Hematopoietic Stem Cell-based Gene Therapy

Clinical Impact and Current Challenges

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Bone Marrow Failure and MDS Curative Therapies Team

Tim Olson MD, PhD
Disclosure Statement

- bluebird bio: Consultancy
## Indications for Stem Cell-Based Curative Therapy in Pediatrics

### Malignant Diseases
- **AML**
- **MDS**
- **JMML**
- **CML**: rarely needed in TKI era
- **ALL**: SCT use ↓ since advent of CAR-T Cell Therapy

### Non-Malignant Diseases

<table>
<thead>
<tr>
<th>BMF/MDS Predisposition Syndromes</th>
<th>Immune Deficiency/Dysregulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquired Aplastic Anemia</td>
<td>Cartilage Hair Hypoplasia</td>
</tr>
<tr>
<td>Amegakaryocytic Thrombocytopenia</td>
<td>Chediak-Higashi syndrome</td>
</tr>
<tr>
<td>Diamond Blackfan Anemia</td>
<td>Chronic Granulomatous Disease</td>
</tr>
<tr>
<td>Fanconi Anemia</td>
<td>Hyper IgM Syndromes</td>
</tr>
<tr>
<td>GATA2 haploinsufficiency</td>
<td>Hyper IgE Syndromes</td>
</tr>
<tr>
<td>MECOM germline syndromes</td>
<td>HLH-primary</td>
</tr>
<tr>
<td>PNH</td>
<td>IL10R Deficiency</td>
</tr>
<tr>
<td>RUNX1 germline syndromes</td>
<td>Interferon-γ receptor deficiency</td>
</tr>
<tr>
<td>SAMD9/SAMD9L syndromes</td>
<td>IPEX</td>
</tr>
<tr>
<td>Severe Congenital Neutropenia</td>
<td>Leukocyte adhesion deficiency</td>
</tr>
<tr>
<td>Shwachman Diamond Syndrome</td>
<td>LCH-Multicentric, refractory</td>
</tr>
<tr>
<td>Telomere Biology Diseases</td>
<td>Omenn Syndrome</td>
</tr>
<tr>
<td></td>
<td>SCID (X-linked, ADA, other)</td>
</tr>
<tr>
<td></td>
<td>Wiskott-Aldrich Syndrome</td>
</tr>
<tr>
<td></td>
<td>XIAP deficiency</td>
</tr>
<tr>
<td></td>
<td>XLP</td>
</tr>
</tbody>
</table>

### Hemoglobin/Heme Disorders
- Beta Thalassemia Major
- Congenital Erythropoietic Porphyria
- Congenital Sideroblastic Anemia
- Pyruvate Kinase Deficiency
- Sickle Cell Disease

### Inborn Errors of Metabolism
- Hurler’s (MPS-I)
- Krabbe (Globoid Cell Leukodystrophy)
- Adrenoleukodystrophy
- Metachromatic Leukodystrophy
- Osteopetrosis
Pediatric BMT Programs: Increased Focus on Non-Malignant Diseases

- **1998**: 31 allogeneic stem cell transplants at CHOP per year
- **2008**: 37 allogeneic stem cell transplants at CHOP per year
- **2018**: 65 allogeneic stem cell transplants at CHOP per year

The pie charts show the distribution between non-malignant and malignant diseases over the years.
Because optimal medical treatment and screening does not always prevent disease progression/complications

- **Hemoglobinopathies**: acute and chronic pain, stroke, chronic lung disease
- **Immune deficiencies**: life-threatening infections, permanent organ damage
- **Neurologic/metabolic**: irreversible disease progression

Because non-curative treatments can cause their own problems

- **Hemoglobinopathies**: alloimmunization, transfusional iron overload, osteopenia
- **Immune deficiencies**: Costs of care, antibiotic side effects

Because curative technologies are now more widely available!!
Curative Therapy Options for Patients with Severe Non-Malignant Diseases

**Matched Sibling Donor (MSD)- BMT**

- Only an option for 15-25% of patients with SCD
  
  (Mentzer et al. AM J Pediatr Hematol Oncol, 1994)

**Historical**

- Unrelated Donor BMT w/wo T cell depletion
- HSC editing Gene Therapy
- Haplo-BMT with PT-Cy or Ex vivo T cell Depletion
- Lentivirus-based Gene Therapy
Milestones in Hematopoietic Stem Cell Based Curative Therapy

Allogeneic Transplant

- Matched Sibling BMT
- NMDP: Matched Unrelated RMT
- URD Cord Blood Banking
- Hi Res HLA Typing + Better Supportive Care
- Disease-specific Protocols
- Ex vivo and In vivo T cell Depletion
- MMUD/Haploidentical SCT


Gene Therapy

- Gene Discovery and Cloning
- γRV Gene Addition Trials in SCID, XCGD, WAS
- LV Gene Addition Trials in SCID, XCGD, WAS, cALD, MLD, SCD, BTM
- CRISPR-Cas9, ZFN Preclinical Development
- Gene Editing Clinical Trials in SCD/BTM
### Active Stem Cell Based Gene Therapy Clinical Trials for Non-Malignant Diseases

#### Hemoglobinopathies

<table>
<thead>
<tr>
<th>Product</th>
<th>Sponsor</th>
<th>Strategy</th>
<th>Diseases</th>
<th>Age (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentiglobin BB305</td>
<td>bluebird bio</td>
<td>LV Gene addition: β-globin</td>
<td>SCD, BTM</td>
<td>2-50</td>
</tr>
<tr>
<td>BCH_BB-LCR shRNA(miR)</td>
<td>Boston Children's</td>
<td>LV Gene addition: shRNA targeting BCL11a</td>
<td>SCD</td>
<td>3-40</td>
</tr>
<tr>
<td>Lenti/G-βAS3-FB</td>
<td>UCLA</td>
<td>LV Gene addition: β-globin</td>
<td>SCD</td>
<td>&gt;18</td>
</tr>
<tr>
<td>GLOBE1</td>
<td>Multiple</td>
<td>LV Gene addition: β-globin</td>
<td>SCD, BTM</td>
<td>5-35</td>
</tr>
<tr>
<td>TNS9.3.55 Mem Sloan Kettering</td>
<td>LV Gene addition: β-globin</td>
<td>SCD, BTM</td>
<td>&gt;18</td>
<td></td>
</tr>
<tr>
<td>CSL200</td>
<td>CSL Behring</td>
<td>LV Gene addition: γ-globin + shRNA734</td>
<td>SCD</td>
<td>18-45</td>
</tr>
<tr>
<td>CTX001</td>
<td>Vertex/CRISPR</td>
<td>CRISPR-CAS9 Gene editing: BCL11a</td>
<td>SCD, BTM</td>
<td>12-35</td>
</tr>
<tr>
<td>OTQ923/HIX 763</td>
<td>Novartis</td>
<td>CRISPR-CAS9 Gene editing: BCL11a</td>
<td>SCD</td>
<td>2-40</td>
</tr>
<tr>
<td>BIVV003</td>
<td>Bioverativ</td>
<td>ZFN Gene editing: BCL11a</td>
<td>SCD</td>
<td>18-40</td>
</tr>
</tbody>
</table>

### Primary Immune Deficiencies

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<thead>
<tr>
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<th>Sponsor</th>
<th>Strategy</th>
<th>Diseases</th>
<th>Age (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTL-101</td>
<td>Orchard</td>
<td>LV Gene addition: ADA</td>
<td>ADA-SCID</td>
<td>&lt; 18</td>
</tr>
<tr>
<td>AProArt</td>
<td>UCSF</td>
<td>LV Gene addition: DCLRE1C</td>
<td>Artemis-SCID</td>
<td>&gt; 2mth</td>
</tr>
<tr>
<td>SIN-LV-RAG1</td>
<td>Leiden Univ</td>
<td>LV Gene addition: RAG1</td>
<td>RAG-1 SCID</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>G2SCID</td>
<td>Boston Child</td>
<td>LV Gene addition: IL2RG</td>
<td>X-linked SCID</td>
<td>≤ 5</td>
</tr>
<tr>
<td>CL200-i4-EF1α-hyc-OPT</td>
<td>Multiple</td>
<td>LV Gene addition: IL2RG</td>
<td>X-linked SCID</td>
<td>varies</td>
</tr>
<tr>
<td>OTL-103</td>
<td>Orchard</td>
<td>LV Gene addition: WAS</td>
<td>Wiskott-Aldrich</td>
<td>&gt; 5</td>
</tr>
<tr>
<td>G1XCGD</td>
<td>Genethon</td>
<td>LV Gene addition: CYBB</td>
<td>X-linked CGD</td>
<td>&gt; 2</td>
</tr>
</tbody>
</table>

### Neurologic, Metabolic, and BMF Disorders

<table>
<thead>
<tr>
<th>Product</th>
<th>Sponsor</th>
<th>Strategy</th>
<th>Diseases</th>
<th>Age (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTL-200</td>
<td>Orchard</td>
<td>LV Gene addition: Arylsulfatase A</td>
<td>MLD</td>
<td>0-7</td>
</tr>
<tr>
<td>Lenti-D</td>
<td>bluebird bio</td>
<td>LV Gene addition: ABCD1</td>
<td>cALD</td>
<td>0-17</td>
</tr>
<tr>
<td>IDUA</td>
<td>IRCCS San Raffaele</td>
<td>LV Gene addition: α-L-iduronidase</td>
<td>MPS-1 (Hurler’s)</td>
<td>0-11</td>
</tr>
<tr>
<td>RP-L401</td>
<td>Rocket</td>
<td>LV Gene addition: TCIRG1</td>
<td>Infantile Osteopetrosis</td>
<td>&gt; 1mth</td>
</tr>
<tr>
<td>RP-L102</td>
<td>Rocket</td>
<td>LV Gene addition: FANCA</td>
<td>Fanconi Anemia</td>
<td>&gt; 1</td>
</tr>
</tbody>
</table>
Timeline of Gene Therapy

- **Stem Cell Collection:**
  - Mobilized PSC preferred
  - Plerixafor only for Sickle Cell
  - BM Harvest necessary in young infants SCID)
  - HSC dose may be lower

- **Conditioning:**
  - Myelablation still preferred
  - Busulfan in most studies
  - Non-genotoxic agents would be preferable
  - Efficacy in Gene Therapy vs allo-BMT?

*Shortening time to treatment a major challenge in many diseases (SCID, cALD)
Cell Manufacturing: Lentivirus Based Gene Addition

Collect stem cells from patient with genetic defect in blood/immunity

GMP* Ex vivo lentiviral transduction → production of gene of interest

Infusion of genetically modified HSC back into patient

Condition Patient with Stem Cell-Directed Chemotherapy

Options for Gene Addition

1. Add a normal copy of the gene associated with the disorder (β-globin in SCD)
2. Add a compensatory gene that fixes phenotype (γ-globin in SCD)
3. Add a Short hairpin(sh)RNA (miR) that impacts gene expression to fix phenotype (shRNA targeting BCL11A enhancer in SCD)

*GMP → Good Manufacturing Practice regulations per Code of Federal Regulations (CFR)
Cell Manufacturing: Gene Editing

Collect stem cells from patient with genetic defect in beta globin

Electroporation of Guided Nuclease constructs to induce strand breaks at site of interest → gene correction or disruption

Infusion of genetically corrected HSC back into patient

Goals of gene editing

1. Gene correction: challenging because needs DNA repair by homologous recombination, which is inefficient (soon to be in trials)

2. Gene disruption by error-prone non-homologous end joining (NHEJ) creation of insertions/deletions (efficient, currently in trials)
Allogeneic HSCT versus Gene Therapy: How to Decide?

- If HLA matched sibling available, MSD-BMT is typically preferred
  - Exception: MSD who are carriers of some genetic conditions

For patients lacking MSD:

<table>
<thead>
<tr>
<th></th>
<th>Allo HSCT</th>
<th>Gene Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloablation Risks</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Speed of Engraftment</td>
<td><strong>Faster</strong></td>
<td><strong>Slower, particularly platelets</strong></td>
</tr>
<tr>
<td>Alloimmunity Risk</td>
<td>Present</td>
<td>None</td>
</tr>
<tr>
<td>Infection Risk</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Medication Burden</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Degree of Phenotype</td>
<td><strong>Typically complete (if full chimerism achieved)</strong></td>
<td>Often partial correction (but this may be ok for many diseases)</td>
</tr>
<tr>
<td>Correction</td>
<td><strong>Early (by a month?)</strong></td>
<td>Late (6-9 months or more)</td>
</tr>
</tbody>
</table>

- Biggest Problem for Gene Therapy: Access
  - Trial slots, post-approval capacity, insurance?
Allogeneic HSCT versus Gene Therapy: How to Decide?

GVHD risk should really no longer be the differentiator….

- TCRαβ⁺ T cell with CD19⁺ Depletion

Johns Hopkins PT-Cy (Bolanos-Meade, Blood 2012)

- No need for serotherapy, fludarabine, cyclophosphamide in Gene Therapy conditioning
- No post-transplant immune prophylaxis (no rejection risk)
- Risk of virus reactivation post-gene therapy minimal (except in PID!)
**Gene Therapy for Hemoglobinopathies: Dr. Janet Kwiatkowski**

**bluebird bio Lentiglobin Studies**

**Gene Therapy for Hemoglobinopathies: Dr. Janet Kwiatkowski**

**bluebird bio Lentiglobin Studies**

**CRISPR/Vertex: CTX001-121**

**Challenges:**
- **Thalassemia**: What Hgb level is enough to prevent ineffective erythropoiesis
- **SCD**: How low does the HbS level need to be to prevent symptoms?
- **Both**: Clonal evolution and MDS/AML risk
Gene Therapy for Primary Immune Deficiency Syndromes: Dr. Sung-Yun Pai

Clinical efficacy of gene-modified stem cells in adenosine deaminase-deficient immunodeficiency

Klit L. Shaw; Elizabeth Carabedian; Suparna Mishra; Prouzzobbo Barman; Alejandro Davila; Denise Carbresne; Sally Shuppler; Christopher Silvén; Sabrine Geciers; Barbara Nowicki; E. Monika Smagorowska; Berkley Brown; Xiaoyan Wang; Satiro de Oliveira; Yeong Cho; Allan Helges; Dayna Terrazas; Pei-Yu Fu; Allen Wu; Beatriz Campo Fernandez; Aaron G. Jaya; Suzani David.

Lentiviral gene therapy for X-linked chronic granulomatous disease


Lentiviral haemopoietic stem/progenitor cell gene therapy for treatment of Wiskott-Aldrich syndrome: interim results of a non-randomised, open-label, phase 1/2 clinical study

Francesca Ferra, Maria Pia Carafa, Stefania Galimberti, Stefania Gennetti, Francesca Dianesi, Fabiennne Besozz, Madalena Miguez, Maria Estrella Bernal, Valeria Galbi, Andrea Angelo Assenatti, Mariacarla Facchin, Claudia Fossali, Elena Alberti, Sumantha Sirtini, Immacolata Brigida, Serena Scoci, Lucia Osso, Antonio Cigna, Miriam Casagrande, Daniela Canarutto, Federica Andreo Sala, Michael H. Albert, Antonello Bartollet, Hermia Keiendro von Rosden, Gi

OCCURRENCE OF LEUKAEMIA FOLLOWING GENE THERAPY OF X-LINKED SCID

Donald B. Kohn, Michel Saudemont and Joseph Girouet.

MECHANISMS OF DISEASE

Activation of the T-Cell Oncogene LMO2 after Gene Therapy for X-Linked Severe Combined Immunodeficiency

Matthew P. McCormack, Ph.D., and Terence H. Rabbitts, Ph.D.

GENE THERAPY

Gene Therapy for Wiskott-Aldrich Syndrome—Long-Term Efficacy and Genotoxicity

Gene Therapy for Leukodystrophies: Dr. Amy Waldman

STARBEAM Study (ALD-102)

- Bluebird Bio led, multicenter study (Boston, Minnesota, GOS, Paris)
- Stabilization of imaging findings in most

TIGET-MLD

- San Raffaele, Milan (Orchard)
- Ad hoc analysis in 2016
- 8/9 subjects showed prevention of disease onset or no progression.

Challenges:
- Biomarkers of clinical activity
- Early identification of patients requiring therapy
- Elimination of neurotoxic conditioning
Current and Future Challenges for Gene Therapy

❖ Availability
   ❖ Current: limited trial slots
   ❖ Future: limited manufacturing capacity?

❖ Exportability
   ❖ Conditioning/infusion straightforward
   ❖ Collection requires specialized expertise

❖ Long Term Follow-up
   ❖ Coordinating network of centers likely needed

❖ In The Future…
   ❖ Head to head comparisons with Allo-BMT
   ❖ Elimination of alkylating agent conditioning?