

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

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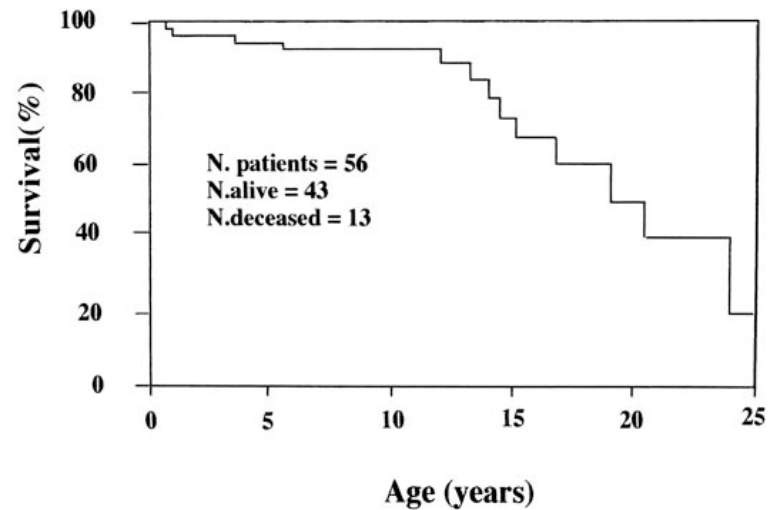
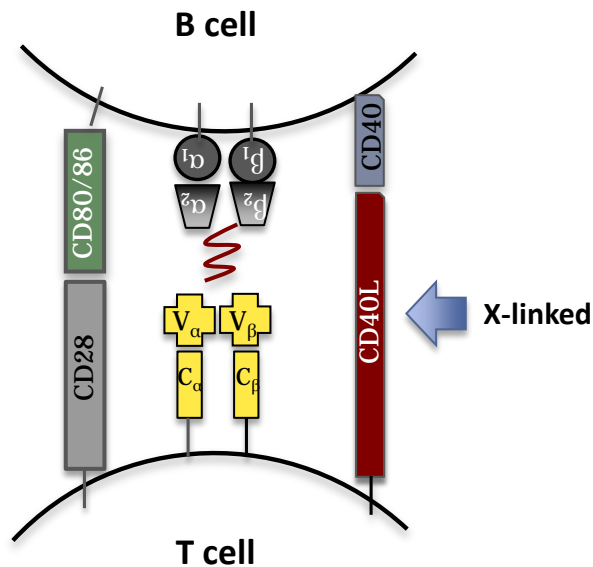


# Disclosures

- None.

# Hyper-immunoglobulin M syndromes

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



# Gene Therapy For XHIM

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

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

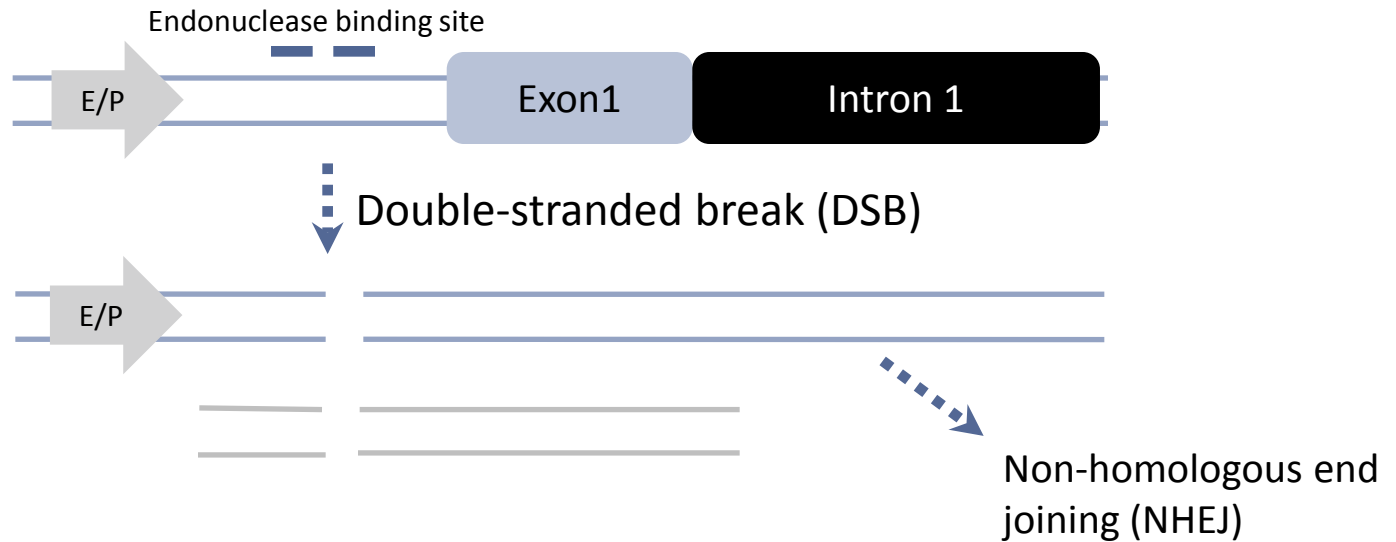
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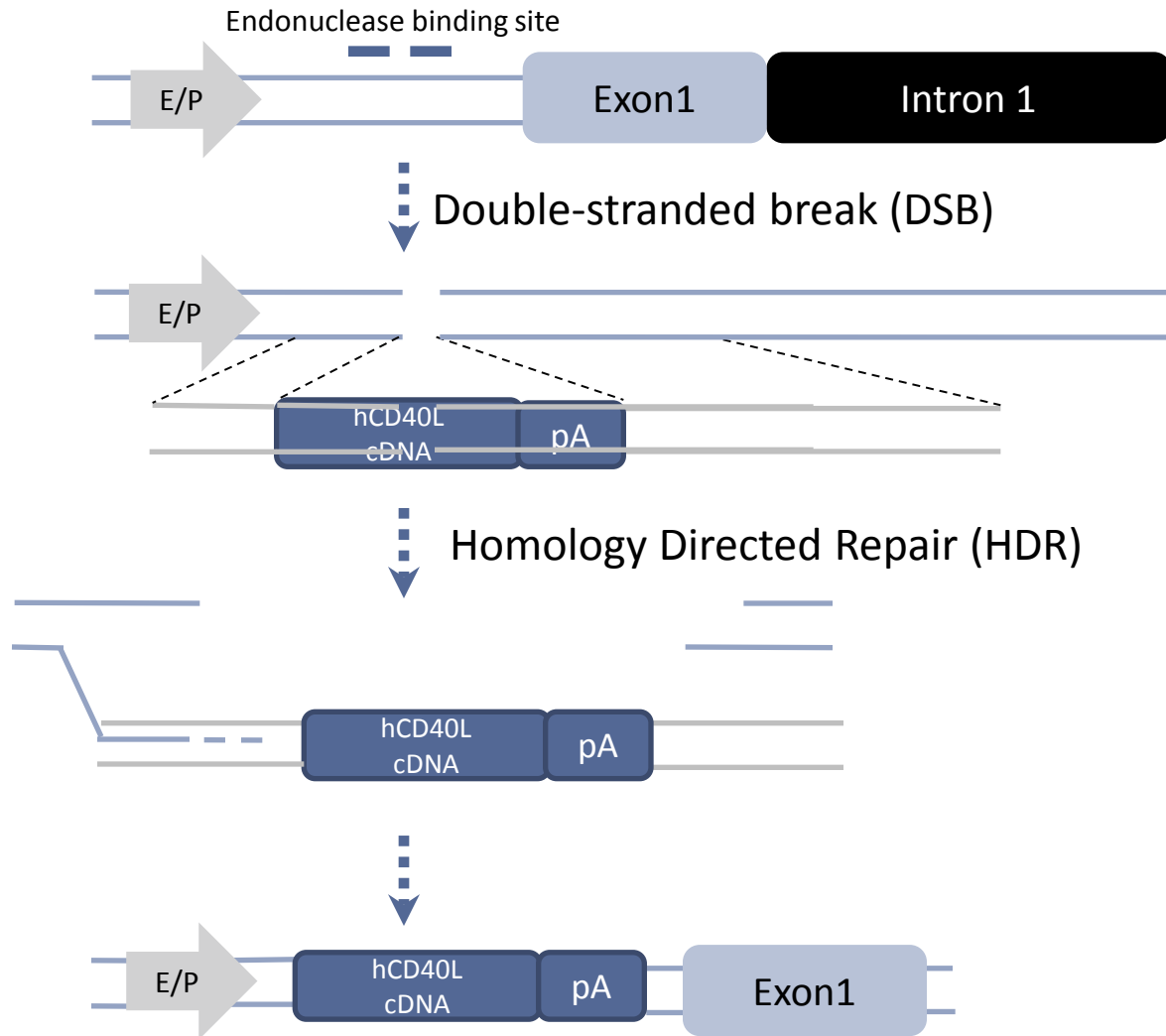
# Rationale

- 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 by autologous transplantation
- Site-specific endonucleases
  - Target specific DNA sequences for gene modification
  - Allow physiologic expression of the corrected endogenous CD40L gene

# Targeted CD40L Gene Insertion

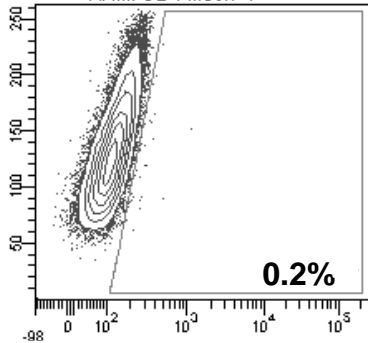


# Targeted CD40L Gene Insertion

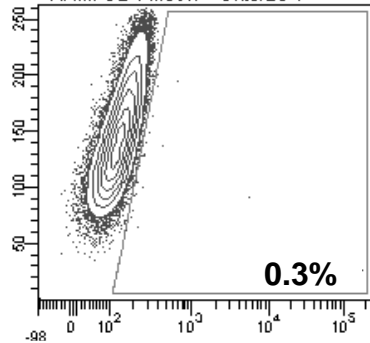


# Gene Correction of XHIM Patient CD4 T cells Using TALENs and an AAV CD40L cDNA donor

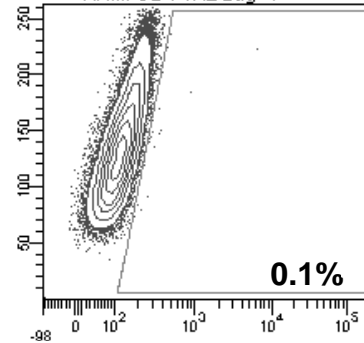
Untreated XHIM T cells



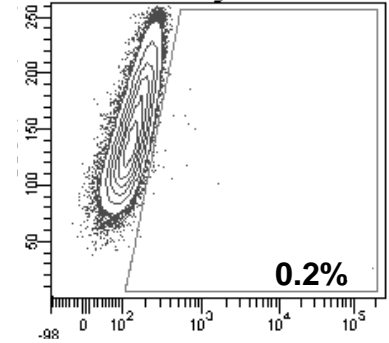
Untreated XHIM T cells (stim.)



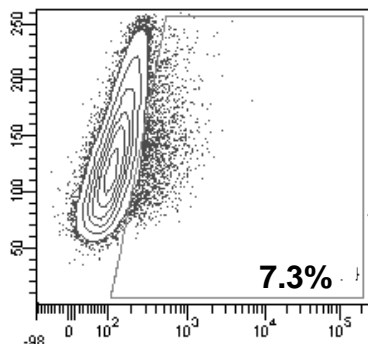
TALEN Only



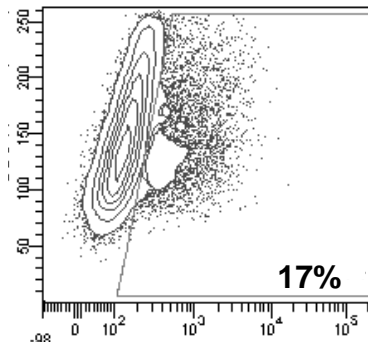
TALEN Only (stim.)



TALEN + AAV cDNA Donor



TALEN + AAV cDNA Donor (Stim.)



% CD40L

Mock

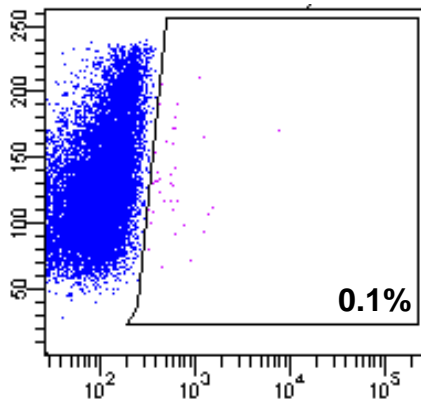
TALEN Only

TALEN + AAV

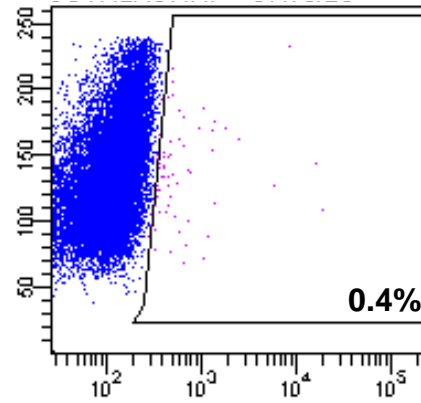


# CRISPR/Cas9 and AAV CD40L cDNA Donor Restores CD40L Expression in XHIM T Cells

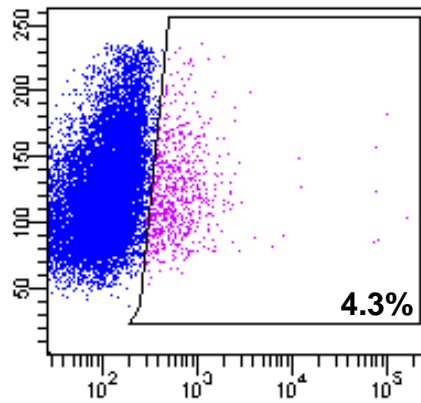
CRISPR Only



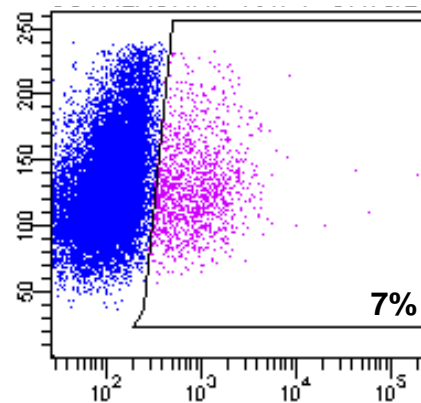
CRISPR Only (Stim.)



CRISPR + AAV Donor



CRISPR + AAV Donor (Stim.)



% CD40L

CRISPR Only

AAV Donor Only

CRISPR + AAV Donor

# Gene Modified XHIM T cells Respond Physiologically to Re-stimulation

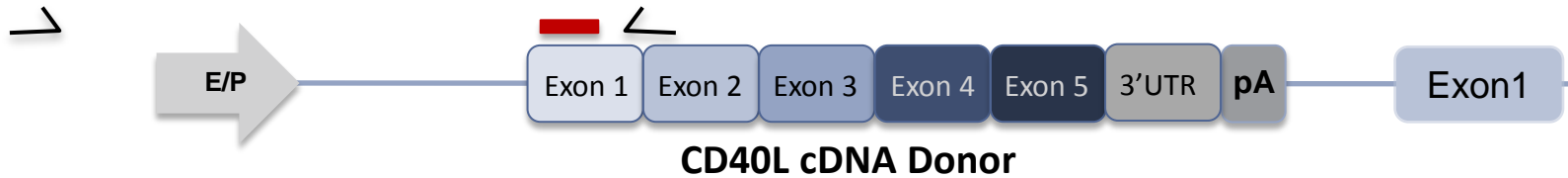
**TALEN**

**% CD40L**

**CRISPR**

**% CD40L**

# Correlation Between Flow Expression and Site-Specific Gene Insertion by ddPCR



%CD40L/Gene Modification

TALEN Only

AAV Donor Only

TAL+AAV Donor

CRISPR Only

CRISPR + AAV Donor

# TALENs and CRISPRs Achieve High Rates of Gene Modification in CD34+ HSC

- Day -2: Prestimulation of CD34+ HSPC
- Day 0: Nuclease electroporation and AAV transduction
- Day 1: Cell counts (survival)
- Day 3-4: Analysis

% Gene Modification by ddPCR

Mock

TALEN Only

TALEN + AAV Donor

CRISPR Only

CRISPR + AAV Donor

# Summary

- Achieved targeted gene modification at the CD40L locus in cell lines, primary XHIM T cells, and primary hematopoietic stem cells
- Corrected XHIM T cells responded physiologically to immune stimuli
- Future Directions:
  - Differentiate gene corrected CD34+ HSCT and demonstrate functional T cell reconstitution in NSG mice and artificial thymic organoids

# Long-Term Goals

- Demonstrate safety and efficacy of site-specific gene therapy for XHIM *in vitro* and *in vivo*
- If sufficient efficacy and safety observed, translate the optimal approach to a clinical trial of autologous transplantation/gene therapy

# Thank you!

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