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.

**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

2. **QC Analytical - LV**

   - LV Titer, TU/mL
   - GFP TU/mL: 9.10E+08, 1.15E+09
   - qPCR TU/mL: 1.00E+09, 1.15E+09

3. **QC Analytical - AAV**

   - AAV Titer, GC/mL
   - GC/mL titers of AAV from day 2 and day 3 harvests.
   - Day 2: 2.27E+11, 1.72E+11
   - Day 3: 2.72E+11, 2.27E+11

**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**

Ibeawuchi Oparaocha, Oxana Slessareva, Rimas J Orentas, Tony Luo, Steven Ly, Ying Xiong, Kim Anthony-Gonda, Boro Dropulić

Caring Cross, Inc., Gaithersburg, MD USA