Targeted Gene Therapy in the Treatment of X-linked Hyper-IgM Syndrome

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Disclosures

• None.
Hyper-immunoglobulin M syndromes

- Heterogeneous group of genetic disorders resulting in defects of immunoglobulin class switch recombination +/- defects of somatic hypermutation

Clinical Presentation
- Bacterial sinopulmonary infections
- Pneumonias
- Gastrointestinal infections
Prognosis & Treatment

- Concerns with HSCT
  - Reactivation of occult cryptosporidial infection
  - Preexisting lung damage
  - Graft-versus-host disease
  - Unstandardized conditioning regimen
  - Timing

Levy et al., 1997.
CD40L Defects as a Candidate for Gene Therapy

Thymic lymphoproliferative disease after successful correction of CD40 ligand deficiency by gene transfer in mice


Lymphoid abnormalities in CD40 ligand transgenic mice suggest the need for tight regulation in gene therapy approaches to hyper immunoglobulin M (IgM) syndrome

Maria Grazia Sacco, Marco Ungari, Enrica Mira Catò, Anna Villa, Dario Strina, Luigi D. Notarangelo, Jos Jonkers, Luigi Zecca, Fabio Facchetti and Paolo Vezzoni

Rationale

• CD40L gene is tightly regulated and requires expression in its normal chromosomal context

• Hypothesis
  • Site-specific gene modification of the CD40L gene in human hematopoietic stem/progenitor cells will correct XHIM

• Site-specific endonucleases
  • Target specific DNA sequences for gene modification
  • Allow physiologic expression of the corrected endogenous CD40L gene
**TALENs**

- TALENs (transcription activator-like effector nucleases)
- Tandem near-identical 34 AA repeats which recognize one base pair via two adjacent AAs (12 and 13) termed repeat-variable diresidues (RVDs)
- Fused to catalytic domain of the FokI nuclease to create targeted DSBs

_Cermak et al., 2011._
Targeted CD40L Gene Insertion

TALEN binding site

Exon1

Intron 1

Double-stranded break (DSB)

Non-homologous end joining (NHEJ)
Targeted CD40L Gene Insertion

- **TALEN binding site**
- **Double-stranded break (DSB)**
- **Homology Directed Repair (HDR)**
TALENs Introduce Site-specific DSBs at the CD40L Locus in K562 Cells

CD40LG Promoter

TSS

5’ UTR

Exon1

Pos. [TALEN] GFP

Neg

22% 31%

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GFP Donor as a Model of Targeted Gene Addition in Jurkat Cells

GFP Donor Only

GFP + TALEN

%GFP

GFP

SSC
PHA-L Stimulation of Electroporated Jurkat Cells Increases GFP Expression

- 0.1 ug/mL PHA: 2.2%
- 0.3 ug/mL PHA: 6.4%
- 1 ug/mL PHA: 11.5%
- 3 ug/mL PHA: 15.8%

% GFP

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Targeted CD40L cDNA Addition in K562 Cells

CD40L cDNA Donor

Exon 1  Exon 2  Exon 3  Exon 4  Exon 5  pA
Targeted CD40L cDNA Addition in K562 Cells

- CD40L cDNA Donor
  - Exon 1
  - Exon 2
  - Exon 3
  - Exon 4
  - Exon 5
  - pA

- GFP
  - E/P

- TALEN + cDNA Donor
  - Exon 3-4 Primer
  - Exon 4-5 Primer

- cDNA Donor Only
- Neg.

- 715 bp
- 652 bp
Targeted Addition of CD40L cDNA in XHIM Primary T cells

Mock

TALEN + Donor
Exon 3-4

TALEN + Donor
Exon 4-5

cDNA IDLV Only

TALEN + cDNA IDLV

- CD40L mRNA
- GFP only

Neg. 33.2%

Pos. 31.1%

30.8%

0.2%

0.1%

0.1%

0.2%

<table>
<thead>
<tr>
<th>CD40L PE-A</th>
<th>CD3 APC-A</th>
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<tbody>
<tr>
<td>Q1</td>
<td>0.1%</td>
</tr>
<tr>
<td>Q1</td>
<td>0.1%</td>
</tr>
<tr>
<td>Q1</td>
<td>0.2%</td>
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Patient Specific Gene Correction

CRISPR binding site

X Patient mutation G → T

CRISPR

GFP

54%
Patient Specific Gene Correction in K562 Cells

CRISPR binding site

Intron 4  Exon3  Intron 3  Exon4

SphI RFLP

Exon3  Introns 3  G  C

RFLP Analysis

CRISPR + Donor  Mock  CRISPR Only  Donor Only

% Correction
Summary

• Targeted gene modification at the CD40L locus in cell lines
• Targeted gene addition of normal codon-optimized CD40L cDNA in cell lines and primary T cells
• Targeted gene correction of a patient-specific splice site mutation in intron 3 of the CD40L gene in cell lines
Future Directions

- Optimize gene addition & correction in patient primary T cells
- Achieve gene modification at the CD40L in CD34+ HSCT
- Transplant corrected CD34+ HSCT into NSG mice
Thank you!

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