OPEN LENTIVIRAL VECTOR AND ADENOVIRAL-ASSOCIATED VIRAL VECTOR MANUFACTURING PLATFORMS (ARP-27)

Ibeawuchi Oparaocha, Oxana Slessareva, Rimas J Orentas, Tony Luo, Steven Ly, Ying Xiong, Kim Anthony-Gonda, Boro Dropulić
Caring Cross, Inc., Gaithersburg, MD USA

TAP HERE TO RETURN TO KIOSK MENU

INTRODUCTION

Lentiviral vectors (LV) and adeno-associated viral vectors (AAV) are key components of currently approved products for cell and gene therapy, such as CAR-T cell therapy for hematologic malignancies and repair of genetic disorders leading to blindness, respectively.

The lack of publically-available standardized methods for creating and quantifying genetic vectors has hindered their broader application to meet important medical needs. This project will create an open platform to address some of the bottlenecks experienced by vector manufacturing organizations that hinder the development of new therapeutic candidates. The development and public dissemination of standard manufacturing and testing procedures for the production of LV and AAV vectors would greatly benefit academic, early-stage biotech, and other organizations with development of their gene therapy or vaccine candidates as clinical products.

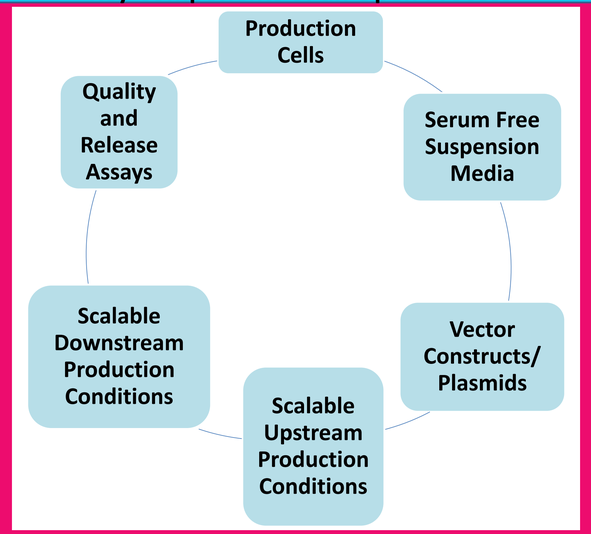
We are developing an open, clinically relevant entry-scale LV and AAV manufacturing platforms that can be used for their manufacture for clinical trials. Such a platform would accelerate development of therapeutic candidates and relieve current bottlenecks.

GOALS

- Develop open entry-scale methods and materials for GMP manufacture of LV and AAV, applicable to academic and commercial organizations
- Use of a common suspension 293 cell platform for both LV and AAV
- Creation of publically available SOPs for the manufacture of LV and AAV vectors, including required assays for release of vector product
- The processes, methods and assays generated in this project will be made publicly available through Caring Cross and NIIMBL – and thus create broadly-shared standards

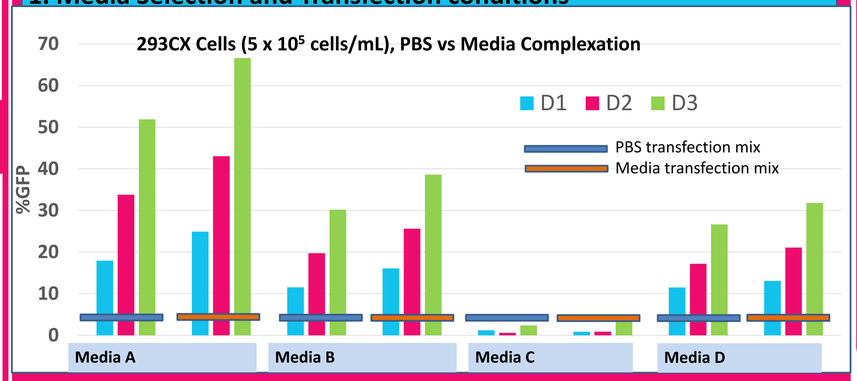
OVERVIEW

Key Components of an Open Process



RESULTS

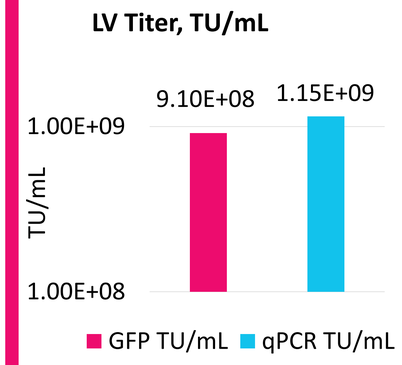
1. Media Selection and Transfection conditions



- Of 15 media screened the top 4 (above) showed consistent GFP expression
- Transfection of plasmids in media was superior to PBS
- GFP expression over 3 days is shown

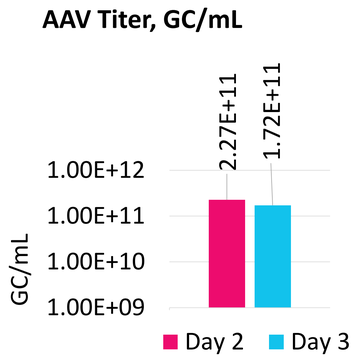
RESULTS

2. QC Analytical - LV



Expression-based (GFP) and qPCR titers of the current LV positive control. Caring Cross LV positive control has been established to support process development. It is characterized by Expression-based (GFP titer) using flow cytometry and qPCR titer.

3. QC Analytical - AAV



GC/mL titers of AAV from day 2 and day 3 harvests. The first AAV production samples were harvested on Day 2 and Day 3. The samples were characterized by Vector Genome Titration assay (GC/mL) using qPCR method.

CONCLUSION

1. Multiple sources of HEK293/HEK293T cell lines were investigated for LV/AAV production. An OS-compliant HEK293 line was identified without T-antigen (thus suitable for clinical AAV production).
2. Media optimization is on-going, with top 4 candidates identified.
3. Caring Cross with its partners, NIIMBL and NIST will complete this project within 2 years, creating publically available materials, protocols, and standards for the field.

ACKNOWLEDGEMENTS