Titer Boosting of HEK293-based AAV Manufacturing Process using Proprietary Small Molecule Booster (SMB) and Successful Scale up to 200L

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Abstract

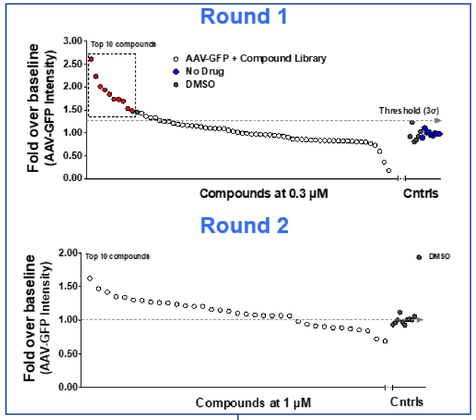
Adeno-associated virus (AAV) is quickly becoming a safe and effective therapeutic modality for the delivery of potentially curative treatments for genetic diseases. One of the major challenges associated with AAV Gene Therapy (GT) is to cost effectively produce AAV Drug Product (DP) with adequate levels of critical quality criteria (safety, identity, strength, purity, potency, and quality). Triple transient transfection using HEK293 process is widely used in both clinical and commercial scale manufacturing. The main limitation of HEK293 is scalability and viral productivity. Using high-content screening leveraging our deep knowledge in the areas of cell biology, metabolic, anti-fibrotic, human genetics, tubulin and histone regulation, and extensive chemistry experience, Tenaya has developed a class of proprietary small molecule boosters (SMB) that can significant increase AAV yield in cell line and cell culture media-independent manner. Additionally, SMB can enhance the scalability of HEK293-process by maintaining consistent yield up to 200L. And finally, SMB has demonstrated to have no impact on purity, quality, safety, and potency of the AAV viral vector and can be readily cleared in standard AAV purification process. This novel and selective class of SMB can potentially be instrumental in debottlenecking AAV manufacturing and decrease of cost of AAV gene therapy.

Tenaya's Manufacturing Capabilities

In-House Screening and Production of SM

Tenaya's proprietary small molecule booster (SMB) was made in-house and screened against other SMBs and its derivatives. Results were compared with AAV-GFP intensity resulting from each culture with SMB.

A.



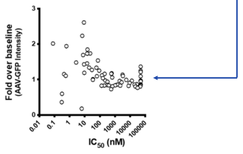
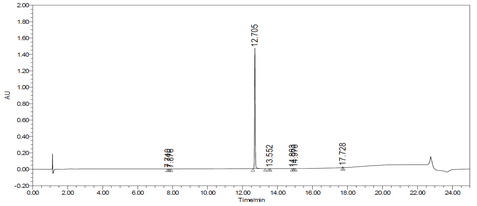


Figure 1. (A) AAV-GFP Intensity Screening and (B) Mass Spectrum of Tenaya's SMB

B. Sample of LC/MS Spectrum of Tenaya SMB compound at >99% purity



SMB Improves Scale-Up Productivity

In order to improve the scalability of HEK293 process, Tenaya screened potential small molecule boosters (SMB) in shake flasks. Tenaya has developed a proprietary SMB to improve yield. SMB. SMB addition process and titer improvements improve productivity at 3L, 50L, and 200L BRX based on power per unit volume (PV)

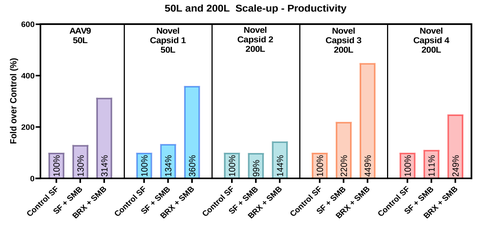
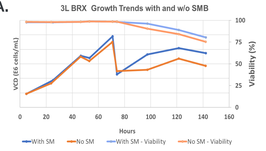


Figure 3: Scale-up to 50L and 200L

Successful SMB scaling was achieved at 50L and 200L bioreactor (BRX) scale. Vg titer boosting is shown in AAV9 along with various other novel capsids at BRX scale

A.



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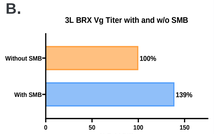

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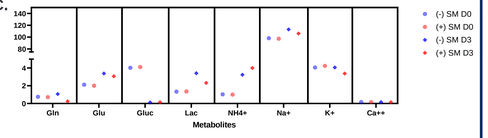


Figure 4. 3L BRX Results with and without SMB

A. Viable cell density and % viable cell density was comparable with and without SMB

B. Vg titer with SMB increased by 39% compared to without SMB in 3L BRX

C. Metabolites were comparable with and without SMB in the shake flask satellites.

No Impact on Product Quality

A.

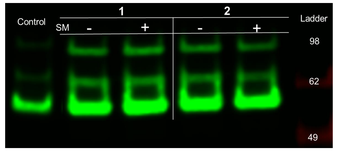
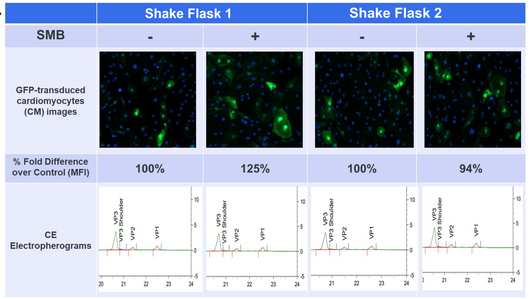


Figure 5. Western blot (A), images of cardiomyocyte transduced with AAV:GFP, and capillary electrophoresis electropherograms (B) to verify the small molecule effects on AAV quality.

B.

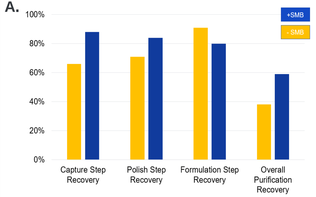


A. Western blot of affinity purified AAVs from small scale shake flask productions (2 replicates – 1 with SMB and 1 without). Consistent ratios of VP1/VP2/VP3 with and without small molecule addition.

B. Based on cardiomyocyte transduction with AAV9:GFP, there is no significant impact of SMB addition on AAV infectivity. Additionally, from the CE electropherograms, VP1/VP2/VP3 ratios are found to be similar with SM added samples vs. no SM.

No Impact on Purification Performance

A.



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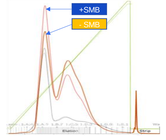
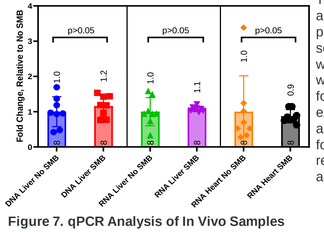


Figure 6. (A) Purification Recovery comparison and (B) Chromatograph of polishing step with and without SMB

SMB Addition - No Impact on *In Vivo* Transduction



The *in vivo* study consisted of two arms, the first injected with AAV produced without SMB and the second injected with AAV produced with SMB. Mice were euthanized 3 weeks post injection and harvested for heart and liver tissue, which were extracted for RNA and DNA and analyzed using qPCR to determine fold change in Gene of Interest (GOI) relative to a process without SMB addition.

Figure 7. qPCR Analysis of In Vivo Samples

No significant differences in seen in mouse study for product made with or without SMB

SMB Improves Lentiviral Titer

Tenaya's SMB has been shown to increase viral titer without impacting quality of product produced. Tenaya's SMB may be effective in increasing viral production of other vector types such as lentivirus.

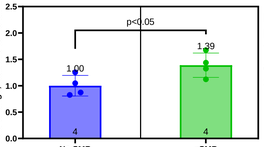


Figure 8. Lentiviral Titer with and without SMB

Lentiviral production with addition of SMB increased titer by >39% over a process without SMB addition

SMB Demonstrates Unique and Novel Mechanism of Action

Tenaya's SMB provides unique and novel mechanisms that affect many different pathways during the production of AAV in HEK293.

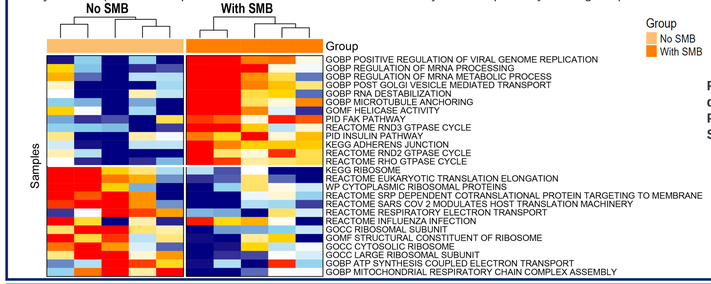


Figure 9. Heatmap of enriched and depleted pathways in SMB Production Culture relative to no SMB from RNAseq analysis

Conclusion and Next Steps

- Tenaya's proprietary SMB consistently shows a substantial increase in vg productivity
- SMB can plug-and-play into any existing HEK-based process
- SMB titer improvements are media, cell-line and scale independent
- SMB has no observable impact on product quality and purity and transduction efficiency (*in vivo* and *in vitro*)

Future SMB Development:

- Demonstration of SMB viral titer improvement with other mammalian cell-produced viral vectors
  - Preliminary results show improvements on lentivirus titer with SMB
- Scaling up to 1kL

- Bioreactor (**BRX**)
- Power per unit volume (**PV**)
- Small molecule booster (**SMB**)
- Small molecule (**SM**)
- Gene of interest(**GOI**)
- Adeno-associated virus (**AAV**)
- Drug product (**DP**)
- Gene Therapy (**GT**)