IPEX Syndrome and Gene Therapy Case Study

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GENE THERAPY

CENTER FOR DEFINITIVE AND CURATIVE MEDICINE

“To cure the incurable”
Immune Dysregulation Polyendocrinopathy Enteropathy X-linked (IPEX) Syndrome

*Prototype of Genetic Autoimmune Disease*

**Genetic disease:** FOXP3 gene mutation resulting in regulatory T cell dysfunction

**Autoimmune Clinical Manifestations:** neonatal diabetes, severe enteropathy and eczema

**Severe onset and outcome:** fatal in infancy

**Current treatments:** only partial efficacy

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Rare disease with wide distribution-Incidence under estimated

*Bacchetta et al, Ann Rev NYAS 2016; Barzaghi et al, JACI 2018*
Immune dysregulation Polyendocrinopathy Enteropathy X-linked Syndrome (IPEX): main features at onset

N=96 patients

Barzaghi F, Frontiers Immun, 2012
Bacchetta R, NYAS 2016
Barzaghi F, JACI 2018
TWO BROTHERS WITH THE SAME FOXP3 MUTATIONS, BUT DIFFERENT CHANCES TO BE CURED

- **Onset at few weeks of life**, with severe enteropathy, severe weight loss and hepatitis

- **Genetic diagnosis of FOXP3 mutation** at about 4mo, after already 3 mo in hospitals

- **Immune suppression over the course of 18 months**: methylprednisolone, tacrolimus, sirolimus, ATG, ATG, tocilizumab, bortezomib, and campath. Overall poor response.

- **αβ T-cell depleted haploidentical HSCT** from the father at 18 months

- **Today**: 3 months post HSCT: alive, 100% donor chimerism, no IS, *he is carrying over gut and liver problems.*

- **Onset at few weeks of life**, admitted to Stanford ER with severe dehydration and weight loss.

- **Immunologic diagnosis within the first month and genetic diagnosis** of FOXP3 mutation confirmed 2 weeks later.

- Presence of autoAb but no clinical manifestations yet.

- **Immune suppression**: methylprednisolone, and sirolimus with good response.

- **αβ T-cell depleted haploidentical HSCT** from the father at 4 months of age.

- **Today** 1 month post HSCT: alive, engrafted, no IS, no autoimmune clinical manifestations.

*.....need for early diagnosis and curative therapy available to every patients*

Stanford, Sept 2018
IPEX syndrome can be identified at birth: facilitate approval of tests that can be broadly used to identify the disease.

- Combined epigenetic quantification of TSDR for regulatory T cell and TLSDR for CD3+ T cells allows discrimination between IPEX and IPEX-like patients.

- Potential for newborn screening

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Barzaghi et al., J Autoim 2012

Baron U, Sci Transl Med 2018
What can be plan to improve current treatment outcome

A. Intervene at onset to bridge to transplant or instead of the transplant

B. Intervene in chronically ill patients to reduce the continue use of immune suppressive drugs

Survival probability (%)

<table>
<thead>
<tr>
<th>Time</th>
<th>6m</th>
<th>1y</th>
<th>5y</th>
<th>10y</th>
<th>15y</th>
</tr>
</thead>
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<tr>
<td>HSCT</td>
<td>82.8</td>
<td>75.5</td>
<td>73.2</td>
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<tr>
<td>IS</td>
<td>96.7</td>
<td>96.7</td>
<td>92.9</td>
<td>86.8</td>
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</table>

Disease free survival probability (%)

<table>
<thead>
<tr>
<th>Time</th>
<th>6m</th>
<th>1y</th>
<th>5y</th>
<th>10y</th>
<th>15y</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSCT</td>
<td>83.9</td>
<td>89.3</td>
<td>71.6</td>
<td>61.0</td>
<td>61.0</td>
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<tr>
<td>IS</td>
<td>90.0</td>
<td>83.1</td>
<td>65.1</td>
<td>50.1</td>
<td>37.1</td>
</tr>
</tbody>
</table>

Barzaghi et al. JACI online publication, Jan 2018
Two possible approaches of cell/gene therapy for IPEX syndrome

Cell therapy with LV-engineered T cells to restore tolerance

- wtFOXP3 over-expression in conventional CD4+ T cells
- in vitro expansion and selection of transduced cells
- Re-infusion of purified autologous CD4LVFOX3 T cells with normal regulatory function

Gene therapy with CRISPR/Cas9 gene corrected autologous HSPCs to preserve endogenous regulation of FOXP3 gene expression

- Ongoing studies on best strategy

Sato Y, Postdoc
Passerini L et al. STMed 2013
Bacchetta Lab, Stanford
CD4^{LV-FOXP3} “CONVERTED” Treg CAN BE OBTAINED FROM FOXP3 null T CELLS

Mutation in FOXP3 gene
Abrogated protein expression

FOXP3 gene protein expression
Restored after LV-FOXP3 “conversion”
DEVELOPING CD4<sup>+</sup>FOXP3 “CONVERTED” TREG CELLS FOR CLINICAL USE

......process is consistent and reproducible among different subjects

Sato Y, Stanford University
DEVELOPING CD4^LVFOX3 "CONVERTED" TREG CELLS FOR CLINICAL USE

Methods: *in vivo* (xeno GVHD model)

- NSG mice (6-8 week, female)
- Effector T cell (left)
- Bidirectional LV construct
- Co-injection (IP)
- CD4+ T cells (xeno GVHD model)
- Allogenic/Autologous FOXP3 overexpressed CD4+ cells (Therapeutic product)

Safely, efficacy, survival tested in hu-mice models

Sato Y, Stanford University
PHASE I CLINICAL PROTOCOL FOR THE CLINICAL USE OF CD4LVFOX3 T CELLS IN IPEX SYNDROME

Primary Objective:
• Asses safety
• Dose ranges and feasibility
• PK and PD of the cell product

Outstanding Questions:
• Patient inclusion criteria?
• Disease status
• Age
• Response to prior therapy

Bacchetta & Roncarolo, Stanford University
KEY QUESTIONS TO EASE THE CLINICAL APPLICABILITY OF PHASE I TRIALS IN IPEX AND SIMILAR BLOOD RARE DISEASES

• CRITERIA FOR PATIENTS ELIGIBILITY FOR RARE DISEASES
  Usual criteria for phase 1 study include the presence of clinical manifestation and failed previous therapy: based on clinical history, tools for early diagnosis and disease measurements, can we aim to treat even before the symptoms arise?

• AGE CONSTRAINT FOR PHASE I TRIAL:
  many of these disease have very early onset, patients can deteriorate very quickly: can we intervene at early pediatric age?

• DISEASE SPECIFIC CRITERIA for evaluating safety and efficacy of a cell/gene product based on preclinical model: what are the expectations from the available preclinical models?

• Need to establish a committee to look at the issues related to clinical research in rare disease.