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A High-Throughput Small Molecule Screen Identifies Targets That Increase AAV9 Production in Suspension HEK293 Cells

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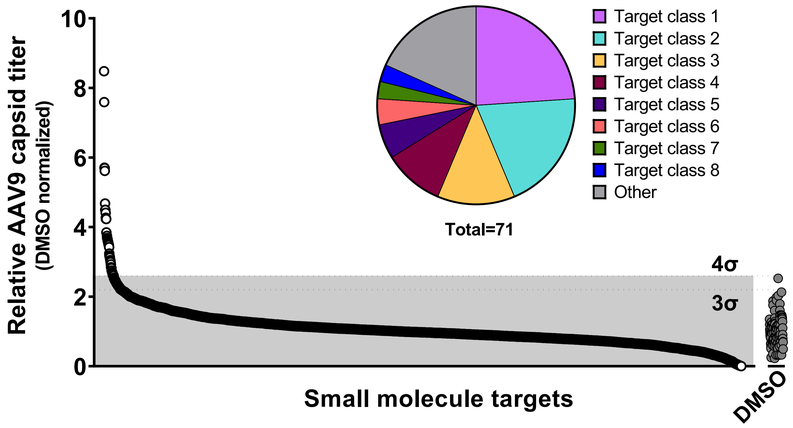
Abstract

The use of recombinant AAV as a vector for gene delivery is widespread, with over 900 pre-clinical and clinical programs underway. However, inefficient manufacturing methods result in high costs, limiting the availability of gene therapies. In this study we describe a high-throughput small molecule screening strategy to identify compounds that increase the capacity of cells to produce AAV9. We used the ATLAS (Arrayed Targeted Library for AAV Screening) platform to perform the primary screen using a library of over 3000 small molecules. Targets identified included transmembrane proteins, DNA repair proteins, cell-cycle regulators, and epigenetic modulators.

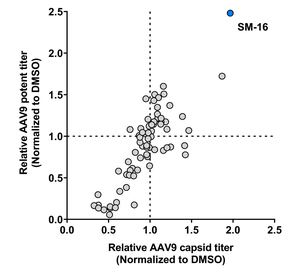
The top 71 performing compounds were re-evaluated in a dose-response manner on our proprietary clonal HEK293 cell line (AC001.230). After a series of studies in small and large-scale shake flasks, we identified a novel compound (SM-016) that increased rAAV9 production in a robust and dose-dependent manner. We have confirmed these findings via capsid titer and vector genome quantification using two reporter constructs. Evaluation of SM-016 is ongoing to apply these findings for production of differently sized AAV vectors with multiple AAV serotypes and in a scale-down model of our large-scale manufacturing platform. We will also analyze any impact of the compounds on key quality attributes of AAV vector batches.

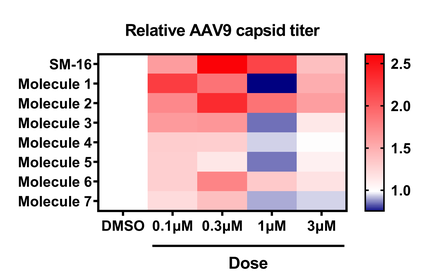
Compound screen identifies pathways improving AAV9 production



- A high-throughput small molecule screen of bioactive compounds was performed on adherent HEK293 cells. AAV9 capsid titer was measured 3 days post triple transfection and normalized to DMSO.
- Top performing compounds increasing AAV9 capsid titer yields consisted of transmembrane and DNA repair proteins, cell-cycle, and epigenetic modulators.

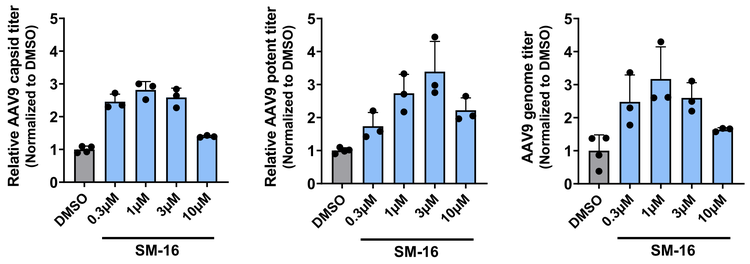
Confirmation studies show SM-16 increases AAV9 titer in adherent HEK293 cells





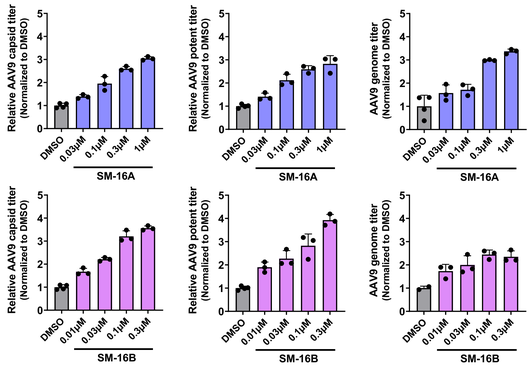
- Top performing AAV9 potentiators from the primary screen were examined in a confirmation study using a 1 μM dose. Capsid titer was measured using ELISA and AAV potency was measured using a proprietary reporter gene assay.
- Data on the top 8 compounds is plotted as a heatmap
- SM-16 performed most consistently, exhibited a dose-dependent response, and was selected for further investigation

Confirmation studies show SM-16 increases AAV9 titer in suspension HEK293 cells



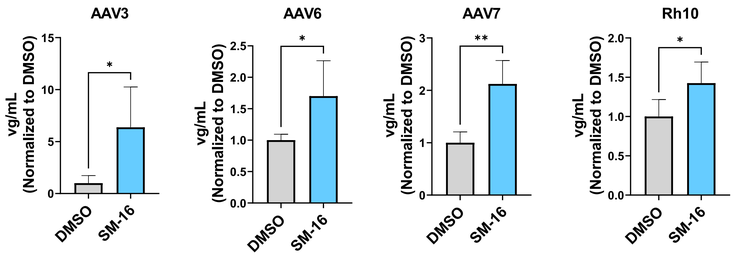
- Studies in Ascend's proprietary, clonal suspension HEK293 cell line (AC001.230) confirm the effects of SM-16 in increasing AAV9 production using capsid ELISA, a cell-based reporter gene and qPCR assay

Two analogs of SM-16 increase AAV9 titer in suspension HEK293 cells



- Studies in Ascend's proprietary, clonal suspension HEK293 cell line (AC001.230) confirm two analogs of SM-16 (SM-16A and SM-16B) increase AAV9 production using capsid ELISA, a cell-based reporter gene, and qPCR assay

SM-16 significantly improves production of additional AAV serotypes



- Efficacy of SM-16 (1 μM) was tested in additional AAV serotypes. Significant improvements were observed in the production of AAV3, AAV6, AAV7 and Rh10

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