Development of a Universal, CGMP-ready, clonal HEK293 Cell Line for the Manufacturing of Adeno-Associated Virus, Lenti- and Adenoviral Vectors

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Abstract

Utilizing the well-established, adherent Frank Graham HEK293 cell line as a starting point, we have successfully adapted cells from a qualified master cell bank to grow in animal component-free, suspension conditions in shake flasks and bioreactors. In head-to-head studies comparing our SKPT-HEK suspension-adapted cells with three commercially available suspension HEK293 cell lines, we demonstrated comparable yields of AAV vectors following the plasmid triple transfection method for virus production. To establish a cell line suitable for CGMP-grade manufacturing, we clonally printed SKPT-HEK293 pool cells using the Cytena f.sight cell printing and CloneSelect imaging platform. Here, we show full traceability of clonal outgrowth from single cell through scale-up and have identified select clones that perform equally well in head-to-head comparison studies with other HEK293 cell lines for virus production. The lead clone, 4G9, supports robust production of all viral platforms tested: AAV, LVV, and adenovirus. Importantly, the 4G9 clone can support production across a diverse panel of natural AAV serotypes evaluated (AAV1, 2, 5, 6, 8, 9) as well as lentiviral CAR-Ts. Establishment of CGMP master cell banks for large-scale AAV manufacturing is underway, with expected availability in 2024. The advancement of this production-ready clone represents significant cost savings for viral vector manufacturing. With integrated process and analytical development, as well as commercial-scale manufacturing, SK pharmteco can streamline the entire manufacturing process.

Results

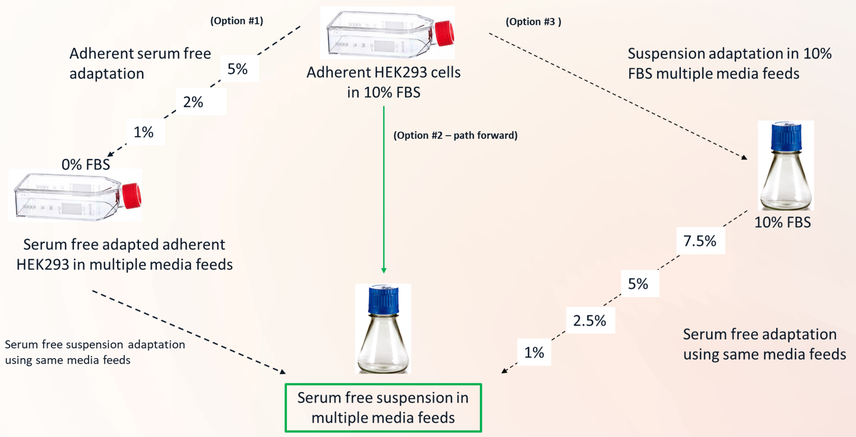

Figure 1. Strategy for generation of serum-free suspension cells. 3-pronged approach to serum-free suspension cells. Option #1- Stepwise adaptation to reduced serum in adherent cells in multiple media feeds. Option #2 – Direct adaptation from adherent cells with serum to serum-free suspension in multiple media feeds. Option #3 – Adaptation to suspension, followed by stepwise adaptation serum-free suspension in multiple media feeds. Option #2 produced the SKPT-HEK293 serum-free suspension pool.

Results

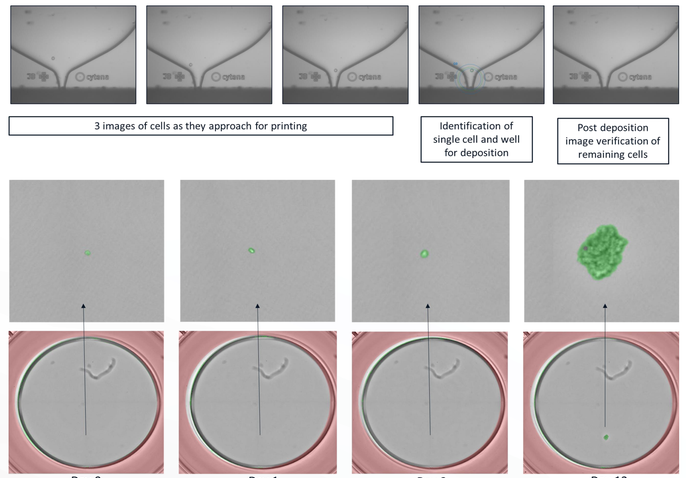

Figure 2. SKPT-HEK293-4G9 (clone) isolation using Cytena f.sight Technology. Monoclonality Imaging Verification. Cytena f.sight single cell printing (top). Imaging of SKPT-HEK293 pool cells prior to printing, identification of a single cell for deposition into a unique well in a 96-well plate, and post-deposition observation of remaining cells. CloneSelect Imaging (bottom). Whole well and magnified imaging documentation of single cell outgrowth to colony formation over time.

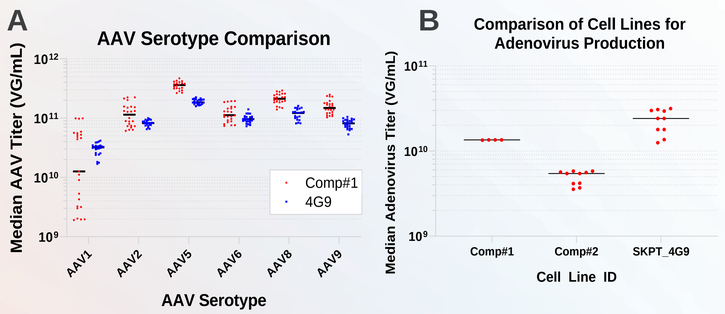

Figure 3. Comparison of AAV Seroptype Panel and Adenovirus Production. A) Small-scale (30mL) shake-flasks transient triple transfection comparison of SKPT-HEK293-4G9 vs competitor cell lines across commonly used AAV serotypes. B) Small-scale (30mL) comparison of SKPT-HEK293-4G9 vs two competitor cell lines for Adenovirus production.

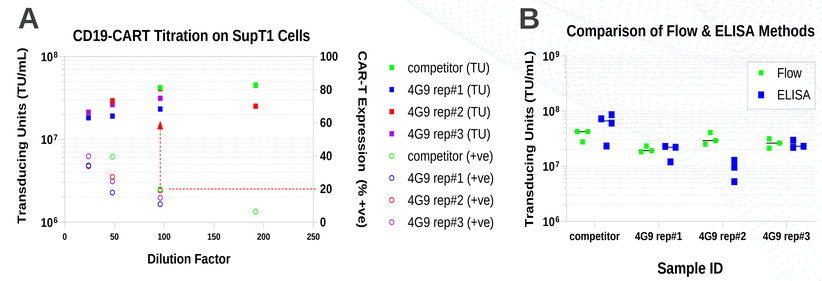

Figure 4. Quantification of CD19 CAR-T Expression on LVV transduced SupT1 cells. A) LVV produced by SKPT-HEK293-4G9 or competitor was purified by precipitation and used to transduced SupT1 cells. CAR-T expression was measured using a CD19 CAR Detection reagent (Miltenyi). 20% of positive cell-expressing cell were used to compare B) ELISA vs Flow cytometry. Comparison analysis shows similar titers.

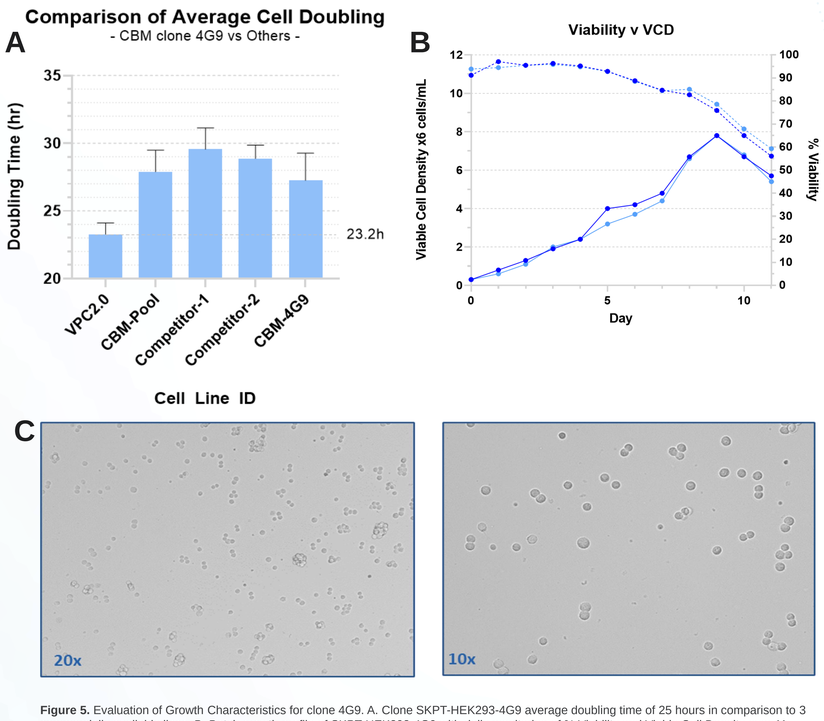

Figure 5. Evaluation of Growth Characteristics for clone 4G9. A. Clone SKPT-HEK293-4G9 average doubling time of 25 hours in comparison to 3 commercially available lines. B. Batch growth profile of SKPT-HEK293-4G9 with daily monitoring of % Viability and Viable Cell Density over 11 days. C. EVOS 7000 brightfield microscopy of Clone 4G9 cell morphology.

Summary

In conclusion, the development of the SKPT-HEK293-4G9 cell line marks a significant advancement in viral vector manufacturing. This clonal, serum-free suspension, T antigen-negative cell line supports universal virus production, offering consistently high titers that are comparable to or exceed those of competitor lines. More importantly, this cell line meets critical quality and scalability standards required for CGMP production, facilitating reliable large-scale manufacturing. Along with cost savings in production, the SKPT-HEK293-4G9 cell line provides a more efficient and universal solution than other commercially available options. These efficiencies and reduced resource demands across various viral platforms translate into substantial cost reductions, enhancing its value as a preferred cell line for both clinical and commercial applications. A master cell bank has been established and is available for manufacturing.