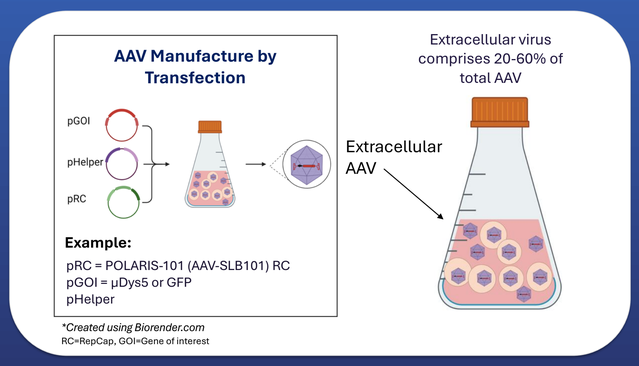
Boosting rAAV Yield and Quality in HEK293 Cells Through Multi-Mechanism Strategies

Xiaofei E[1], Jishwa Shah[1], Neil Templeton[1], Patrick Starremans[1], Ben Wright[1], Paul Herzich[1], Glen B. Banks[1]

[1]*Solid Biosciences Inc., Charlestown, MA, USA*

INTRODUCTION

Recombinant adeno-associated virus (rAAV) vectors are widely used for gene therapy; however, large-scale manufacturing remains constrained by biological processes that limit productivity. Producer-cell transduction by extracellular rAAV, activation of innate immune pathways, and cell-cycle dysregulation can reduce vector yield and consistency by diverting cellular resources away from vector assembly and packaging. This study evaluated whether targeted modulation of these pathways could improve rAAV manufacturing performance and product quality.



METHODS

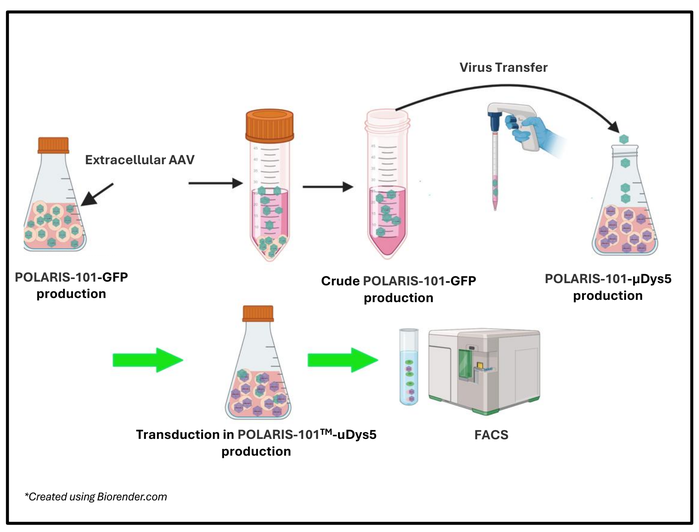
HEK293-based rAAV production cultures were treated with small-molecule modulators targeting three biological pathways associated with manufacturing limitations:

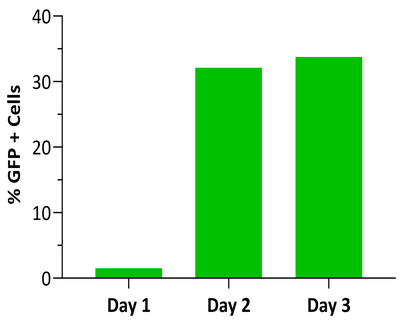
- **Endocytosis inhibition:** Brefeldin A was added 21 hours post-transfection to reduce re-entry and transduction of producer cells by extracellular rAAV.
- **Cell-cycle modulation:** RevIT™ (Mirus) was added at transfection to promote G2/M cell-cycle arrest.
- **Innate immune pathway modulation:** BX795 was added 4 hours prior to transfection to inhibit interferon signaling and reduce antiviral responses.

Individual treatments were evaluated alone and in combination. Vector productivity was assessed by measuring rAAV yield and product quality attributes, including full capsid percentage and residual host-cell DNA. Lead conditions were subsequently evaluated at the 400-mL scale to assess scalability.

RESULTS

Figure 1: Extracellular AAV can transduce HEK293 cells during production



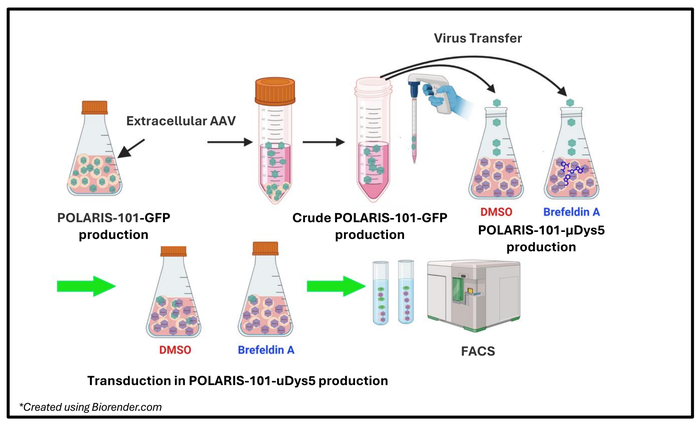


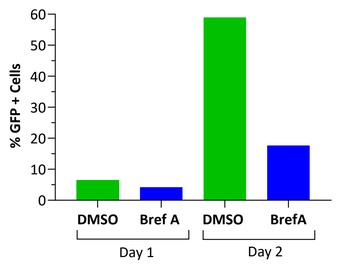
- **Figure 1:** Transferred POLARIS-101-GFP virus transduces HEK293 cells actively producing POLARIS-101-µDys5

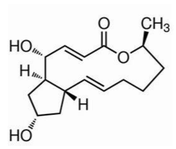
ACKNOWLEDGMENTS
These studies were sponsored by Solid Biosciences Inc. (Charlestown, MA, USA).

RESULTS

Figure 2: Brefeldin A Reduced POLARIS-101-GFP Transduction of HEK293 cells Actively Producing AAV

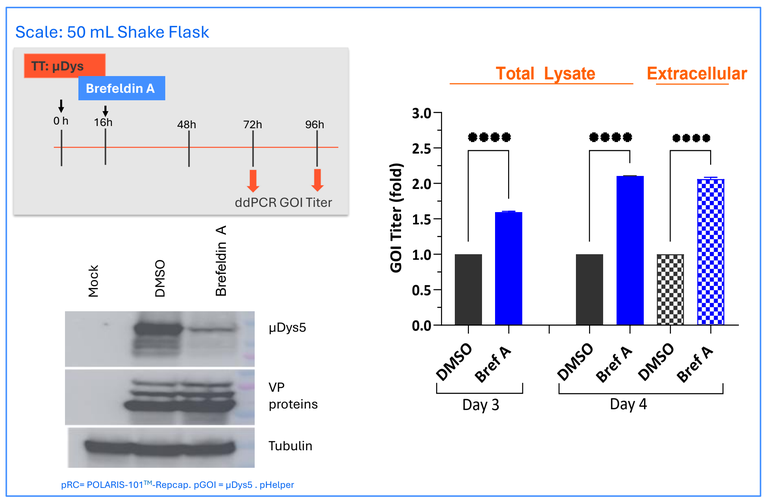






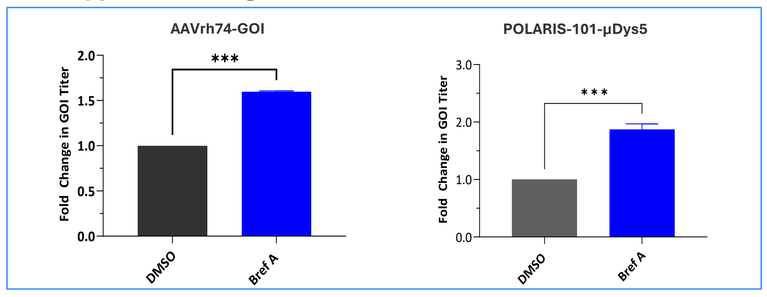
- **Figure 2:** Brefeldin A (Bref A) significantly reduced POLARIS-101TM-GFP transduction of HEK293 cells during AAV production.

Figure 3: Brefeldin A Reduced POLARIS-101 Transduction of HEK293 cells Actively Producing AAV and Increased AAV Yield



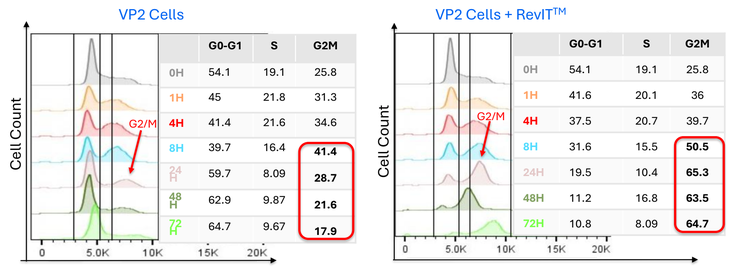
- **Figure 3:** Brefeldin A (Bref A) significantly reduced POLARIS-101TM transduction of HEK293 cells during AAV production.

Figure 4: Brefeldin A Increased AAV Yield Across Capsid Serotypes & Transgenes



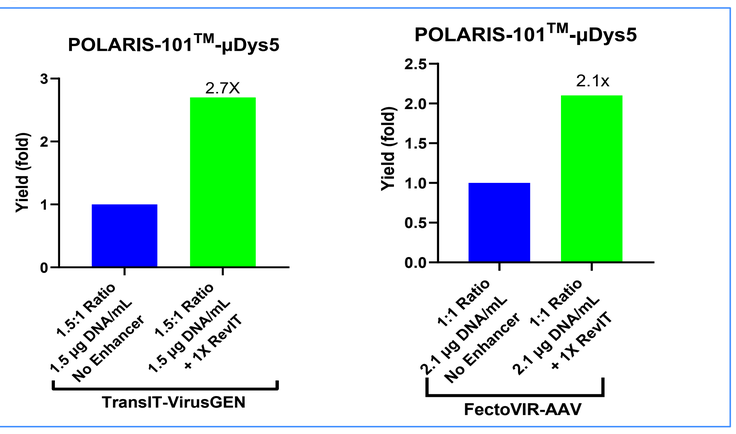
- **Figure 4:** Addition of Brefeldin A increased AAV yield for rh74 and AAV9-based POLARIS-101

Figure 5: RevIT™ Arrests the Cell Cycle in G2/M phase



- **Figure 5:** More cells are in the G2/M phase after 8, 24, 48, and 72 hours with RevIT™. This suggests that RevIT™ functions by arresting the cell cycle at the G2/M phase.

Figure 6: Impact of RevIT™ on AAV Production



- **Figure 6:** Representative experiment, RevIT™ (Mirus) can increase AAV yield 2-3 fold in 30ml shake flasks.

Figure 7: RevIT™ and Brefeldin A together is better than individual RevIT™ or Brefeldin A

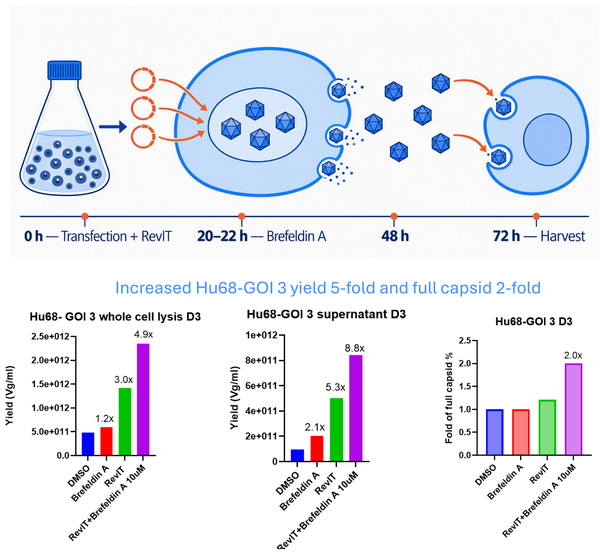


Figure 8: BX795+RevIT™ increase AAV yield 3.2 fold

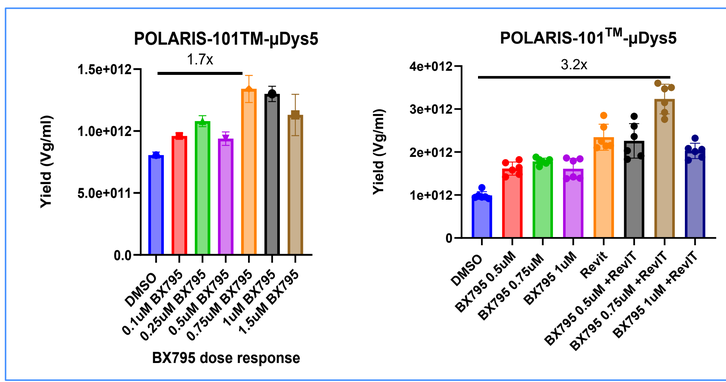
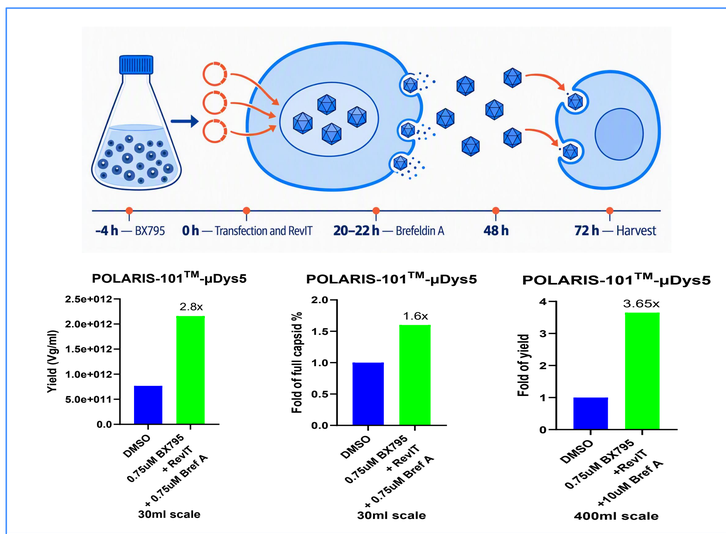


Figure 9: BX795, RevIT™ and Brefeldin A together increase POLARIS-101-µDys5 yield 3-fold and full capsid 1.6-fold



CONCLUSIONS

- AAV excreted into the supernatant from HEK293 cells can re-transduce cells within the same culture.
- Transduction also reduces rAAV yield.
- Small molecule inhibitors of endocytosis (Brefeldin A) reduce this transduction and increase AAV yield 1.5~2 fold.
- RevIT™ can arrest cell cycle in G2/M phase and increase AAV yield ~2 fold
- BX795, an interferon signaling pathway (IFN) inhibitor can increase AAV yield 1.7 fold.
- Combination of RevIT™ with Bx795 can increase AAV yield 3.2 fold.
- Combination of RevIT™ with Brefeldin A can increase AAV yield 3~5 fold and AAV full capsid 2 fold in multiple capsids and GOI.
- BX795, RevIT™ and Brefeldin A together increase POLARIS-101TM-µDys5 yield 3-fold and full-capsid 1.6-fold.

Presented at The 33rd Annual European Society of Gene & Cell Therapy, October 27-30, 2026, Hamburg, DE