Small Molecule Induced G2/M Arrest Boosts Recombinant AAV Production and Preserves High Quality

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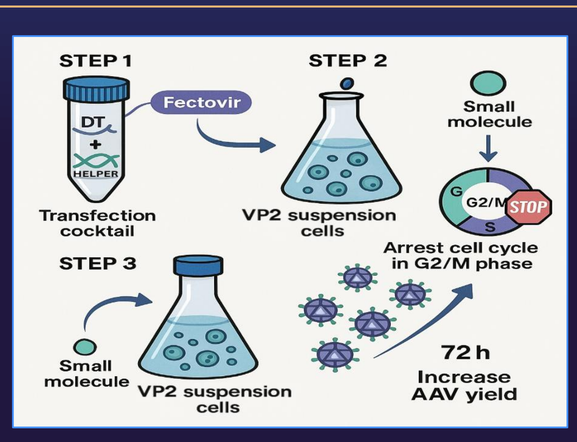
INTRODUCTION

Recombinant adeno-associated virus (rAAV) vectors are widely used for gene therapy, but manufacturing scalability is limited by low production yields. Understanding cellular mechanisms that drive efficient vector production can inform strategies to enhance yield without compromising product quality. Thermo Fisher Expi293F™ and Viral Production 2.0 (VPC 2.0) cells differ markedly in rAAV productivity, with VPC 2.0 achieving approximately four-fold higher yields. This study aimed to elucidate transcriptomic differences between these cell lines and to evaluate small molecule interventions that could modulate relevant pathways to improve rAAV production.

RNA-Seq analysis was performed on Expi293F™ and VPC 2.0 cells across multiple post transfection time points to identify differentially regulated pathways associated with rAAV production. Flow cytometry was used to assess cell cycle distribution and confirm RNA-Seq findings. Guided by pathway enrichment analyses, small molecule inhibitors targeting G2/M cell cycle progression were evaluated, including docetaxel and alisertib. Their effects on crude genome titers, capsid quality attributes, and cellular phenotypes were measured. rAAV vectors produced in the presence of docetaxel were further evaluated in vivo for biodistribution and toxicity following systemic delivery in mice.

PICTURE ABSTRACT



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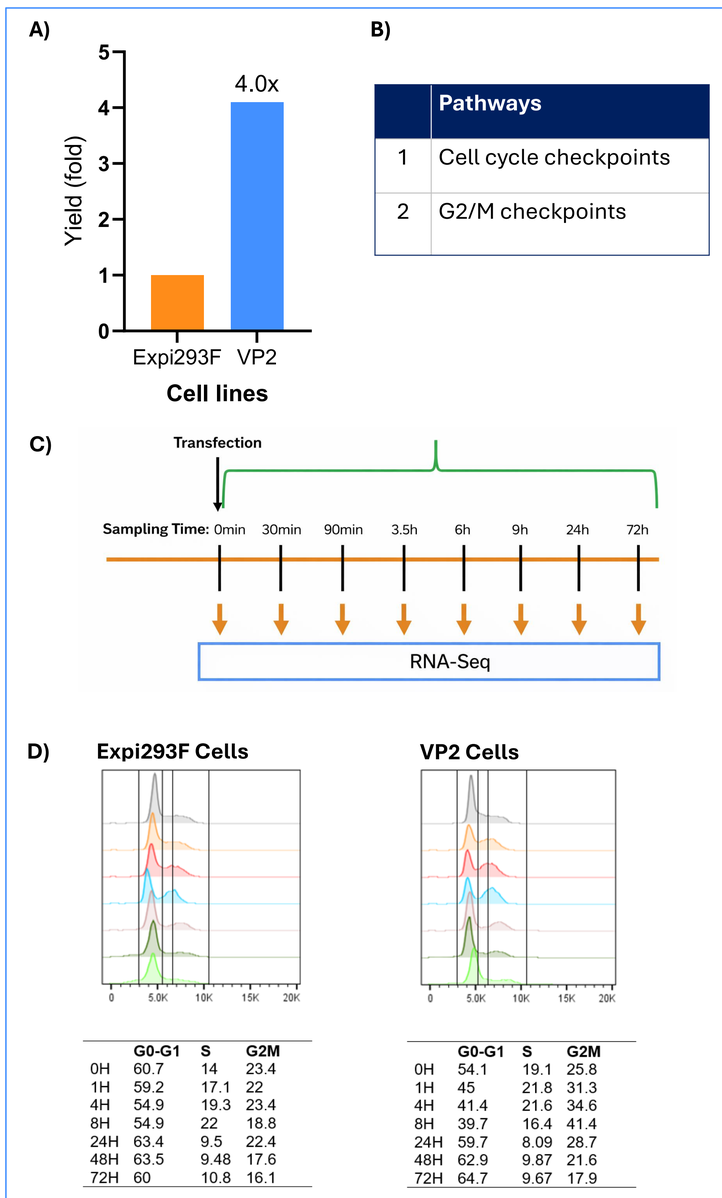
For POLARIS-101™ (AAV-SLB101) vector; Dual Rep/cap-GOI plasmids

RESULTS

Figure1: Enhanced rAAV Production in VP2 Cells Is Associated with G2/M Cell Cycle Regulation



	Pathways
1	Cell cycle checkpoints
2	G2/M checkpoints

	G0-G1	S	G2M
0H	60.7	14	23.4
1H	59.2	17.1	22
4H	54.9	19.3	23.4
8H	54.9	22	18.8
24H	63.4	9.5	22.4
48H	63.5	9.48	17.6
72H	60	10.8	16.1

	G0-G1	S	G2M
0H	54.1	19.1	25.8
1H	45	21.8	31.3
4H	41.4	21.6	34.6
8H	39.7	16.4	41.4
24H	59.7	8.09	28.7
48H	62.9	9.87	21.6
72H	64.7	9.67	17.9

RESULTS

Figure 2: Cellular Characterization after Docetaxel Treatment at Day 3 Post-Transfection.

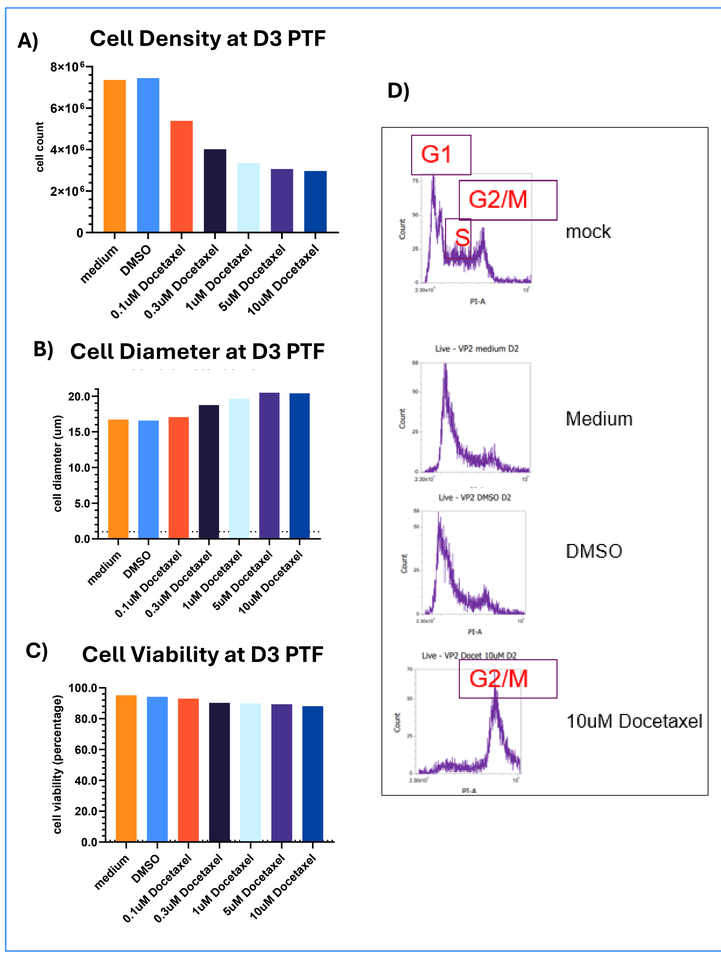


Figure 3: Dose-Dependent Effect of Docetaxel on Viral Titer

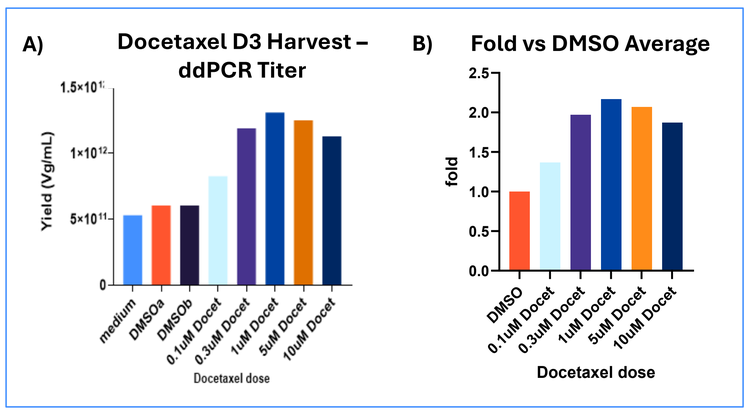


Figure 4: Evaluation of Alisertib on AAV Titer at Different Shake Flask Scales.

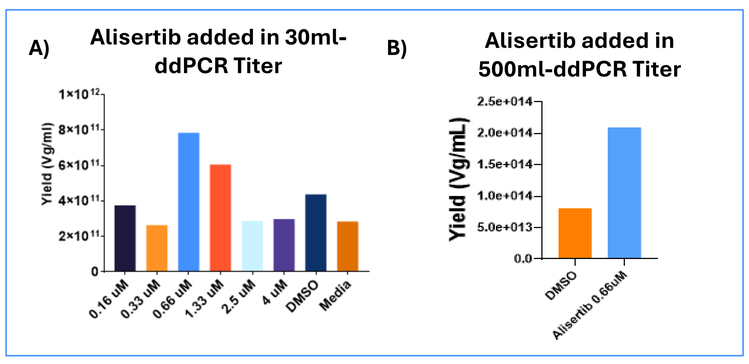
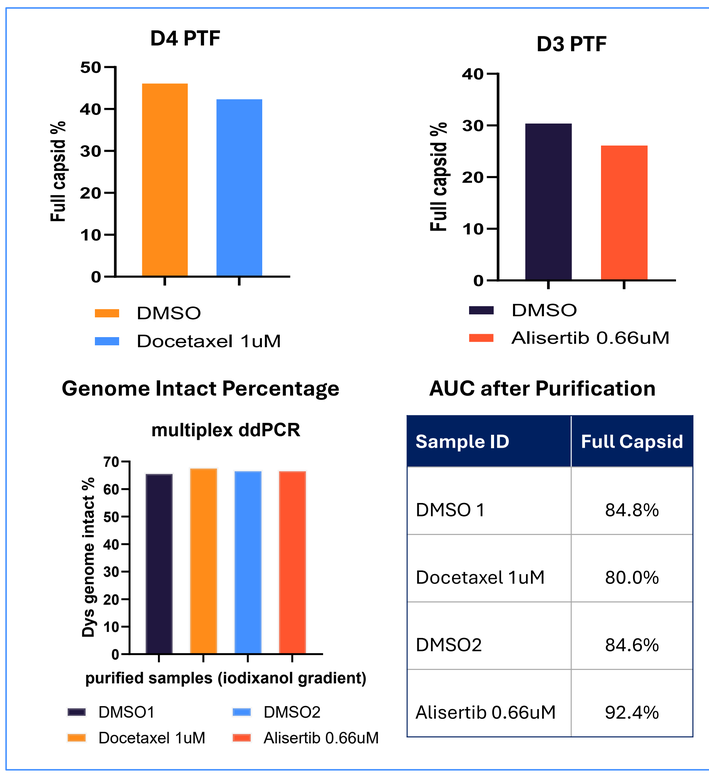


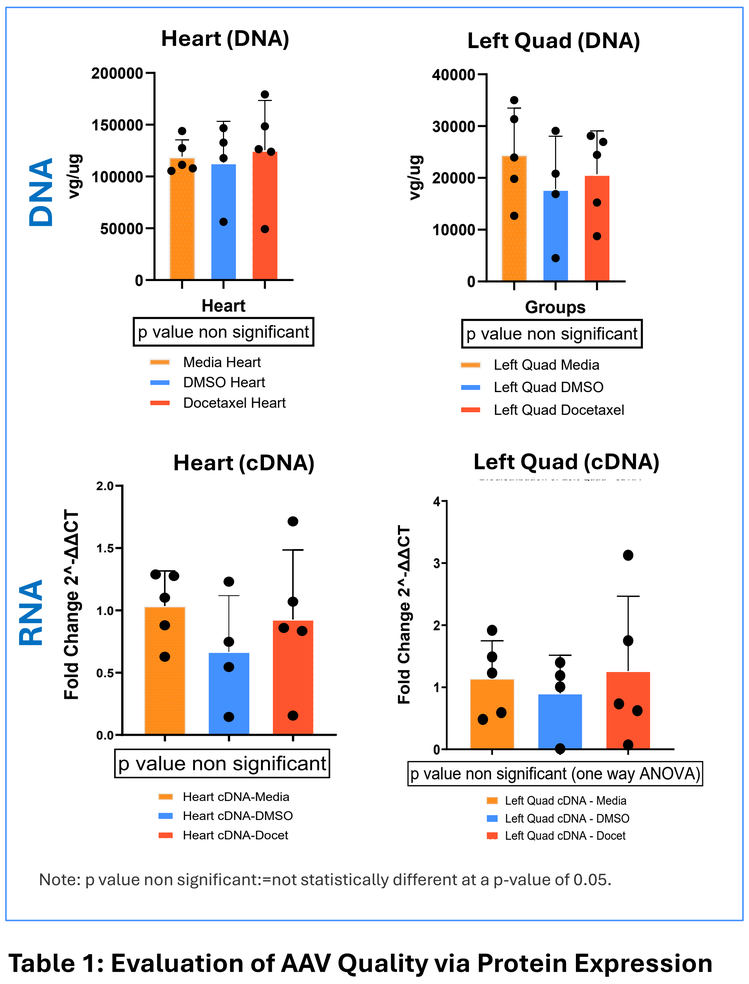
Figure 5: AAV Quality (pre-purification AAV material)



AUC after Purification

Sample ID	Full Capsid
DMSO 1	84.8%
Docetaxel 1uM	80.0%
DMSO2	84.6%
Alisertib 0.66uM	92.4%

Figure 6: In Vivo: Biodistribution and RNA Expression

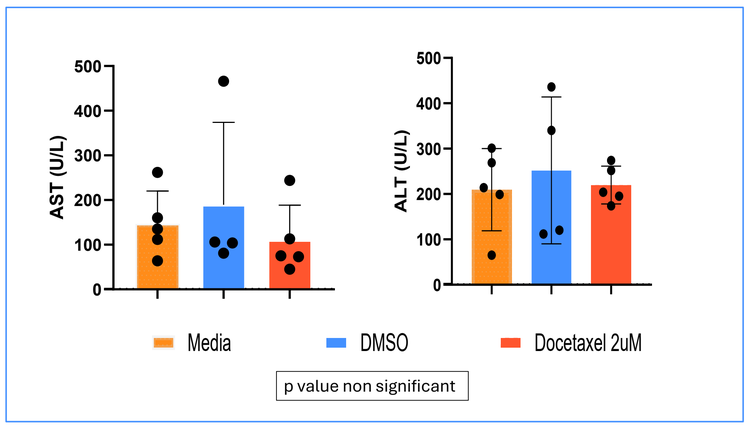


Note: p value non significant:=not statistically different at a p-value of 0.05.

Table 1: Evaluation of AAV Quality via Protein Expression in C2C12 Cells.

Plate #	Sample	Sample Name	ddPCR titer (vg/ml)	Relative Potency
1	1	DMSO-AAV	6.23E13	100%
	2	Docetaxel 1uM-AAV	2.78E14	97.0%
2	3	DMSO-AAV	8.01e13	100%
	4	Alisertib 0.66uM-AAV	2.09e14	108.9%

Figure 7: Serum AST and ALT Levels Following Systemic Administration of rAAV Produced with Docetaxel



CONCLUSIONS

- This study identifies cell cycle modulation—specific enhancement of G2/M arrest—as a promising strategy to boost rAAV yield while maintaining high vector quality and safety.
- Transcriptomic profiling revealed intrinsic differences between high and low yield producer cell lines that guided the selection of small molecule interventions.
- Docetaxel and alisertib reproducibly increased rAAV production without compromising product attributes or in vivo safety.
- These findings advance understanding of the cellular drivers of rAAV manufacturing efficiency and support the development of scalable, high yield production platforms for gene therapy applications.

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