Successful scale-up validation of a small-molecule compound for increased AAV yields

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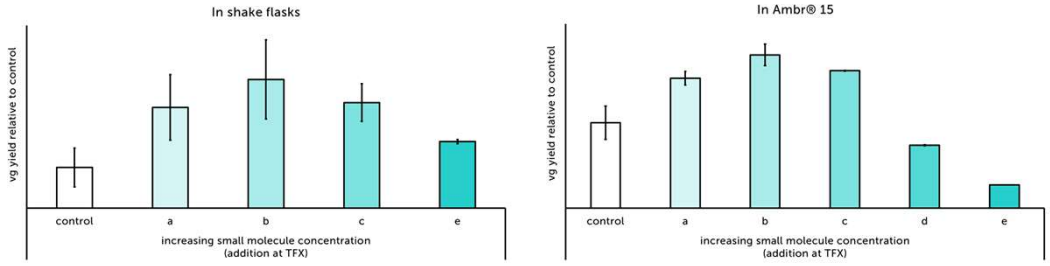
Abstract

Recombinant adeno-associated virus (rAAV) is the most widely used viral vector for in vivo gene therapy today. One of the major challenges associated with AAV gene therapy is the cost-efficient production of high-quality vectors meeting safety and efficacy requirements in a rapidly evolving regulatory environment. To address this, we applied a high throughput small molecule screening strategy in HEK293 cells using the ATLAS (Arrayed Targeted Library for AAV Screening; see also poster P345) platform to identify compounds that demonstrate enhanced rAAV production. After studies in shake flasks, we identified compound SM-016 that increases rAAV production in a robust and dose-dependent manner.

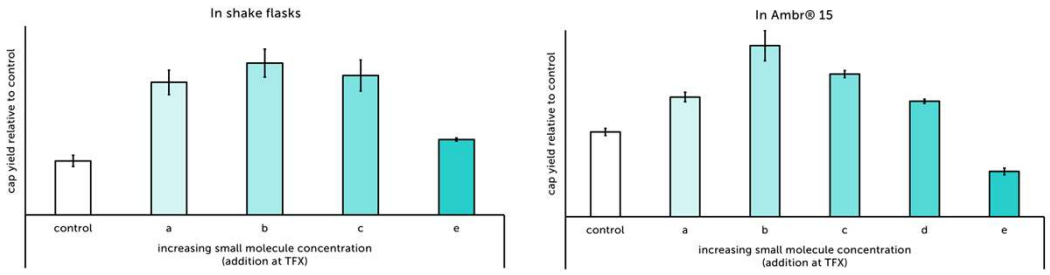
We present here validation data generated in a controlled bioreactor predictive of our large-scale manufacturing platform supporting the scalability of this compound. Using the Ambr® 15 bioreactor system, we confirmed the robust and dose-dependent increase of rAAV yields previously demonstrated in shake flask format. We additionally analyzed the impact of small molecule addition on selected quality parameters. Our data show that vector quality is not significantly compromised after an optimized compound supplementation strategy, indicating analytical comparability of vectors produced with the next generation platform.

AAV yield boost by small molecule SM-016 addition is scalable across production systems

Vector genome yield



Capsid yield



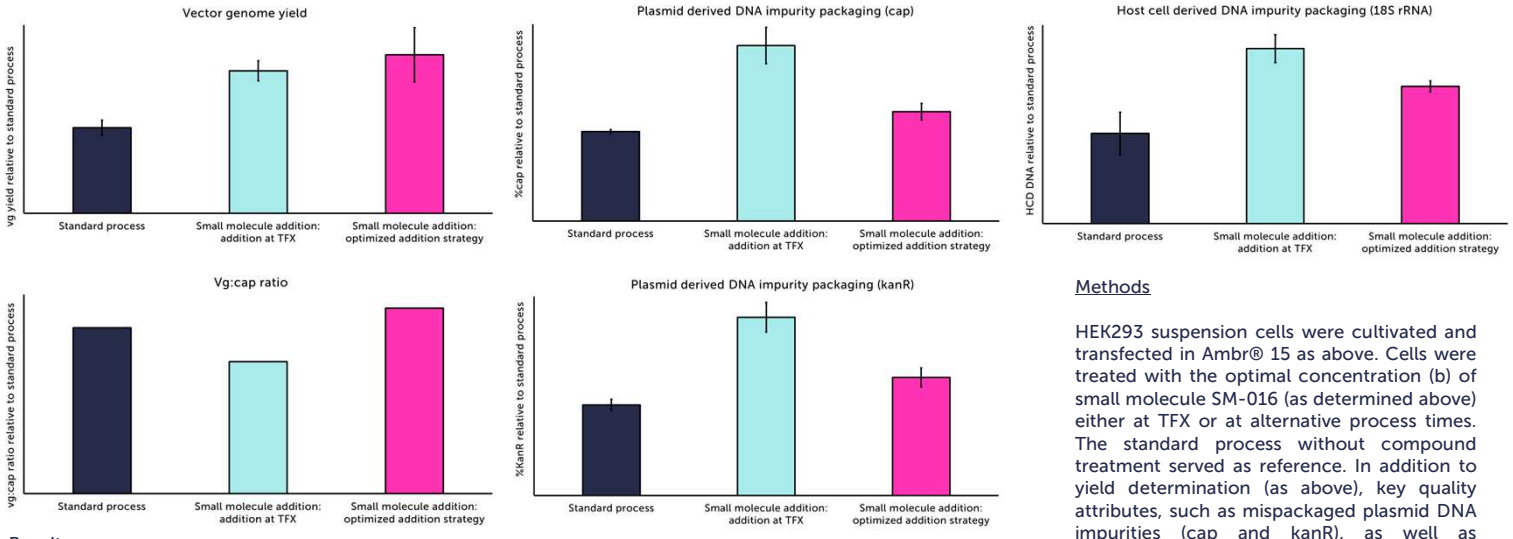
Methods

HEK293 suspension cells were transfected either in shake flasks or in an Ambr® 15 system with our proprietary 2-split plasmid system (capsid/transgene & rep/AdV). Increasing concentrations of small molecule SM-016 were added at transfection (TFX). Solvent only (DMSO) served as control. Vg yields in the harvest were determined by qPCR. Capsid yields were quantified by rAAV capsid specific immunoassay.

Results

Vg and capsid yields were increased in a dose dependent manner in the shake flask and Ambr® 15 based production system, both reaching maximum yield increase at concentration (b) of the small molecule SM-016. Concentrations above (b) led to a decrease in cell viability and rAAV productivity.

Timing of small molecule SM-016 addition is critical to boost yield, and thereby retain high vector quality



Methods

HEK293 suspension cells were cultivated and transfected in Ambr® 15 as above. Cells were treated with the optimal concentration (b) of small molecule SM-016 (as determined above) either at TFX or at alternative process times. The standard process without compound treatment served as reference. In addition to yield determination (as above), key quality attributes, such as mispackaged plasmid DNA impurities (cap and kanR), as well as mispackaged HCD (host cell DNA) impurities were determined by qPCR or ddPCR, respectively.

Results

Addition of the small molecule SM-016 to the production process at TFX led to improved yields but had an impact on key quality attributes of the rAAV product (vg:cap ratio; cap, kanR DNA and HCD mispackaging). Optimization of small molecule supplementation resulted in the same yield increase as addition at TFX while retaining high quality.

Summary

We have successfully validated a small molecule compound (SM-016) that we previously identified in screenings to boost AAV production in a scale-down bioreactor system (Ambr® 15) that is predictive for our large-scale manufacturing platform.

We thoroughly assessed the quality of the produced AAVs and identified an optimized timing of small molecule supplementation to retain high AAV product quality.

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