Manufacturing Considerations

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Advancing knowledge, awareness, and education of gene and cell therapy
Disclosures

• Own equity in and am an employee of Audentes Therapeutics, a publicly traded biotechnology company developing rAAV gene therapies for serious diseases with unmet medical need

• Receive royalty income from St. Jude Children’s research hospital resulting from licensing of gene therapy technology to commercial entities
Appropriate manufacturing controls for genetic/biological starting materials and process intermediates (i.e., plasmids, producer viruses)
The specific language used to describe materials used during production of the final product has great impact on the regulatory burden associated with generation and use of those materials:

- Plasmids used for transfection to make vectors – referred to as ‘process intermediates’ in the guidance, which could encumber plasmid manufacturers with process validation requirements that would be burdensome.
- Viral vectors used for *ex vivo* modification of cells – referred to as ‘drug substance’ in the guidance, which encumbers production facilities with additional regulatory requirements and contradicts with EMA acceptance of classification of such vectors as ‘starting material’, and not ‘DS’.
ASGCT perspective on the classification of plasmids used for the manufacturing of viral vectors

• Improving specifications and traceability for raw materials used in plasmid manufacture are minimizing or eliminating the risks of adventitious infectious agents (some processes are completely animal product free)
• QC assays for plasmids are robust and reliably predict performance in transfection processes
• Testing the activity of plasmids produced from bacterial Master Cell banks in the vector production process, as per the draft guidance (line 911 of CMC Draft Guidance, July 2018) should capture deviations of any additional unknown quality attributes
• Although certain research production facilities have generated material that is cross-contaminated with other plasmids, and this represents a supply chain risk, process segregation measures should control this problem
ASGCT Recommendations

• The statement in the draft CMC guidance, (line 1042-1044, July 2018), “manufacturing intermediates should be defined by the manufacturer” is ambiguous and fails to clarify the status of plasmids used for viral vector manufacture.

• ASGCT recommends guidance documents stipulate that if plasmids do not directly become part of the drug substance or drug product, then they may be defined as raw materials or reagents, and their quality ensured by supply chain control.
ASGCT Perspective on the classification of viral vectors used for the *ex vivo* production of genetically modified cells

- Consistent language from regulators is essential for manufacturers to establish worldwide supply for advanced medicines

**ASGCT Recommendation**

- ASGCT recommends guidance documents stipulate that viral vectors manufactured for *ex vivo* modification of cells may, with proper supply chain control, be defined as raw materials
Suitability of certain cell lines for manufacturing specific vector classes and recommendations to address risk
Issue #2 raised by the ASGCT

The World Health Organization’s standard of 10 ng host cell DNA/dose is not supported by experimental data quantitating the oncogenic risk associated with contaminating host cell DNA in rAAV preparations, and may be a gross overestimation of the true risk.
Historical Perceptions of Oncogenic Risk

• 1986: WHO Study group calculated that 1 ng residual cellular DNA (rcDNA) from cells containing 100 copies of an oncogene could give rise to one transformational event in \(\sim 10^9\) recipients

• 1998: adjustment to 10 ng based on data generated more recently reflecting difficulty of exogenous DNA to be integrated

• 2010-2014: Studies in mice with cell line DNA and control oncogene DNA (Sheng-Fowler et al., 2010, 2014):
  • In young NIH Swiss mice, 1 µg pure oncogene DNA induced tumors
  • In CD3 epsilon transgenic mice, which are defective in T- and NK-cell function, <1 ng oncogene DNA induced tumors (3-log higher sensitivity, likely due to lack of immune surveillance)
  • In CD3 epsilon mice, 100 µg genomic DNA from HeLa, A549, HT-1080, and CEM do not cause observable tumors
Mechanisms of Virus Induced Oncogene\sis


Additional Lessons from Retrovirally Induced Leukemias

• LMO2 proto-oncogene locus integrations seen in gene therapy patients with other diseases (e.g., ADA-SCID) with no oncogenicity, emphasizing the importance of factors other than oncogene activation in the transformation process (i.e., proliferative environment in disease patients, profound immunodeficiency limiting immune surveillance, etc.) (Aiuti A, et al., J Clin Invest. 2007 Aug;117(8):2233-40. Cooper AR, et al., Blood. 2017 May 11;129(19):2624-2635.)

• Mature, differentiated cells, even when transduced with oncogene expressing retroviral vectors (LMO2, etc.) do not give rise to tumors (Newrzela et al., Blood 2008; 112(6): 2278-2286.)
Mechanisms of rAAV Induced Oncogenesis


Nature of Contaminating DNA in rAAV

- Some random incorporation, some selective based on Rep protein binding, fragmented to fit in rAAV particle
Counting Copies  
(via NGS, not validated)

<table>
<thead>
<tr>
<th>DNA</th>
<th>% Vector Genome (reads)</th>
<th>Copies / $10^{14}$ vg</th>
<th>Max Mass / $10^{14}$ vg</th>
<th>Max Fraction by Copy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector Genome</td>
<td>96.7%</td>
<td>$10^{14}$</td>
<td>241 µg</td>
<td></td>
</tr>
<tr>
<td>Whole HEK Genome</td>
<td>0.3% – 1.5%</td>
<td>$3.7 \times 10^5$</td>
<td>3.6 µg</td>
<td>$3.7 \times 10^{-9}$</td>
</tr>
<tr>
<td>Kan$^{r}$</td>
<td>0.3% – 0.7%</td>
<td>$10^{12}$</td>
<td>1.7 µg</td>
<td>0.003 – 0.007</td>
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<tr>
<td>Proto-oncogenes*</td>
<td>0.01% – 0.05%</td>
<td>$\sim 10^9$</td>
<td>121 ng</td>
<td>$10^{-5}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DNA</th>
<th>Copies/Cell in Liver</th>
<th>Copies/Mouse Liver**</th>
<th>Copies/kg Human Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector Genome</td>
<td>100-1000</td>
<td>$10^{11} – 10^{12}$</td>
<td>$10^{14} – 10^{15}$</td>
</tr>
<tr>
<td>Whole HEK Genome</td>
<td>$4 \times 10^{-7} – 4 \times 10^{-6}$</td>
<td>400 – 4000</td>
<td>$10^5 – 10^6$</td>
</tr>
<tr>
<td>Kan$^{r}$</td>
<td>0.3 – 7</td>
<td>$10^9 - 10^{10}$</td>
<td>$10^{12} - 10^{13}$</td>
</tr>
<tr>
<td>Proto-oncogenes</td>
<td>$10^{-4} – 10^{-3}$</td>
<td>$\sim 10^5 – 10^6$</td>
<td>$\sim 10^8 – 10^9$</td>
</tr>
</tbody>
</table>

*Proto-oncogenes defined as COSMIC Tier 1 genes; n=572; mean size = 120 kb
** Mouse liver ~ $10^9$ cells
Counting Copies: Consequences

- After dosing neonatal mice with $10^{14}$ vg/kg of a weak promoter rAAV vector, no increase in tumor formation was observed, even though > 100 cells would be expected to have received E1 genes from HEK293, and > $10^5$ cells would have received proto-oncogene sequences.

- When animals were similarly dosed with a strong promoter vector, HCC tumors were induced.
  - Molecular characterization of the tumors showed that the genotoxic insult derived from insertion of the strong promoter into proto-oncogene loci in the mouse liver cells.

- Sheng-Fowler study showed that 100 µg genomic DNA (~$10^6$ genome equivalents) from several immortalized lines was also not tumorigenic after subcutaneous injection in a highly sensitive mouse model.
The Unproven Hypotheses

• Transfer of cellular proto-oncogenes via rAAV preparations will not be a significant oncogenic risk in immune competent individuals, due to the rarity of the events, the need for high copy transmission or activating mutations, and the likelihood that tumor suppressive processes will counteract progression (relevant to all production cell types).

• In the context of HEK293 cell production, the rare transfer of single copies of adenoviral serotype 5 E1 genes will also be inconsequential for some if not all of the above reasons.
Questions not answered

• Sensitivity of animal models and very low abundance of HC DNA in rAAV preparations prevent assessment of relative risk in animal models

• Degree to which packaging biases of the AAV system influence the likelihood of this outcome are still unknown (‘random fraction’ vs. Rep mediated cross-packaging)

• Degree to which fragmentary, single-stranded structure of the genomic DNA contaminants influences likelihood of full length, stable oncogene transmission unknown

• Degree to which risk derived from vector DNAs supersedes risk from HC DNA is unknown, and may depend on the strength of the transcriptional regulatory elements

• Factors related to disease and tissue states cannot be predicted or simply categorized
ASGCT Recommendations

• Caution that strict 10 ng HC DNA/dose standard may be a gross underestimation of the safe dose of host cell DNA when delivered by rAAV, and may prevent beneficial therapies from reaching patients that need them

• Recommend higher doses of HC DNA be allowed immediately in severe diseases with significant unmet medical need

• Recommend that regulatory strategy reflect the fact that the risk of host cell DNA remains unsubstantiated, by requiring sponsors to document levels of contaminating host cell DNA and strongly transforming oncogene DNA in products as opposed to complying with an arbitrary set limit
One Last Comment

• As gene therapy applications and testing expand, greater numbers of individuals will contain cells that are permanently marked with fragments of DNA that can be detected with profound sensitivity.

• At some point, one of these patients will develop a tumor as part of natural processes, and that tumor could contain one of those marks.

WE MUST NOT JUMP TO CONCLUSIONS ABOUT CAUSALITY AND THE RELATIVE RISK OF THE ORIGINAL GENE THERAPY, JUST BECAUSE THE TECHNOLOGY IS POWERFUL AND LEAVES HERITABLE MARKS IN CELLS.
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