

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

Caroline Kuo, MD

Pediatric Allergy & Immunology

Clinical Instructor



David Geffen
School of Medicine

Mattel Children's Hospital 



Disclosures

- None.



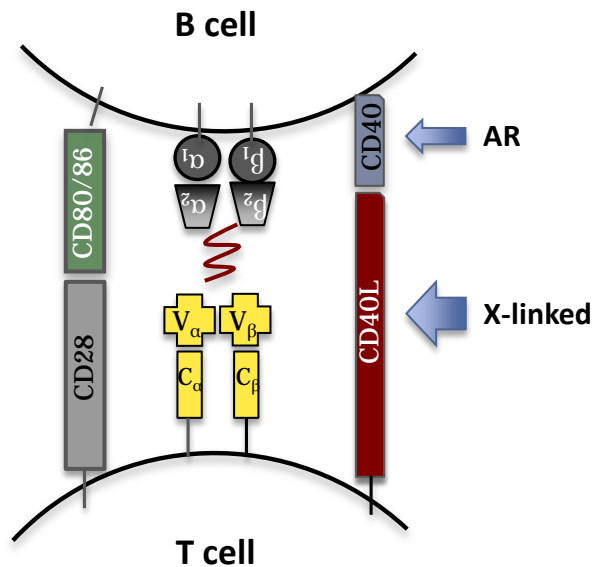
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Hyper-immunoglobulin M syndromes

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

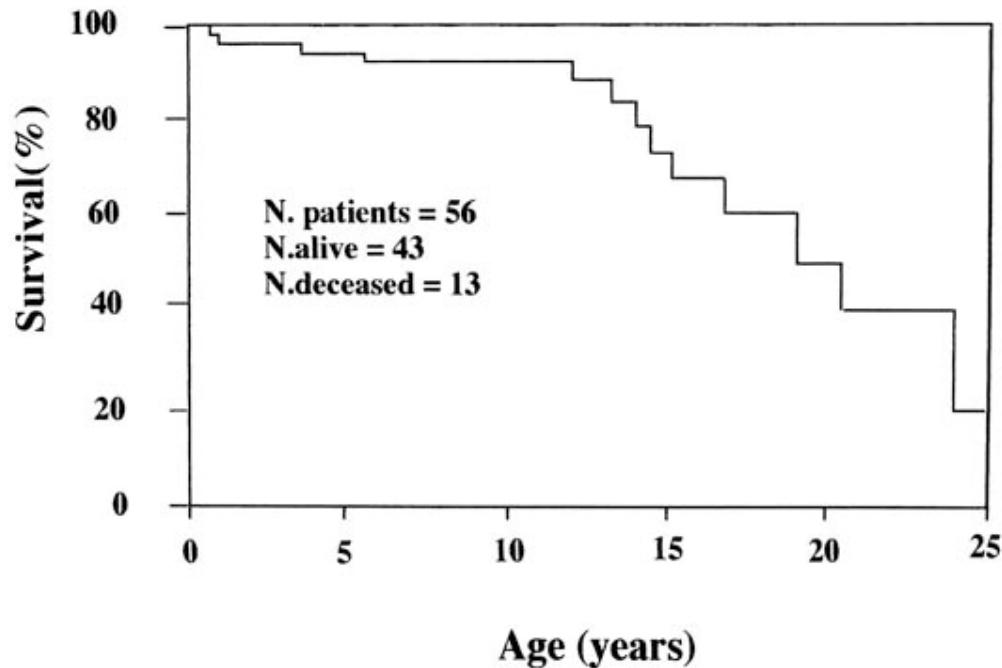


- Clinical Presentation

- 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.

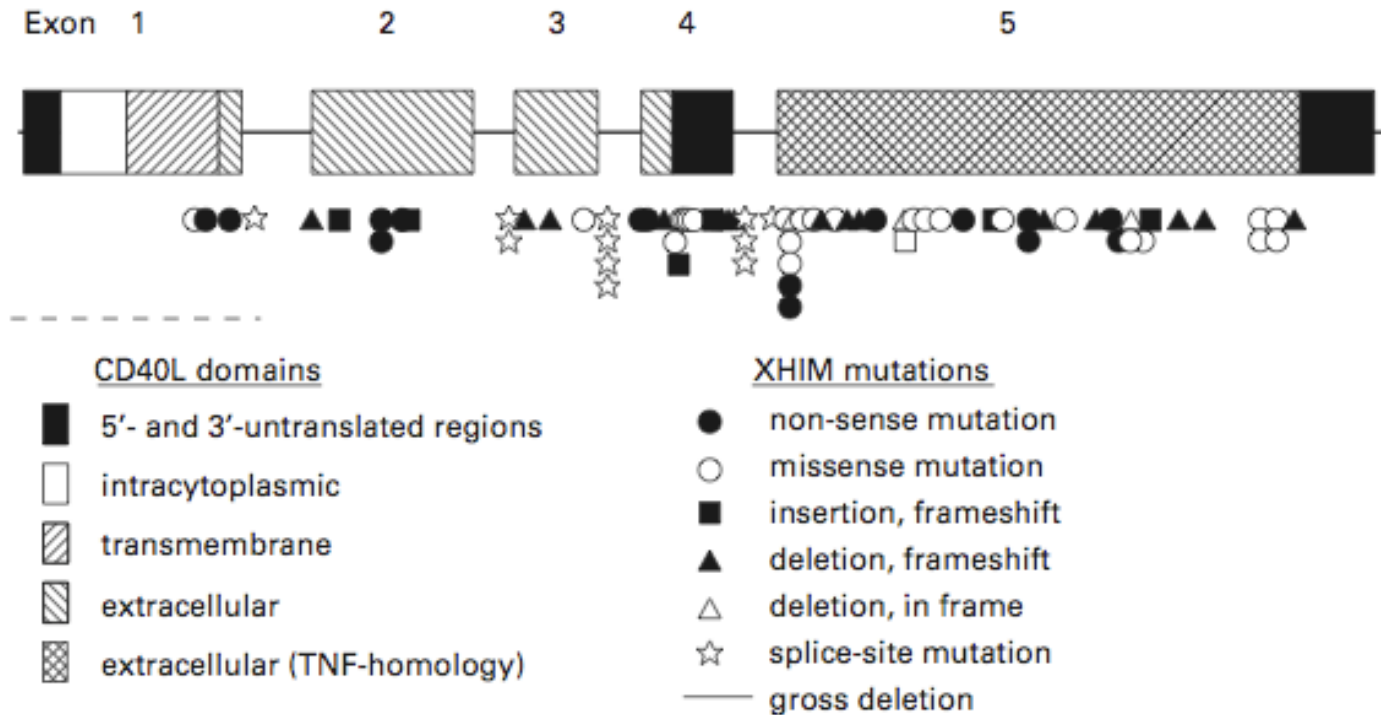


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CD40L Defects as a Candidate for Gene Therapy



Notarangelo L & Hayward A, 2000.



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Gene Therapy For XHIM

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

MICHAEL P. BROWN¹, DAVID J. TOPHAM², MARK Y. SANGSTER², JINGFENG ZHAO¹,
KIRSTEN J. FLYNN², SHERRI L. SURMAN², DAVID L. WOODLAND², PETER C. DOHERTY²,
ANDREW G. FARR³, PAUL K. PATTENGALE⁴ & MALCOLM K. BRENNER⁵

NATURE MEDICINE • VOLUME 4 • NUMBER 11 • NOVEMBER 1998

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,¹ Marco Ungari,² Enrica Mira Catò,¹ Anna Villa,¹ Dario Strina,¹
Luigi D. Notarangelo,³ Jos Jonkers,⁴ Luigi Zecca,¹ Fabio Facchetti,² and Paolo Vezzoni¹

Cancer Gene Therapy, Vol 7, No 10, 2000: pp 1299–1306



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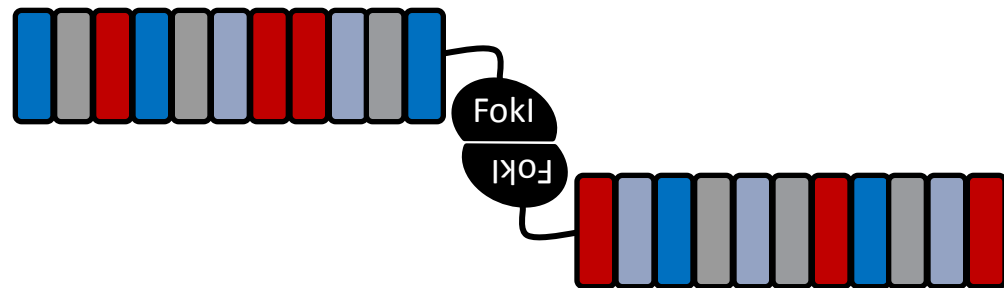
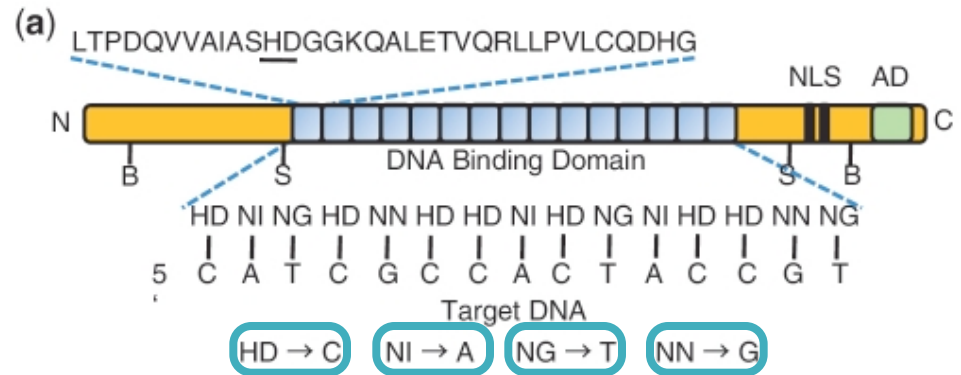
Rationale

- 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
- 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.

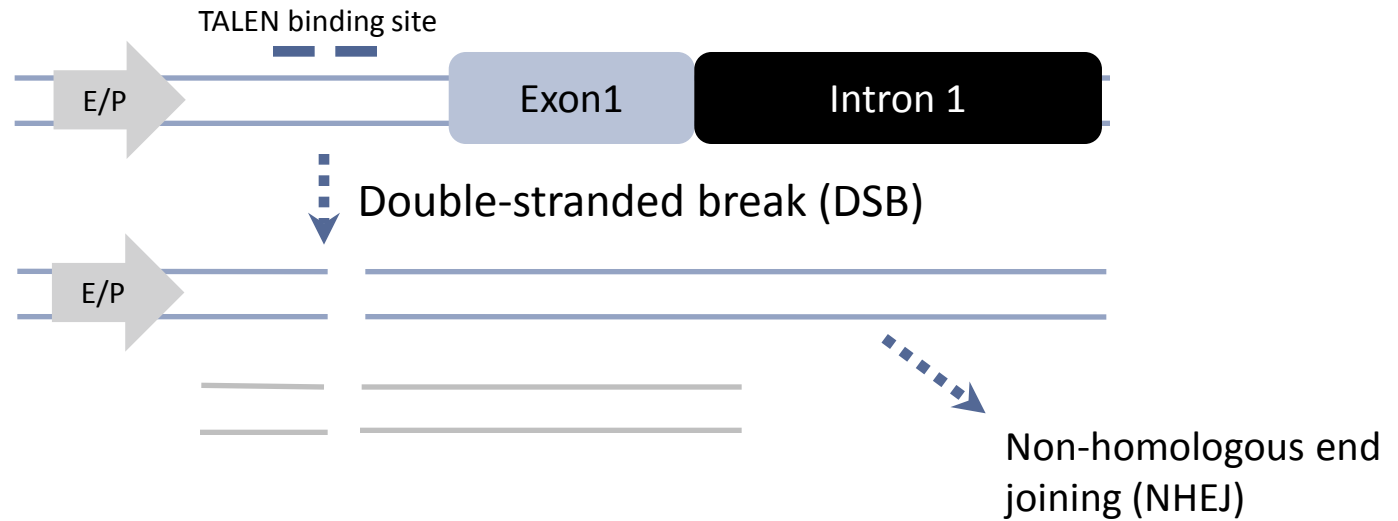


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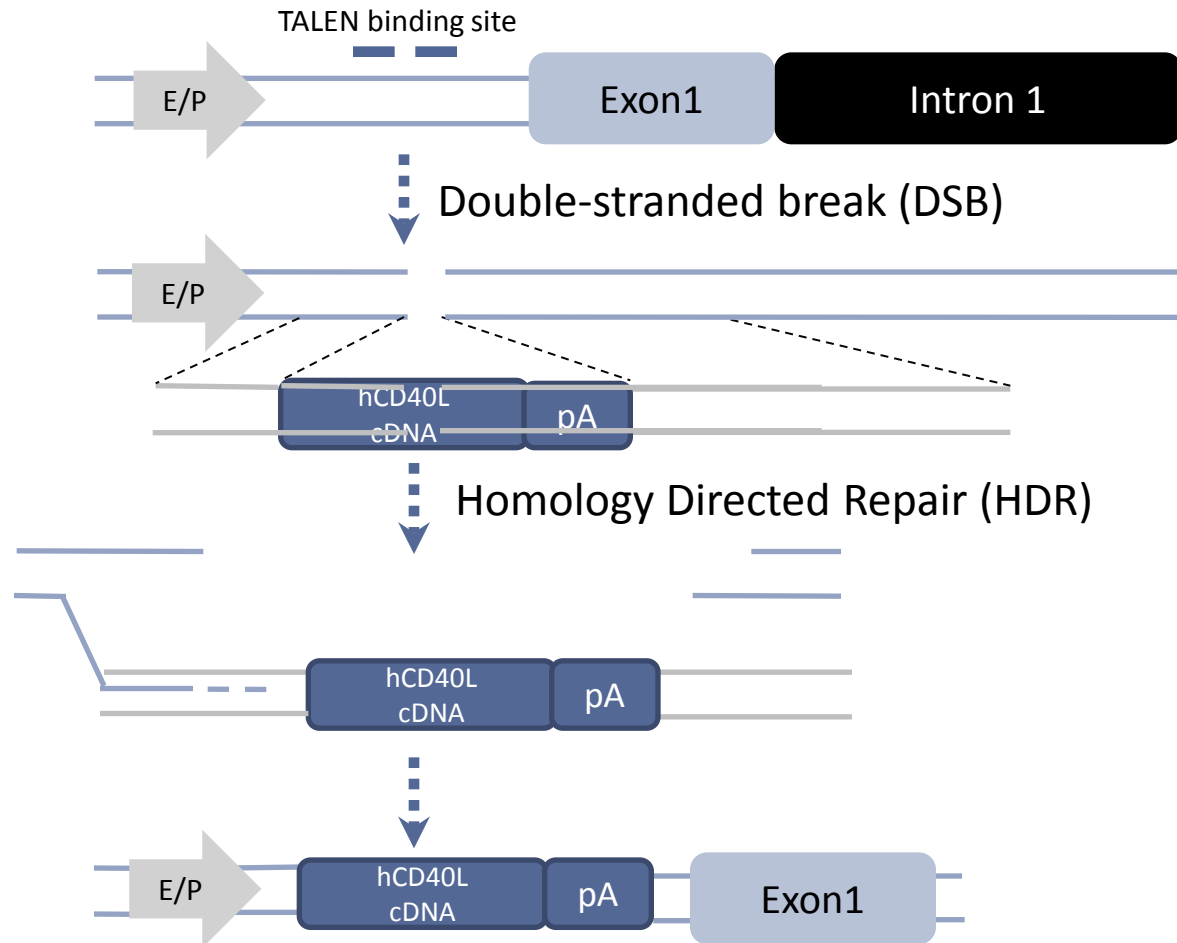
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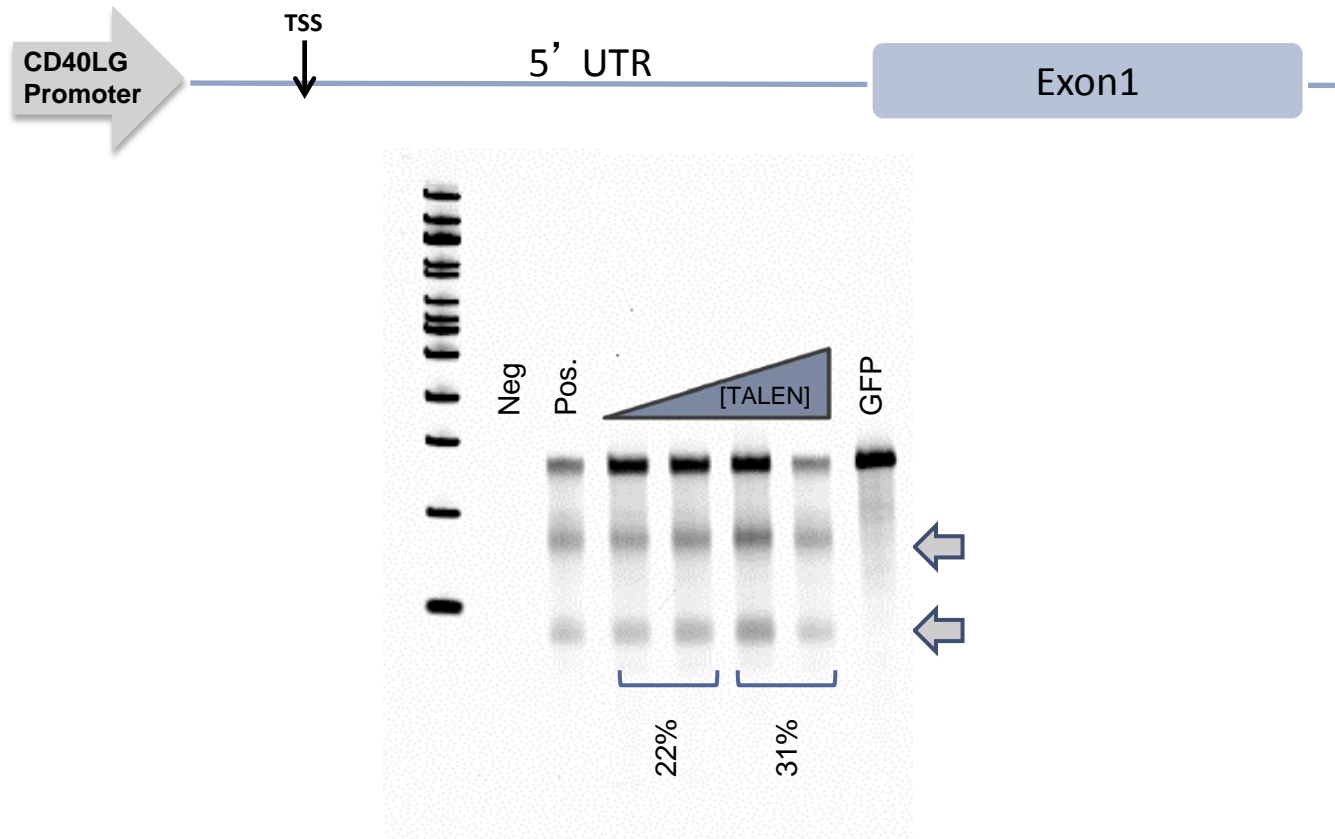
Targeted CD40L Gene Insertion



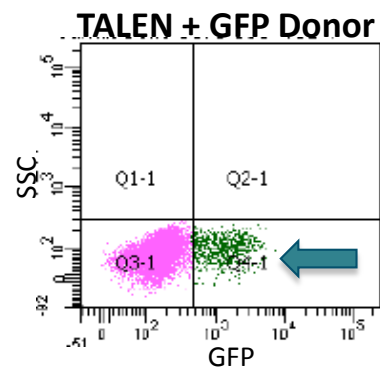
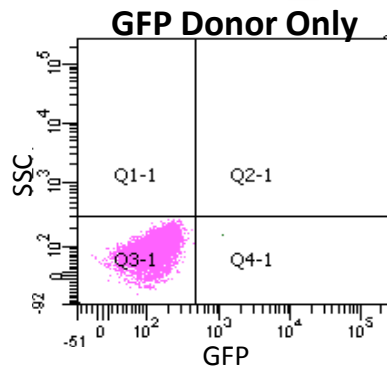
Targeted CD40L Gene Insertion



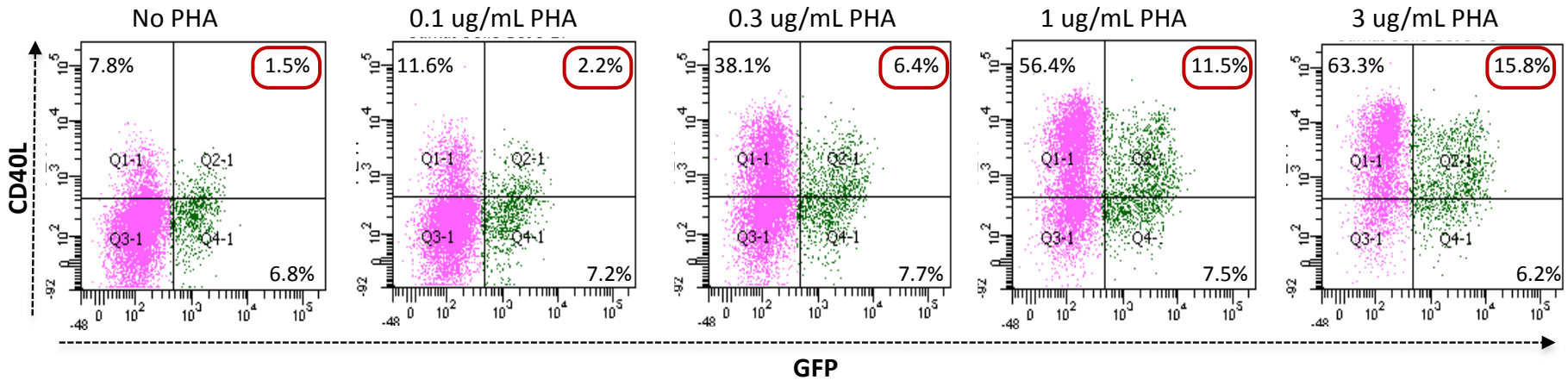
TALENs Introduce Site-specific DSBs at the CD40L Locus in K562 Cells



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



PHA-L Stimulation of Electroporated Jurkat Cells Increases GFP Expression



% GFP



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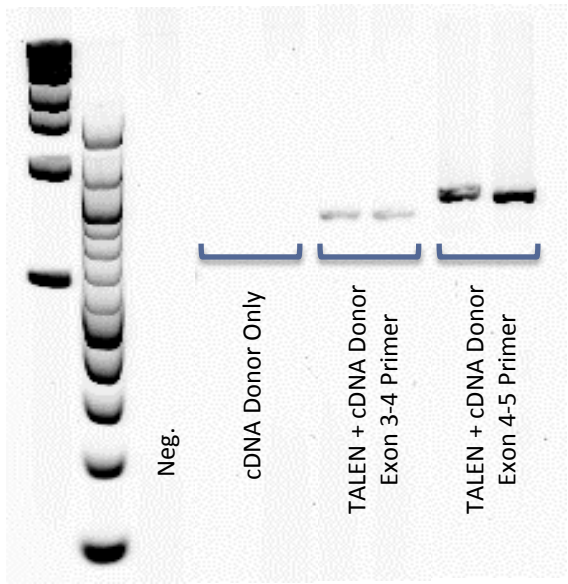
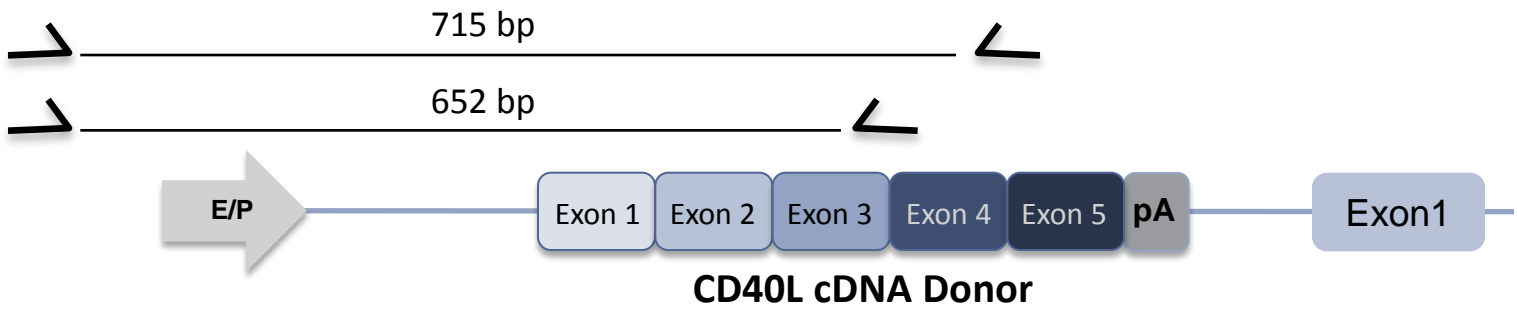


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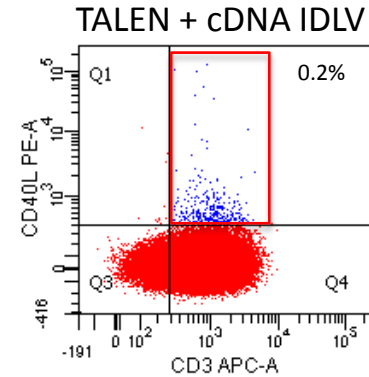
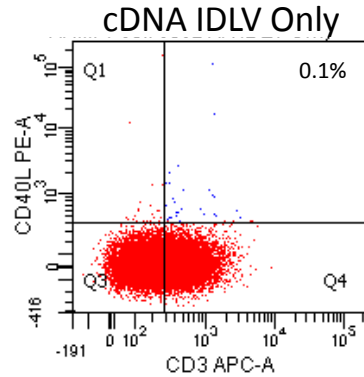
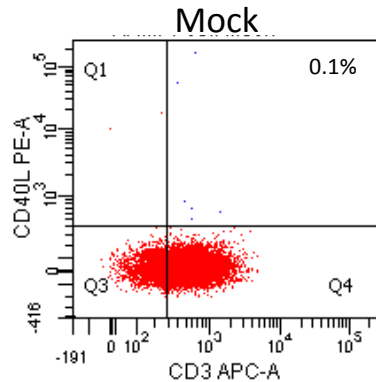
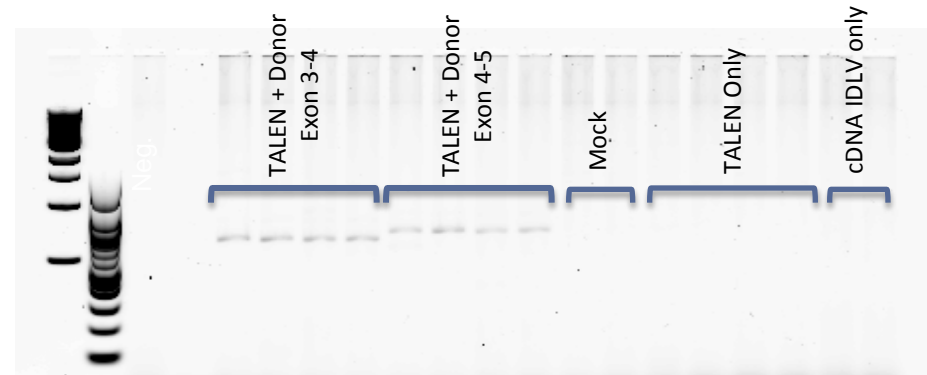
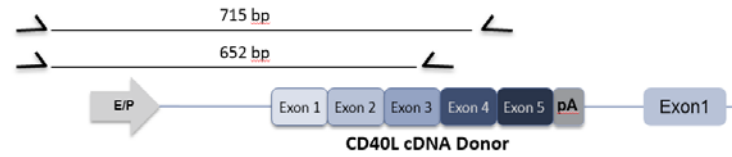
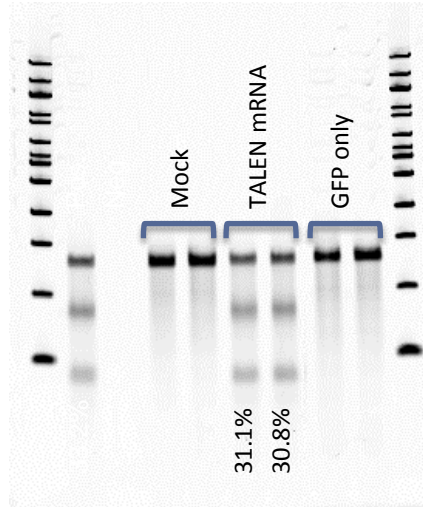
Targeted CD40L cDNA Addition in K562 Cells



Targeted CD40L cDNA Addition in K562 Cells



Targeted Addition of CD40L cDNA in XHIM Primary T cells

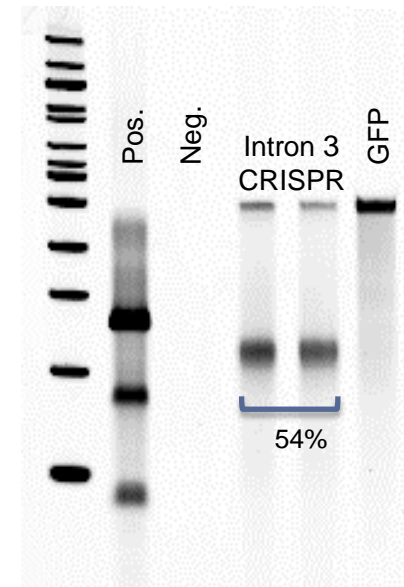
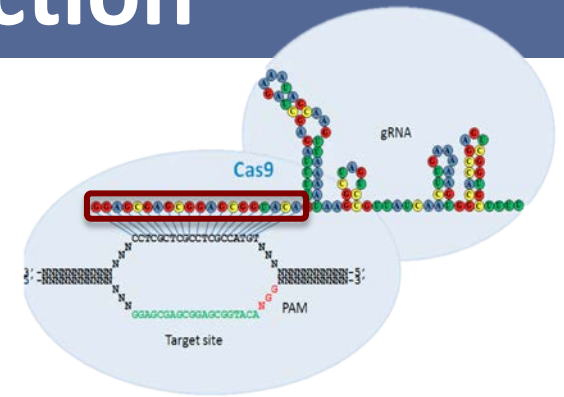
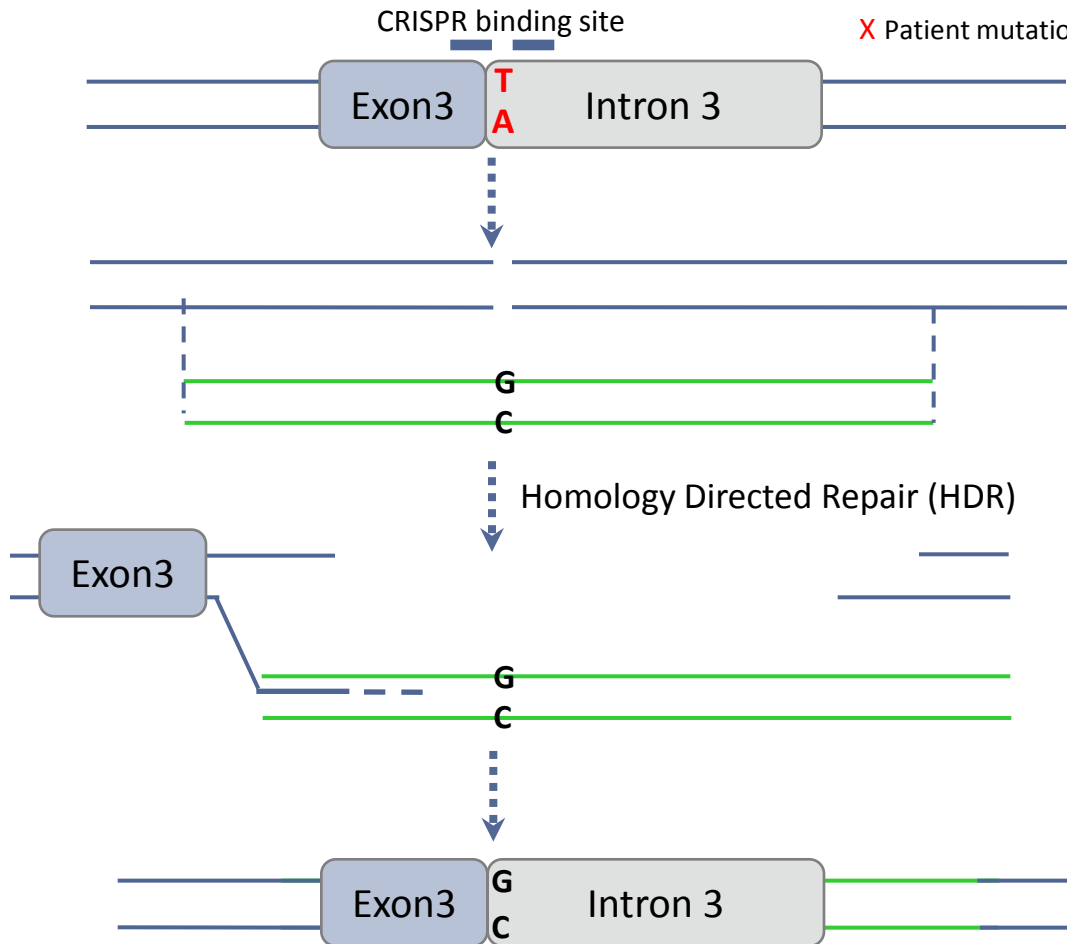


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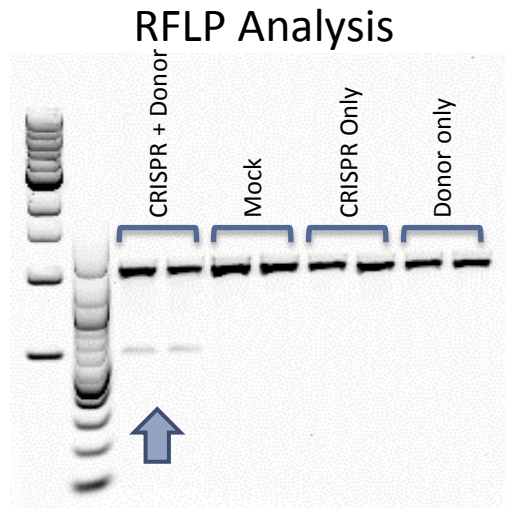
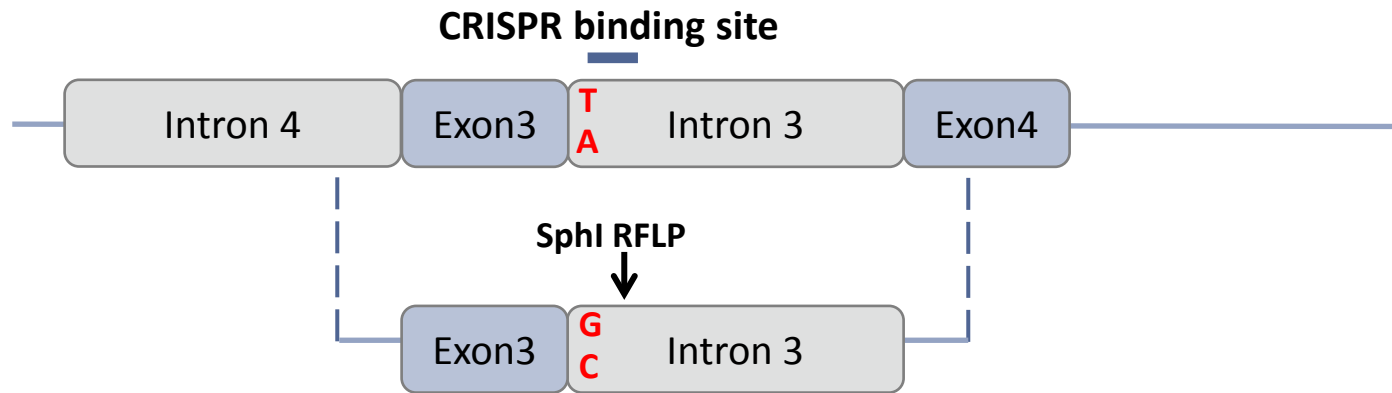
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Patient Specific Gene Correction



Patient Specific Gene Correction in K562 Cells



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!

Donald Kohn, MD

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