Roundtable Session 1 – Table 17–
Host Cell Proteins and Host Cell DNA Risk Assessment for Gene Therapies

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Abstract:
Residual host cell DNA and protein are critical quantity attributes routinely evaluated during the release testing of biological products, including gene therapies. This emphasis stems from longstanding concerns regarding:

1. The oncogenic risk associated with residual DNA, which may lead to insertional mutagenesis or oncogene activation.
2. The immunogenicity risk associated with residual host cell proteins that could elicit immune responses in patients; and
3. The overall potential negative impact on the final drug product's quality, safety and efficacy.

Discussion:
DNA

- AV manufacturing generates large amounts of residual DNA. Potential risks include integration into the host genome, oncogenic activation, and disruption of cellular pathways. The clinical safety relevance of these risks remains debated but it is no longer theoretical.
- Recent Nature publication reported hepatotoxicity associated with plasmid DNA sequences (https://www.nature.com/articles/s41591-025-04073-z).
- Residual DNA concerns are sequence-specific (e.g., E1A when HEK293 cells are used). Thermo Fisher–based assays are commonly used to quantify host cell DNA. Experiments can be designed to detect integration within the genome. Most AV products cannot meet historical WHO DNA limits. FDA has communicated expectations to control levels but has shown flexibility due to technical feasibility challenges. A recent BioPhorum publication discusses acceptable levels and reporting practices.
- Fragment size considerations: for host cell DNA, a target fragment size should be defined. ddPCR and next-generation methods are under development; short-read NGS methods are not currently suitable for release testing. Alternative DNA fragment analysis methods (e.g., capillary-based approaches) can be used. Characterization of fragmentation profile and size distribution is recommended. DNA fragments >200 bp are considered higher risk due to potential protein expression.
- Residual DNA should be defined and assessed in the context of patient dose.
- Acceptable limits: justification should be risk-based, leveraging toxicology batch data and literature references; USP guidance is needed to support alignment.
- Dosing considerations include single-dose versus repeat/weekly dosing regimens.
- AV assembly inherently packages DNA fragments during capsid nucleation; this is part of the manufacturing process, and current processes cannot fully eliminate DNA. Understanding which sequences are preferentially packaged may enable deletion or mitigation strategies.
- Mitigation of DNA risk includes plasmid and starting material design, manufacturing process design, and cell line development. Sequence-specific host DNA and host cell proteins may be targeted for reduction.
- Components of risk include cell source (e.g., Sf9, HEK293), plasmid design, patient dose, route of delivery, level of host cell DNA, and fragment size (>200 bp).
- Mitigation strategies include process design, plasmid and transgene cassette design, improving AV product potency per vector genome (increasing full capsids and reducing partials/empties), and cell line development.
- Development of a qualified NGS-based assay for host cell DNA would provide valuable data on DNA type, sequence, and length to support risk assessment.
- Improved understanding and application of endonucleases is needed.
- Development of stable producer cell lines remains technically challenging.

HCP

- Availability of affinity ligands has improved the ability to remove host cell proteins. Limited feedback is typically received on HCP data submitted to regulators.
- AV products are associated with a high incidence of clinical adverse events, making attribution to product-related impurities challenging.
- Many AV therapies target severely ill patient populations, complicating interpretation of immunogenicity risk.
- Collaboration with purification teams is critical to identify and remove problematic host cell proteins.
- Components of HCP risk assessment should be clearly defined.

Overall Conclusion: The risk of insertional activation due to residual DNA is no longer merely theoretical, as published reports have demonstrated integration of foreign DNA into the host genome. Given that it is extremely difficult to definitively attribute serious adverse safety events to a specific product, it is advisable for sponsors to minimize the presence of residual

DNA in the final product and to conduct a detailed, risk- and science-based assessment to justify appropriate and meaningful acceptance criteria for residual DNA and residual proteins.