USP Biologics Stakeholder Forum 2022: Collaborating to solve CMC challenges and support efficient development of lentiviral-mediated CAR T cell therapies

Executive Summary

The USP Biologics Stakeholder Forum (Bio SF) series is one of many USP programs designed to bring together stakeholders to exchange ideas and identify current trends where solutions might be needed. The third meeting of the Bio SF series, "Collaborating to solve CMC challenges and support efficient development of lentiviral-mediated CAR T cell therapies," was held at USP headquarters in Rockville, MD, on October 26th, 2022.

Multiple CAR T cell therapies are already approved for patients with multiple myeloma, large B-cell lymphoma, and mantle cell lymphoma. But many challenges in product characterization still hinder development even though some regulatory and industry-wide guidance is available on the topic. With this forum, we sought to engage with industry stakeholders to help shape the future of quality standards to support the development and characterization of genetically modified cell-based therapies and identify problems and solutions that can help the industry move forward. The forum was a success on both goals.

The presentations by both industry and regulatory leaders laid out many of the pitfalls that await CAR T therapy developers. The most significant challenges include appropriately characterizing raw and starting materials, drug substances, and drug products. Also, limited manufacturing experience among developers results in poorly defined process variables and critical process parameters, making the characterization of these therapies more difficult. Regulators expect manufacturers to have a controlled manufacturing process, as demonstrated by defining and applying appropriate control process parameters. There is also an expectation that the manufacturer has qualified and validated analytical assays for drug substance and product characterization. Developing and validating suitable analytical methods to ensure the quality of these products during and after manufacture is challenging.

Another barrier to commercialization is developing and validating a suitable potency assay, which should be amenable to quality control, reflect the product's mechanism of action, and be easily transferred to a contract manufacturer. There are multiple different types of assays that a developer can choose from, including cytokine release, cell killing, T-cell activation, or antigen-specific T-cell

expansion. However, choosing suitable reference materials and standards for an assay can be complicated. For example, manufacturers might use healthy donor materials when defining potency early in development, but non-patient material may not correctly reflect patient responses. The early choice of suitable reference materials and standards can be critical because they determine how easy it is to introduce changes to the potency test later in development.

Speakers shared best practices that can be used to mitigate risks. Recommended approaches include implementing analytical quality by design principles into potency assay development and following a matrix approach of assays early in development to ensure that at least one can be tied to clinical efficacy and successfully implemented in a quality control laboratory. Following these principles can guide a developer in creating the analytical target profile or the criteria that must be satisfied by an analytical procedure used to measure a specific quality attribute, such as potency. The future of potency testing was also discussed. Methods such as flow cytometry are being leveraged to assess the tumor-killing potential of CAR T products by using markers of cell death. Critical parameters such as the co-culture ratio of effector and target cells were discussed, along with techniques for quantifying the biological activity of CAR T therapies.

Overall, the forum provided valuable insights and recommendations that will inform USP on the development of documentary and physical reference standards for the characterization of lentivirus manufacturing and CAR T drug products. The discussions led to the realization that standards would benefit many aspects of manufacturing, including titer, infectivity, cytotoxicity assays, particle size and number, and phenotypic characterization. USP has already made progress on these needs by developing several informational general chapters[1] that support this industry as well as a CD34+ cell enumeration standard for system suitability. This USP CD34+ RS has a highly defined range and is a dependable calibrator for ensuring correct gating for data acquisition during flow cytometry.

USP has many opportunities for collaboration and invites qualified candidates to apply to serve as scientific experts on the Lentivirus Cell Therapy Expert Panel. The expert panel aims to develop a new *USP-NF* General Chapter that includes best practices for lentivirus vector design, manufacturing, and release testing. The expert panel will also investigate suitable physical reference standards that accelerate therapy development by industry and academic laboratories. For more

[1]For example, USP chapters <1047> *Gene Therapy Products*; <1046> *Cell-Based Advanced Therapies and Tissue-Based Products*; <1043> *Ancillary Materials for Cell, Gene, and Tissue-Engineered Products*; <1044> *Cryopreservation of Cells*; <1032> *Design and Development of Biological Assays*; <1033> *Biological Assay Validation*; and others.

information or to apply to join the panel before the January 31st, 2023, deadline, go to https://callforcandidates.usp.org/node/32101.