Gene Therapy Vector Platforms share common safety and efficacy issues when deployed across groups of similar orphan diseases

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Disclosures

• ReGenX Biosciences and Dimension Therapeutics
  – Founder and equity
  – Recipient of a grant
  – Chair Scientific Advisory Board
• Ad hoc consultant to Alexion and several investment entities
• Inventor on multiple patents licensed to gene therapy and vaccine companies
Therapeutic Gene Delivery Approaches

**Ex Vivo**
- Each patient treated with unique product

**In Vivo**
- Single administration
  - One product for all patients

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Ex Vivo / Lentiviral System</th>
<th>In Vivo rAAV System</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Bone Marrow Harvest</td>
<td></td>
<td>One-time Injection or Infusion</td>
</tr>
<tr>
<td>(2) Isolation of Stem Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) Cell Culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4) Ex Vivo Viral Vector Transduction of Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5) Conditioning of Patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6) Infusion of Modified Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7) Engraftment and Expansion</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Time to Max Expression             | Months to Year                                                                             | Days to Weeks                                                                      |
| Genome Persistence                 | Chromosomal Integration                                                                     | Extrachromosomal                                                                   |
| Persistence of Expression (observed)| 10+ years                                                                                  | 10+ years                                                                           |
| Outstanding Challenges             | Consistent Cellular Engraftment Insertional Mutagenesis                                     | Host Immune Response                                                               |
Retrovirus Vectors

Virion structure

Vector genome (1st generation)

LTR — NuGene — LTR
Efficacy of Gene Therapy for X-Linked Severe Combined Immunodeficiency

Frequency of lymphocytes in blood

Figure 3
Structure of vector integration sites in blast cells of P7 and P10. (A) Sequence mapping to the human genomic database indicated a 100% match to the 5’ *CCND2* genomic DNA locus on chromosome 12p13.32 at position 4250758. (B) Sequence mapping to the human genomic database indicated a 100% match to the *LMO2* genomic DNA locus on chromosome 11p13 at position 33859782. (C) Sequence mapping to the human genomic database indicated a 100% match to the *SPAG6* genomic DNA locus on chromosome 10p12.31 at position 22699645. Integrated retrivorus vectors and genes are represented by white and gray boxes, respectively; numbered boxes represent exons; right- and left-facing arrows indicate forward and reverse orientation, respectively, of the inserted vector.

# Summary of HSC-Gene Therapy Trials

<table>
<thead>
<tr>
<th>Hematologic</th>
<th>Target</th>
<th>Vector</th>
<th>Efficiency</th>
<th>Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA-SCID</td>
<td>lymphoid</td>
<td>γ-RV</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>X-linked SCID</td>
<td>lymphoid</td>
<td>γ-RV</td>
<td>yes</td>
<td>leukemia</td>
</tr>
<tr>
<td>X-linked CGD</td>
<td>myeloid</td>
<td>γ-RV</td>
<td>transient</td>
<td>myelodysplasia</td>
</tr>
<tr>
<td>WAS</td>
<td>multi</td>
<td>γ-RV</td>
<td>yes</td>
<td>leukemia</td>
</tr>
</tbody>
</table>
## Retroviral Vectors

<table>
<thead>
<tr>
<th></th>
<th>Mouse γ-leukemia</th>
<th>Lentiviral (HIV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gag-pol-env</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Accessory genes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Pseudotype</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Integration cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dividing</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Quiescent</td>
<td>no</td>
<td>yes</td>
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<td>multi</td>
<td>γ-RV</td>
<td>yes</td>
<td>leukemia</td>
</tr>
<tr>
<td>WAS</td>
<td>multi</td>
<td>lenti</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>β-thal</td>
<td>erythroid</td>
<td>lenti</td>
<td>+/-</td>
<td>clonal expansion</td>
</tr>
<tr>
<td>Fanconi’s Anemia</td>
<td>stem cell</td>
<td>lenti</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Storage disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALD</td>
<td>myeloid</td>
<td>lenti</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>MLD</td>
<td>myeloid</td>
<td>lenti</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>
Figure 1: **Ex vivo** transduction and transplantation of *Cdkn2a*−/− HPCs.
(a) Experimental strategy. Schemes of the proviral form of the vectors used are shown.
Improvements in Retrovirus Gene Therapy for Bone Marrow Diseases

Mouse model of HSC gene therapy: tumor formation

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<tr>
<th>Vector</th>
<th>VCN</th>
<th>Median survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mock</td>
<td>0</td>
<td>248</td>
</tr>
<tr>
<td>RV</td>
<td>1-6</td>
<td>192</td>
</tr>
<tr>
<td>LV</td>
<td>1-6</td>
<td>215</td>
</tr>
<tr>
<td>LV &gt;6</td>
<td></td>
<td>172</td>
</tr>
<tr>
<td>SIN-LV</td>
<td>&gt;6</td>
<td>238</td>
</tr>
</tbody>
</table>

Montini, et al., JCI 119; 964 (2009)
Summary of Retro/Lenti BMT GT Programs: Insertional Mutagenesis

- Lenti with SIN vectors mitigate but may not eliminate risk
- Anticipate impact of disease and transgene
- *In vitro* and mouse carcinogenesis/transformation assays
  - stratify relative risk
  - do not predict toxicity
- Cross reference of data sets of similar vectors (env and promoters) may diminish need for pre-clinical studies. *In vitro* assay more practical.
Novel AAV Platform – Industry Standard

PCR recovery of latent virus

high transduction *in vivo*

large family of endogenous virus

Clade E (AAV8) - Hu, Rh, Bb, Pi
Clade D (AAV7) - Rh, Cy
Clade F (AAV9) - Hu
Clade A (AAV1/AAV6) - Hu
Clade B (AAV2) - Hu
AAV3
AAV3B
ch.5
AAV4
rh.34
rh.32
rh.33
AAV5
Avian AAV (Chiorini et al.)
Goose Parvovirus

Brain:
rAAVrh.10,
rAAV9
Heart:
rAAV9
Lung:
rAAV8
Pancreas:
rAAV8
Liver:
rAAV8
Skeletal Muscle:
rAAV8, AAV9
Systemic Gene Transfer:
rAAV8, AAV9

Brain:
rAAV8
Heart:
rAAV9
Retina:
rAAV8
Pancreas:
rAAV8
Liver:
rAAV8
Skeletal Muscle:
rAAV8, AAV9
Systemic Gene Transfer:
rAAV8, AAV9

distribution

PENN VECTOR CORE

0.05
Structure of AAV

Genome
- Stable in post mitotic cells
- Episomal concatamers (linear and circles)

Immune Responses
- Innate - limited
- Adaptive
  - Vector Ab pre-existing and induced – relevant (ROA)
  - Vector and transgene T cells – limited
  - Transgene Ab – possible
- Factors
  - Vector – capsid, ROA and dose
  - Host – memory, target organ, genetic
Long-term Correction in NHPs and Dogs

**IM – AAV2-Epo**

- **cFIX in plasma (ng/ml)**
- **Year**
- **Limits of detection**
- **Days**
- **Years**

**cFIX antigen**

- **cFIX in plasma (ng/ml)**
- **Year**
- **AAV2**
- **AAV8**
The Role of Innate Immunity in Driving Immune Toxicity: Antigen Presenting Cells

Ad

AAV

inflammation

T and B cell responses

NO inflammation

NO T and B cell responses
Transgene T cell Response in Mouse vs. Monkey (AAV8.TBG.EGFP)

**Mouse** (5x10^{12} GC/kg)
- d7
- d90

**Monkey** (3x10^{12} GC/kg)
- d7
- d35

**Graphs**
- ALT
- GFP T cell
- LFT (ALT, AST)
- SFU/10^6 Lymphs

**Days**
- d7
- d35
- d90

**Organs**
- PBMCs
- Spleen
- Liver
Adaptive Immune Responses to In Vivo Gene Therapy in α-sarcoglycan -/- Mice: Intramuscular AAV.CMV.lacZ

Biodistribution of AAV vector DNA in tissues of rhesus macaques following intravenous infusion of AAV3B, LK03, rh10, and AAV8 vectors
Summary of AAV *In Vivo* Safety Studies: Immune Toxicity

- **Bio-distribution**
  - Dependent on capsid, dose and ROA
  - Cross reference data sets from similar vectors (capsid, dose and ROA) should eliminate need for repeat studies

- **Toxicity (immune and transgene) – acute and chronic**
  - Immune toxicity influenced by transgene and disease
  - Mice less predictive than large animals for immune toxicity
  - Challenge of human transgenes in non-human models
  - ERT + GT creates complexities: +/- immune memory
  - **Suggestions**
    - Animal models of disease preferred (FDA Guidance)
    - Large animals more relevant than mice
    - Use species specific transgenes when possible
    - Data sets of similar vectors helpful but not sufficient