-fold difference between the lowest and highest harvest titers for AAV8 variants (Fig. 5a).*
- *Although affinity product yield showed variation, all top capsid candidates were able to be captured using platform affinity resin (Fig. 5b).*
- *The ion exchange chromatography (IEX) step showed differences in binding strength and elution patterns for certain AAV8 and AAV3B variants. All AAV8 variants eluted earlier and all AAV3B variants eluted later when compared to AAV8 and AAV3B parental controls.*

For the small pool of variants produced at 10L production scale for round 5 screening, some initial process development was implemented as needed to demonstrate that all the variants can be manufactured. The observations are:

- *Satellite shake flask material was produced alongside the 10L material, and the titers showed similar trends for both scales. AAV3B variants have higher harvest titer than AAV8 variants. All the variants are at least 2-fold lower in titer than the AAV8 control capsid (Fig. 6a).*
- *All the variants exhibited comparable clarification and tangential flow filtration (TFF) behavior.*
- *Step yields for all the assessed variants within each downstream step are comparable to each other and to the AAV8 parental control (Fig. 6b).*
  - *Two AAV8 variants showed less yields for two different steps, which will require further development.*
- *Temperature ramping on DLS separated the assessed AAV variants into two temperature groups (Figure 6c). However, DSF demonstrated comparable melting temperature for all the capsids tested.*
- *All the AAV3B variants have good fitness to the internal platform IEX polishing process. However, the IEX process fitness for AAV8 variant showed variation (Fig 6d, e). Some initial development was able to improve separation for empty and full capsid peaks.*

All BDS material had acceptable product qualities based on data in viral protein (VP) ratio, genome integrity, DSF thermal stability, and % of full capsids present in solution. Development work required for upstream titer, downstream process, and formulation vary in degree of efforts (Table 2).

Conclusions

To increase the value of an ocular route of administration of gene therapy for patients, REGENXBIO designed a directed evolution platform, NAVIGATE, to discover potential AAV variants that have improved transduction efficiency and minimized off-target effects. Although biological activity data is critical in identifying the top variants, it is as important to evaluate if they are manufacturable and their fitness to the NAVXpress™ platform to reduce the process development effort and drug development risks.

We incorporated a manufacturability assessment at an earlier time after biological activity data narrowed down the AAV variants to a manageable number to be processed on our high throughput bioprocessing platform. Throughout the manufacturability assessment, we discovered that all the AAV3B variants performed similarly to the parental control, while the AAV8 variants had mixed manufacturing performances when compared to their parental control.

In summary, via our high throughput toolbox we ensured optimal production efficiency to provide material for all the animal studies, while discovering the potential process and product differences to better understand manufacturability for these variants. This has aided in selecting a novel capsid that has an enhanced biological activity profile and that possesses the desirable manufacturability attributes. Thus, saving time, cost, effort by reducing drug development risks.