Highly Efficient CRISPR/Cas9 Gene Editing and Long-Term Engraftment of Human Hematopoietic Stem and Progenitor Cells

Disclosure:
Jennifer Gori and Co-Authors are Full-time Employees of Editas Medicine
Gene-Modified Autologous Hematopoietic Stem and Progenitor Cell Therapy

*ex vivo* approach to gene correction of hematopoietic diseases
Rationale for Delivery of Cas9 RNP for Gene Editing in HSCs

Effective gene editing and transient nuclease expression

- **Hypothesis**
  - Cas9 RNP would support gene editing in HSCs without impacting viability or functionality *in vivo*

- **Electroporation of Cas9/gRNA ribonucleoprotein (RNP)**
  - High efficiency
  - Limited exposure

**WT Cas9**

5’
Blunt

PAM

**D10A Nickase**

5’
Overhang

PAM

*Effective for NHEJ*  *Effective for HDR*

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Efficient and Reproducible Editing in HSCs

Comparison of Wild-Type and D10A SpCas9 at β-hemoglobin locus (HBB)

- Reproducible gene editing across 20 donors
- Maintenance of viability of RNP treated HSCs
Gene-Edited HSCs Maintain Erythroid and Myeloid Multipotency *ex vivo*

Analysis of gene editing in clonal derivatives of edited HSCs

- RNP treated HSCs
  - Differentiate into erythroid and myeloid colonies
  - Monoallelic and biallelic gene disruption detected in HSC clones
Long-Term Engraftment of Cas9/gRNA RNP Treated Human HSCs

Compare engraftment of RNP treated and control human CD34+ cells in mouse xenograft model

- Reconstitution of human hematopoiesis in vivo (4+ months)
- Gene editing in marrow, spleen, blood (human subsets)

<table>
<thead>
<tr>
<th>Parameter</th>
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<tbody>
<tr>
<td>Busulfan (mg/kg)</td>
<td>25</td>
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<tr>
<td>Control HSC mice</td>
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<td>RNP HSC mice</td>
<td>7</td>
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<tr>
<td>CD34+ cell dose</td>
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Gene Edited Cells Reconstitute Peripheral Blood

Human CD45⁺ lymphoid and myeloid cells at 4 months

85% human CD45⁺

 Subset within human CD45⁺ gate

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Gene Edited Human Blood Cells and HSCs Repopulate the Bone Marrow and Spleen

5 million HSCs recovered from bone marrow of each recipient

### Bone Marrow

- **70% human CD45**
- **18% HSC**

### Spleen

- **73% human CD45**
- **13% HSC**

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Efficient Gene Editing Detected in Human Blood Cells in the Bone Marrow and Spleen

Gene editing in vivo equal to editing in pre-infusion product

- 50% gene editing HSC before transplantation
- 50% gene editing in engrafted cells in the blood, marrow, and spleen 4 months after transplantation
Gene Editing is Maintained in HSC Progeny Differentiated *in vivo*

Gene editing in engrafted HSCs is maintained in progeny *in vivo*

*Sorted Bone Marrow Fractions*
Summary and Conclusions

- Cas9/gRNA RNP supports efficient and reproducible gene editing in human HSCs across donors (57% ± 8)

- Gene edited HSCs retain phenotype, viability, and differentiation potential \textit{ex vivo}

- Gene edited human HSCs retain long-term engraftment and multipotency \textit{in vivo} (50% editing and 85% human blood reconstitution)
Electroporation of D10A RNP with Donor Supports Homology Directed Repair in HSCs

- 12% homology directed repair achieved after co-delivery of D10A RNP and single strand oligonucleotide donor