Roundtable Session 2 - Table 15 - Host Cell Proteins (HCP) and Host Cell DNA Risk Assessment for Gene Therapy

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Abstract:

As gene therapy continues to evolve, with growing clinical data and broader regulatory oversight, the safety of these innovative treatments is under increasing scrutiny. Host cell proteins (HCPs) and host cell genomic DNA (HCDNA) are two critical process-related impurities that pose potential risks to patient safety, such as oncogenicity risk, untargeted immune responses, decreased product stability, or reduced efficacy. Due to these risk factors, regulatory agencies have implemented clearance requirements, making quantitation of HCPs and HCDNA critical quality attributes for all gene therapy products. Because of the complexity and single-use potential of these treatments, much of the regulatory guidance is loosely defined without fixed specifications. To ensure accurate quantification, validated analytical methods must be implemented into the release panel of all gene therapy products.

In this roundtable, participants will discuss current trends in the evolving landscape of HCP and HCDNA analysis in gene therapy. Participants will discuss strategies for setting impurity thresholds and conducting risk assessments, particularly for customized therapies that may require a single-dosed treatment. Additionally, emerging analytical methods, such as HCP detection using mass spectrometry, will be discussed. The session aims to create an open dialogue amongst participants to align on best practices and identify gaps in current regulatory frameworks.

Discussion Questions and Notes:

1) How do HCP and HCDNA risk assessments differ between gene therapies and traditional biologics, such as mAbs?
    - For HCP, it was noted that human derived cell lines are utilized for gene therapy manufacturing, thus presenting potentially higher safety (i.e., immunogenicity/product tolerability) in patients. Like mAbs, high levels of HCPs in gene therapy products could also contribute to negative product efficacy impacts, including aggregation formation, product degradation, formulation stability issues. It is important to link these impacts to process, and product, critical quality attributes (CQAs) and optimize HCP removal during process development. It is known that some HCPs are “hard to remove” as the AAV capsid can act as a carrier throughout the purification process. There is not a defined limit for HCPs in AAV products provided by regulatory agencies, and although the mAbs defined 100ppm is typically the accepted rule for the upper limit, it is not generally relevant to AAV gene therapy. Risk assessments generally involve HCP clearance studies through the purification process, the early identification of high-risk HCPs in the product, and a release test to monitor and report HCP levels in AAV products.

- For gene therapies, hcDNA contaminants can be packaged in the capsid, which creates unique challenges when compared to mAbs. First, nuclease treatments typically used during purification will not remove these process-related impurities as the DNA is protected by the AAV capsid. Second, it is hard to separate these impurities from the final desired product as column-based purification and enrichment targets the AAV capsid. As AAV has a theoretical packaging limit of ~4.7 – 5.0kb, it is difficult to remove the majority of hcDNA contaminants by density gradient techniques (i.e., cesium-chloride ultracentrifugation) as well; this is typically the size of the desired AAV transgene cassette containing the therapeutic gene of interest, providing co-sedimentation of packaged hcDNA impurities and the final AAV product. The accepted World Health Organization (WHO) recommendations of 10 ng/dose and a size of 200 base pairs for residual host cell DNA content in final products are not generally obtainable for gene therapy products. Host cell DNA content can be de-risked through process clearance studies (i.e., starting at harvest material), as well as characterizing the encapsidated species in the final product using technologies like sequencing (i.e., identification of preferentially packaged genes and species sizing). A comment was made that most companies are not employing long-read sequencing to look at hcDNA sizing distribution and packaged gene distribution, however there have been an increased number of recent publications speaking to this characterization.

2) What role does dosing frequency and total dose amount play in risk assessment?
   - Dosing frequency and amount plays a big role in establishing a risk assessment. Most gene therapies are a single administration of the dose, thus limiting exposure to HCP and hcDNA contamination. If levels are low (i.e., well controlled during manufacturing process), this significantly helps to de-risk these impurities, especially if these doses are well tolerated in Toxicology / other animal studies. Total dose also plays a major role in measuring risk. For systematic deliveries, the patient is exposed to a high vector genome load, increasing the exposure to low levels of HCP and hcDNA impurities. Route of administration will also impact risk assessments as this ties together both dosing frequency and total dose amount.

3) When doing an HCP analysis, have we moved beyond total HCP quantification being suitable as a standalone method? How close are we to implementing targeted HCP analysis as the primary method?
   - While it is still critical to include total HCP quantification as a release method for final product, it has become more common to have an enhanced understanding of individual proteins through techniques like mass spectrometry (MS). There have been an increasing number of publications regarding the use of multiple MS techniques for AAV characterization, including, but not limited to, intact mass analysis and peptide mapping. The use of MS as a characterization throughout process development and early batch analysis helps de-risk HCP impurity levels; MS coupled with total HCP assessments (i.e., ELISA) is a comprehensive approach to HCP detection and quantification throughout the lifecycle of a gene therapy program.

4) What improvements or additional guidance would you like to see from regulatory agencies or pharmacopeias regarding HCP and HCDNA assessments in gene therapies?

- Although the table agreed that relevant, established regulatory guidelines / recommendations should exist for HCP and hcDNA in the context of gene therapies, it was acknowledged that many factors, including, but not limited to, manufacturing platform and purification process, will ultimately determine the levels of these impurities. The table discussed the potential benefits of an organization like the USP investigating these assessments in a whitepaper with everyone being favorable of this idea; a whitepaper would help justify elevated, compared to mAb products, HCP and hcDNA levels in gene therapy products. Further discussions on impurity level justifications recommended a strong risk assessment, tied to process and product critical quality attributes, with transparency to detected levels and method performance in submissions to regulatory agencies.

5) Given the genomic copy number variability in HEK293 and other aneuploid cell lines, how should we interpret (d)dPCR HCDNA quantification data, which only reports copy number values?
   - The table acknowledged that this was a hot topic currently in industry as more companies are moving towards including (d)dPCR for final product testing. The discussion focused on the use of qPCR versus (d)dPCR. It was stressed that, regardless of the technology, it is important to pick a repetitive target throughout the human genome (i.e., 18S) for representative packaging across the genome of the cell. There were different opinions as to which qPCR standard curve would be most suitable for use in a hcDNA method. While some favored the use of synthetic oligos for this work (i.e., PCR target specific DNA fragments), others recommended using sheered DNA from the cell line used in your manufacturing process. The latter would allow for a ng/mL result without the need to convert copies/mL to ng/mL. For (d)dPCR, the best way to perform this conversation was discussed at length, but ultimately the mass conversing equation was considered an acceptable method. It was mentioned that a conversion factor due to genomic copy number variability of cell lines used during the manufacturing process may be required to bridge qPCR values. Ideally, the parental cell line used for manufacturing could be used as a qPCR standard to bridge to a (d)dPCR method, however if this is not possible, the conversion factor could be determined empirically for alignment to qPCR. Further, it was mentioned that working with computation biologists to determine the copy number of a specific gene target within a cell line is helpful. There was a brief discussion on the use of short-read NGS for hcDNA detection, however bridging these values to a PCR-based method is slightly more difficult; while PCR methods target a single, repetitive gene sequence, NGS will provide all hcDNA sequences detected in a sample. A discussion around the establishment of a host cell DNA standard to calibrate the performance of hcDNA analytical methods was discussed, and the table agreed this would be helpful.

Other Discussion Topics:

- Sensitivity of HCP and hcDNA methods – are they too sensitive? False positives can be controlled by assay controls (i.e., negative process controls and negative template controls)

- Gene therapy material limits for analytical development / new technology implementation – when is the best time to switch to (d)dPCR from qPCR? Easiest when starting a new program, however bridging data between methods is an absolute necessity.
- Sample preparation considerations for hcDNA quantitation – is an official DNA extraction required? Most discussion participants stated that DNA extraction (i.e., column purification / bead cleanup) is required for qPCR methods and not for (d)dPCR. Other discussion points included +/- benzonase treatments to distinguish between free and encapsidated DNA species in final product. There was a discussion about creating a common protocol/standard for hcDNA from an organization like the USP.
- Use of commercial HCP and (d)dPCR detection kits: most table participants agreed HCP kits are generally robust, however there was not a lot of experiencing using qPCR / (d)dPCR kits.