Safety Considerations for Gene Editing and Other Gene Therapy Products: An FDA Perspective

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Gene therapy products regulated by OTAT

- Plasmids: 22%
- Adeno: 13%
- γ-retro: 20%
- Lenti: 15%
- AAV: 12%
- Pox: 6%
- Bacterial / yeast: 4%
- Other: 4%
- 4HSV: 2%

560 active Gene Therapy INDs (March 9, 2017)

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Clinical applications of lentiviral vectors

**Ex vivo applications**
- T cells to treat HIV infection
- CAR T cells to treat leukemias and lymphomas
- CD34+ hematopoietic progenitor/stem cells to treat blood disorders, metabolic disorders, immunodeficiencies

**In vivo applications**
- Ocular diseases
- CNS disorders
- Immunotherapy applications
Safety issues with HIV-1-based lentiviral vectors used in clinical trials

- Potential to form replication-competent lentivirus
- Potential for insertional gene activation/inactivation
- Potential for off-target transduction \textit{in vivo}
Approaches to improve the safety of lentiviral vectors

- Reducing the likelihood to form replication-competent lentivirus
- Narrowing the vector’s tissue tropism
- Directing vector integration to genomic “safe harbor sites”
- Modifying the cell substrate for lentiviral vector production
Approaches to improve the safety of lentiviral vectors

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Emergence of partial recombinants

A. Packaging construct

B. Vector construct

C. Partial recombinant

Replication-competent lentivirus?
Assay to assess formation of partial recombinants

Transduction of HEK 293 cells

Blasticidin (BSD) selection

Characterization of resistant cell clones by PCR and DNA sequencing
### Reducing sequence overlaps

<table>
<thead>
<tr>
<th>Vector Type</th>
<th>sequences overlap (%)</th>
<th>No. of BSD-resistant colonies per $10^6$ TU</th>
</tr>
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<tbody>
<tr>
<td>NL-EGFP(MSCV) vector</td>
<td>13.3</td>
<td>63.4 ± 15.4</td>
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<tr>
<td>NL-SRRE-EGFP(MSCV) vector</td>
<td>9.8</td>
<td>25.6 ± 5.3</td>
</tr>
<tr>
<td>NL-SRRE/ΔORI-EGFP(MSCV) vector</td>
<td>9.1</td>
<td>9.5 ± 2.4</td>
</tr>
<tr>
<td>NL(CMV)EGFP/CMV/WPREΔU3 vector</td>
<td>15.6</td>
<td>3.2 ± 0.5</td>
</tr>
</tbody>
</table>
Conclusions

• Partial recombinants are rare

• The frequency of partial recombinants is dependent on vector design
Approaches to improve the safety of lentiviral vectors

- Reducing the likelihood to form replication-competent lentivirus

- Narrowing the vector’s tissue tropism

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Strategy to target lentiviral vector transduction
Strategy to target IL-13Rα2-positive cells using lentiviral vectors pseudotyped with measles virus-derived hemagglutinin (H) and fusion (F) proteins

- Measles virus F protein
- Receptor-blind measles virus H protein fused to IL-13
- IL-13Rα2
Targeting human osteosarcoma cells that conditionally overexpress IL-13Rα2

Large-scale manufacturing of lentiviral vectors pseudotyped with measles virus H and F proteins
Large-scale manufacturing of lentiviral vectors pseudotyped with measles virus H and F proteins

Mustang Q anion exchange membrane chromatography

ÄKTA pure chromatography system

<table>
<thead>
<tr>
<th>Samples</th>
<th>Transducing units, TUs (%)</th>
<th>TUs/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input vector samples</td>
<td>100</td>
<td>$2.43 \times 10^4 \pm 1.21 \times 10^4$</td>
</tr>
<tr>
<td>Vector eluate</td>
<td>$64.36 \pm 11.43$</td>
<td>$2.87 \times 10^6 \pm 0.15 \times 10^6$</td>
</tr>
<tr>
<td>Samples after desalting using Amicon units</td>
<td>$60.45 \pm 27.64$</td>
<td>$2.85 \times 10^6 \pm 0.33 \times 10^6$</td>
</tr>
</tbody>
</table>

100-fold purification

Depiction of a lentiviral vector targeting system involving RNA aptamers

Advantages of aptamer approach:

- RNA sequence space is large
- High affinity aptamer variants are available/can be obtained
- Ease of design/selection/production
Binding of boxB RNA to lentiviral vectors displaying an \( \lambda N2-H \) fusion protein
Binding of boxB RNA to lentiviral vectors displaying an λN2-H fusion protein

A

B

A431 cells
Transduction of human epidermoid carcinoma A431 cells using lentiviral vector particles displaying an EGFR-specific RNA aptamer

<table>
<thead>
<tr>
<th></th>
<th>H-λN</th>
<th>Scaffold</th>
<th>Aptamer</th>
<th>RNase</th>
<th>EGF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
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</tbody>
</table>

2.6% 0.5% 0.5% 0.9%
Conclusions

• Strategy involving measles virus H and F proteins allowed selective transduction of IL-13Rα2 positive cells

• Aptamer strategy looks promising but needs optimization
Approaches to improve the safety of lentiviral vectors

- Reducing the likelihood to form replication-competent lentivirus
- Narrowing the vector’s tissue tropism
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Nuclease-mediated integration of integrase-defective lentiviral vectors (IDLVs) at the AAVS1 “safe harbor” site on chromosome 19

AAVS1 locus

Puromycin cassette integrated at AAVS1 site
Double nick strategy involving the AAV2 Rep protein to promote “homologous recombination” at the AAVS1 locus

Rep-mediated integration of IDLVs at the AAVS1 “safe harbor” site

AAVS1 locus

IDLV donor vector

IDLV encoding Rep 78

Puromycin cassette integrated at AAVS1 site

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Rep and ZFN-mediated insertion of transgene sequences at the AAVS1 site mediated by IDLVs

Primers used:
- AAVS1-Puro
- Puromycin-Puro

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of puromycin-resistant clones*</th>
<th>PCR positive clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>66 ± 12</td>
<td>0% (0/10)</td>
</tr>
<tr>
<td>Donor + Rep</td>
<td>275 ± 14</td>
<td>84% (21/25)</td>
</tr>
<tr>
<td>Donor + ZFN</td>
<td>200 ± 10</td>
<td>100% (19/19)</td>
</tr>
</tbody>
</table>

*10^5 cells used for transduction
Conclusions

• The overall efficiencies of the nuclease/nickase approaches need to be improved

• ZFNs and Rep can be toxic if expressed at high levels
Approaches to improve the safety of lentiviral vectors

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Design of a HEK 293T cell line bearing a genomic deletion of the SV40 T antigen coding region

## PCR screens

<table>
<thead>
<tr>
<th>Description</th>
<th>Count</th>
</tr>
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<tbody>
<tr>
<td>Total # of clones screened by PCR</td>
<td>176</td>
</tr>
<tr>
<td>• # of clones negative both for SV40 T-ag and Km/Neo resistance gene sequences</td>
<td>55</td>
</tr>
<tr>
<td>• # of clones negative for Km/Neo sequence</td>
<td>37</td>
</tr>
<tr>
<td>• # of clones negative PCR result for SV40 T-ag sequence</td>
<td>3</td>
</tr>
<tr>
<td>• # of wild type clones</td>
<td>81</td>
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</table>
Lentiviral vector production using modified HEK 293T cell clones
Conclusions

• Three out of the 176 cell clones screened revealed deletions of the T antigen sequence

• Vector titers for the three T antigen knock out clones were lower than those obtained using HEK 293T cells but higher than those obtained using HEK 293 cells
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Questions?