CASSS CGTP 2024: Manufacturing, Quality, and Regulatory Considerations
Plenary Session 4 - Potency Assays for Gene Edited Products
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This session reviewed the Food and Drug Administration's (FDA) recently released draft guideline on potency assurance for cell and gene therapy products. Additionally, it covered issues that should be considered during potency assay design for genome-editing products. Challenges of developing a potency assay for these products were also discussed.

Dr. Andrew Byrnes from the Center for Biologics Evaluation and Research (CBER) at the FDA began the discussion with his presentation, "Potency Assurance for Cellular and Gene Therapy Products". He described updates that were made to the 2011 FDA guidance for potency assurance for cellular and gene therapy products. This updated guidance now covers all aspects of potency, not just assays, and aimed to give industry and regulators a science-based and risk-based framework to discuss potency. Dr. Byrnes went over key aspects of a good potency assurance strategy. This includes an understanding of potency-related characteristics of a product, which can be done by developing a Quality Target Product Profile (QTPP) and identifying potency-related Critical Quality Attributes (CQA's) of a product. For example, there are potency-related CQAs specific to gene therapy vectors, such as the ability to deliver nucleic acids to cells. Nucleic acids potency-related CQA's include length, sequence, and activity. Potency assurance strategies should include multiple release assays, including a bioassay that measures a relevant biological activity of the product. However, redundant assays that measure multiple steps of a biological cascade should be avoided. Progressive implementation of a potency assurance strategy was also discussed. Potency assurance strategies may not be fully mature during early development stages, but they should still be defined, and by later stages they should be comprehensive. Post-release testing and some of the challenges involved were also mentioned. One potential challenge is a short self-life of a product, such as a "fresh" cell therapy product. In this case, a drug product can be released based on physiochemical potency assays, then a potency bioassay can be done post-release.

The second presentation, "Potency Development for an in Vivo AAV Gene Editing Therapy", was given by Dr. Debaditya Bhattacharya from ElevateBio. She spoke about LETI-101, ElevateBio's investigational *in vivo* gene therapy for Huntington's Disease, and described its mechanism, which involves using a nuclease and gRNA to introduce nucleotide insertions/deletions (indels) in the *HTT* gene and trigger loss of the mutant protein through the nonsense-mediated decay pathway. Strategies for potency assay development were then discussed, particularly selection of a suitable cell line. Attributes of suitable cell lines for indel potency include permissiveness to transduction, ease of growth and maintenance, commercial availability, low variability, good target tissue representation, and presence of target SNPs. Methods to evaluate these attributes were also reviewed. Transduction efficiency, for instance, can be tested using a reporter and flow cytometry. Growth in culture can be assessed by measuring doubling time and viability. Screening for SNPs can be done by establishing isolated clonal populations and conducting sanger sequencing. A suitable cell line was identified for an indel detection potency assay for LETI-101 using these approaches. A potency method measuring indels by ddPCR was then developed. The initial method development used synthetic gBlock DNA that was designed to mimic indels expected from nuclease cutting. The assay showed good accuracy and precision, and its good performance was confirmed using the identified cell line.

Dr. Kristy Wood from Intellia Therapeutics, Inc. delivered the final talk of the session, titled "Approaches to Potency Assays for CRISPR Genome Editing Therapeutics". She first gave a brief overview on the design and mechanism of CRISPR/Cas9-mediated genome editing, as well as some of the advantages of this approach to treat genetic diseases, such as permanent knockout or gain of function of the target gene. The design of Intellia's platform was then described. This included sgRNAs 80-120 nucleotides in length, with the gRNA target site specificity defined by the 20mer at the 5' end, packaged into a lipid nanoparticle (LNP) along with *Cas9* mRNA. These three components (sgRNA, *Cas9* mRNA, and LNP) each need to be supported by a slew of analytical methods, including multiple cell-based potency assays. One type of potency assay that can be utilized is a relative potency assay, which measures a shift in biological response compared to a reference represented by a dose-response curve. This can be helpful because biological systems may respond differently day to-day, plate-to-plate or analyst-to-analyst, and with this type of assay the reference standard/test sample will shift together, which improves robustness. Intellia has used a protein reduction assay as one of their potency assays, using ELISAs to measure readouts. Challenges encountered with this

approach include cell culture time, which is several days post-treatment, as well as optimization of each step and culture condition.