Lentiviral Vector Manufacturing Process
January 17, 2023

Q: When you mentioned titering the LVV on "primary cells", does that mean a standard cell for infectious titer (which can be a cell line) or an actual primary cell type? Is there a standard cell type or cell line to determine infectious titer?

A: For release testing of the finished LVV, infectious/functional titer assays are routinely performed using common analytical cell line such as Jurkat, HT1080, or HEK293.

However, in terms of establishing the Multiplicity of Infectivity (MOI), correlation between the *in vitro* LVV infectious titer as compared to the LVV transduction capability in primary cells should be determined. This can be done by performing scale-down model and/or confirming the candidate MOI in full-scale development runs. The goal is to implement an MOI parameter in the CAR-T batch record such that routine testing on “primary cells” isn’t required, and *in vitro* titer can be leveraged.

Q: In LVV manufacturing with CAR/TCR proteins, have you experienced the presence of precipitates or visible aggregates, either in the bulk vector or in the final concentrated vector?

A: Viruses inherently form aggregates via electrostatic and hydrophobic interactions. Formation of visible particulates resulting from issues of cell-related impurities, processing pH, temperature, etc. is highly process-specific. My recommendation is to evaluate the LVV bulk and final concentrated vector during process development to get an early read on the nature of visible particulates present (inherent, intrinsic, foreign). This will allow for possible process optimizations to reduce aggregation, kick off particulate characterization, and to build appropriate visual inspection processes for GMP.

Q: For CAR-T products in EU, do we need to provide the potency assay for the LVV because it is considered as starting material?

A: A biologically relevant potency assay for LVV is expected for the EU since it is considered a starting material/drug substance. The 2018 EU Guideline on the Quality, Non-clinical and Clinical Aspects of Gene Therapy Medicinal Products applies to the vectors used in the modification of allogeneic or autologous somatic cells modified *ex vivo*. The guidance states: *A suitable measure of the potency of the DS should be established. At least one biological potency specification should be established, the attribute(s) reflecting the physiological mode of action and / or the pharmacological effects of the GTMP.*

Additionally, FDA 2022 Draft Guidance Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products recommends: *Potency will likely be requested for clinical study(s) intended to provide primary evidence of effectiveness to support a marketing application.* i.e., by the time of pivotal clinical trials, characterization of the biological potency conveyed by LV should be demonstrated.

Q: Is there any testing requirement to show that there are no residual HIV-1 elements, or is this wrapped up as part of plasmid residuals?

A: There are no specific HIV-1 residual tests for LVV called-out in regulatory guidances. The overall control encompasses purity testing (i.e., residual DNA), Replication Competent Lentivirus (RCL) testing schema, and viral safety strategy. Some key elements include:

- Master cell banks should be tested for retroviruses and adventitious viruses via *in vitro* and *in vivo* methods (see Q5A(R1)).
- RCL is tested on MCB (for stable cell lines), Vector Supernatant, Vector End of Production Cells, and the *Ex Vivo* transduce drug product.
- Additionally, patient monitoring for RCL is advised pre-treatment, followed by testing at three, six, and twelve months after treatment, and yearly for up to fifteen (15) years. Active testing of patient samples for RCL may be discontinued if sufficient data is available.

Q: Is host cell DNA fragment size of <200 bp (per FDA CMC guidance) a CQA expectation for LVV for *ex vivo* gene therapy?

A: Yes. The ability of the manufacturing process to reduce the amount, size, or activity of residual host cell and plasmid DNA should be demonstrated. In general, reduction of host-cell DNA to <10 ng per dose with fragments smaller than 200 bp is recommended. Measuring the size distribution shows that a) endonuclease treatment is performing as expected, and b) smaller size fragmentation reduces risk of oncogene transmission to the primary cell genome.

See: Establishing Acceptable Limits of Residual DNA, Harry Yang, *PDA J Pharm Sci and Tech*, 2013, 67 155-163

Q: Are there any concerns regarding the potential risk of LV-derived impurities on patient cells (i.e., DS/DP process)?

A: There are concerns on how the LV-derived impurities impact: 1) the cells during DS/DP manufacturing, 2) the DS/DP analytics, and 3) the patient exposure limits.

In terms of impact to cells during manufacturing, risks would be highly process specific. Depending on the MOI (transduction volume), transduction day, and levels of LVV impurities, there could be an impact to the T cell recovery, expansion, phenotype, and possibly/theoretically an immune response against host cell proteins.

Q: Are stability studies required for the plasmids used in transfection?

A: Plasmids are considered starting material per European Commission Directive 2001/83/EC. Stability of the material is one of many risk factor related to GMP principles that should be evaluated and mitigated. Although regarded as highly stable, plasmid shelf life/expiry should be verified through a real-time stability program. I recommend paying particular attention to the supercoil attribute.
See: 24 February 2021 EMA/246400/2021 Questions and answers on the principles of GMP for the manufacturing of starting materials of biological origin used to transfer genetic material for the manufacturing of ATMPs.