Poster #: 508

Efficient Targeted Integration in Human T Cells with CRISPR-Cas9 for the Treatment of X-Linked Hyper-IgM Syndrome

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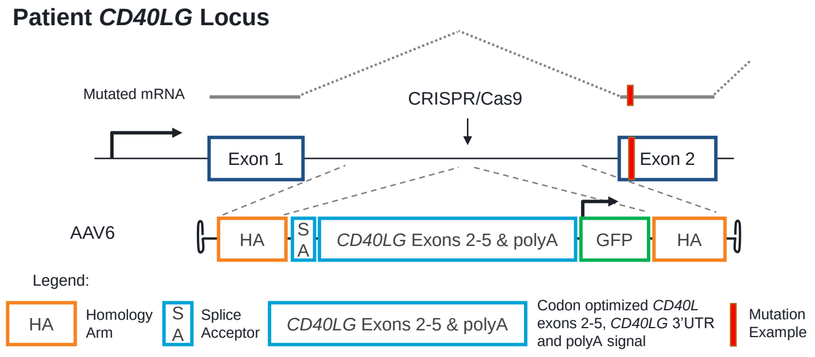
Introduction

- X-linked Hyper-IgM Syndrome (X-HIGM) is a rare primary immunodeficiency characterized by recurrent infections.
- X-HIGM is caused by mutations in the *CD40LG* gene, with >90% of the patient mutations in exons 2-5 [1-3].
- Expression of a corrective gene must be under the endogenous *CD40LG* promoter since constitutive expression of CD40L is causative of a lymphoproliferative phenotype in mice[4].
- To address this unmet medical need, we have developed a cDNA replacement strategy to introduce a corrected cDNA of *CD40LG* exons 2-5 into intron 1.
- The objective of this study was to identify gRNA in *CD40LG* intron 1 that would allow for efficient integration and optimal expression of the corrective cDNA.

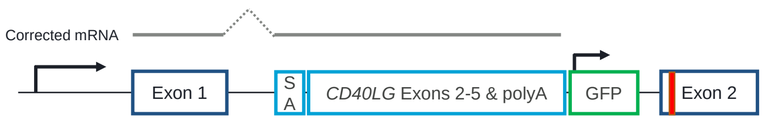
CD40LG Editing & Experimental Strategy

Introduction of a cDNA in primary T cells containing a splice acceptor and codon optimized *CD40LG* exons 2-5 into intron 1 will allow for utilization of the *CD40LG* endogenous promoter and correction of any of the diverse patient mutations found in exons 2-5.

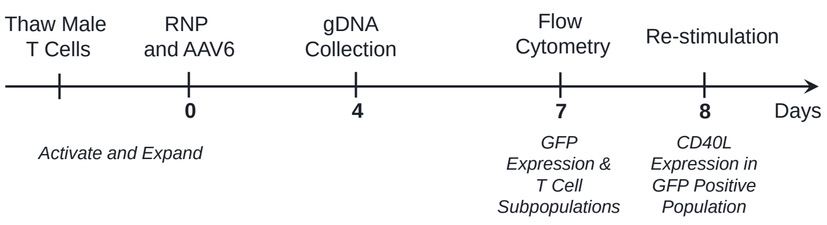
Patient *CD40LG* Locus



Corrected *CD40LG* Locus

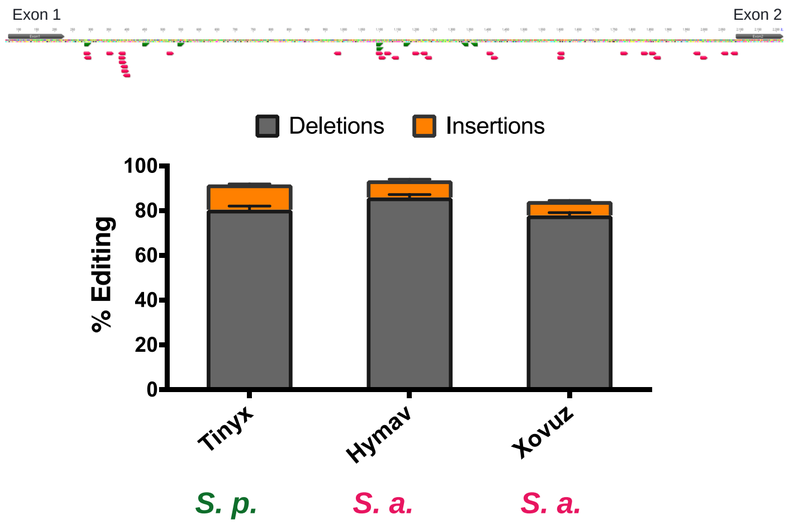


Experimental Timeline



gRNA Screen and Confirmation

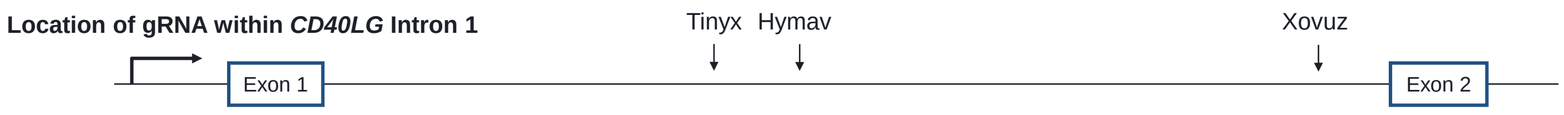
S. pyogenes ***S. aureus***



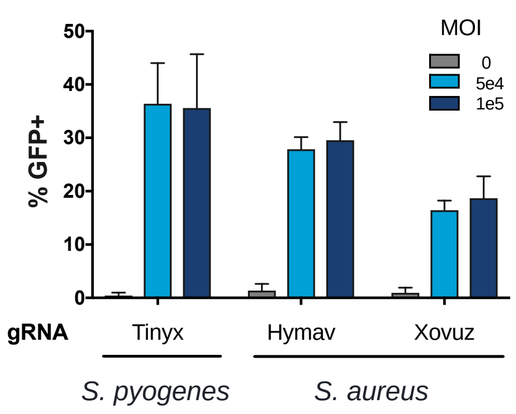
- Among the 8 *S. pyogenes* and 30 *S. aureus* gRNA screened within *CD40LG* intron 1, 3 gRNA were identified and confirmed as top hits
- Top 3 gRNAs achieved >85% editing

References: (1) *Nature* **361**, 539-541, (2) *J Clin Immunol* **36**, 490-501, (3) *J Clin Immunol* **34**, 146-156, (4) *Nature Med* **4**, 1253-1260, (5) *Nat Biotech* **33**, 187-197, (6) *Nat Methods* **12**, 237-243

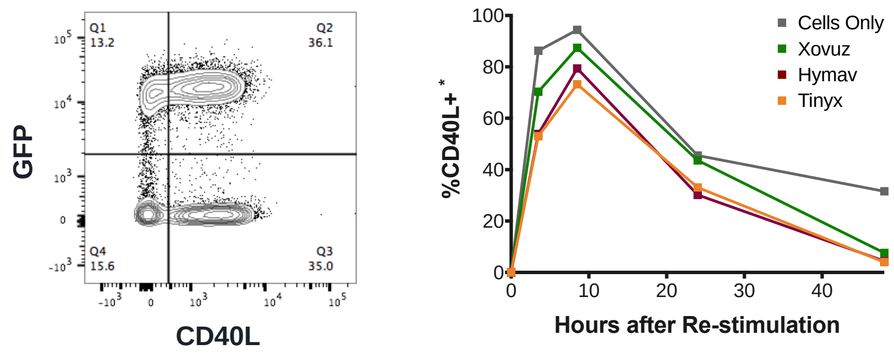
Evaluation of gRNAs for Targeted Integration and CD40L Expression



Targeted Integration Using Top gRNAs



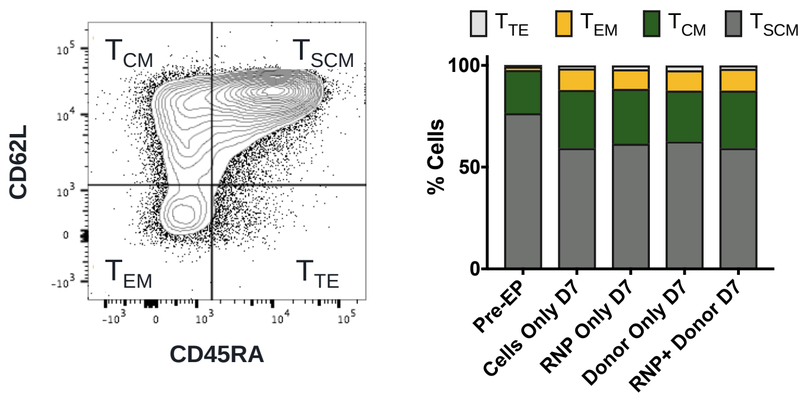
Expression of Integrated cDNA



*Expression of the integrated cDNA was determined as the ratio of the CD40L+ GFP+ cells to all GFP+ cells

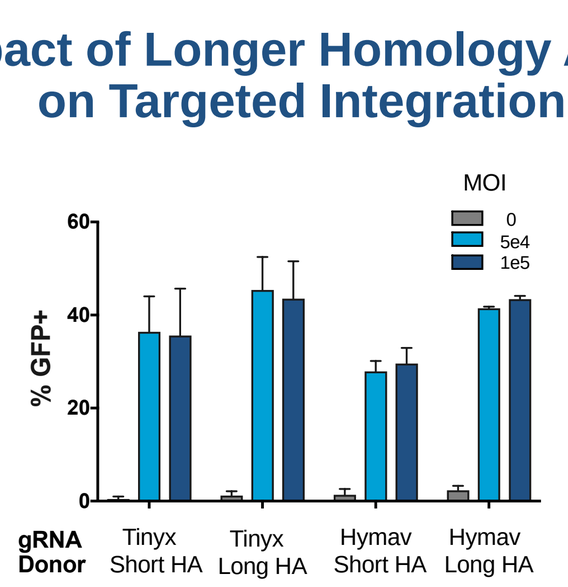
- Tinyx (*S.p.*) and Hymav (*S.a.*) exhibited comparable and higher targeted integration than Xovuz (*S.a.*)
- There were no significant differences in expression of the integrated CD40L cDNA depending on location within Intron 1 and cDNA expression was comparable to endogenous expression

Impact of Editing on T Cell Subpopulation Distributions



- A similar distribution was observed 7 days post nucleofection of T cell terminal effector (T_{TE}), effector memory (T_{EM}), central memory (T_{CM}) and stem cell memory (T_{SCM}) subpopulations between edited cells and control

Impact of Longer Homology Arms on Targeted Integration



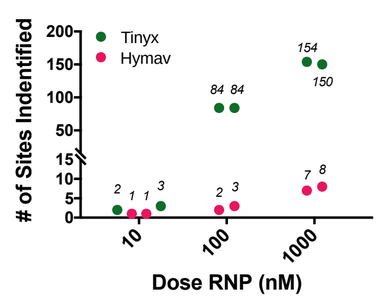
- Longer homology arms showed a trend towards improved targeted integration compared to shorter homology arms at *CD40LG* locus

Evaluation of Specificity of Optimal gRNA

Guide-Seq Analysis

gRNA	Cas9	Average On-Target Editing	Average On-Target Guide-Seq Reads	# Off-Targets Detected	Average Off-Target Editing	Average Off-Target Guide-Seq Reads
Tinyx	*S.p.*	90%	9173	1	0.3%	116
Hymav	*S.a.*	90%	9287	0	0	0

Digenome Analysis



- Guide-Seq[5], an unbiased cell-based assay, identified 1 off-target cut site for Tinyx and none for Hymav
- Digenome[6], an *in vitro* cutting assay, revealed 205 potential off-target sites for Tinyx (*S.p.)* and only 10 for Hymav (*S.a.)*

Conclusions

- 3 highly-effective gRNA were identified in intron 1 of *CD40LG,* 2 demonstrated high levels of targeted integration, and 1 showed an optimal specificity profile
- We achieved CD40L expression under the endogenous promoter at levels comparable to unedited healthy T cells by developing a targeted integration donor comprising an optimal splice acceptor and properly recoded cDNA
- **Overall, we identified the optimal therapeutic candidate gRNA and donor configuration that allows for efficient targeted integration and expression of a corrective *CD40LG* cDNA, demonstrating its potential utility in treatment of patients with X-HIGM**

Conflicts of Interest

CMM, FB, GG, CW, KG, FH, CA, VM, and CCR are shareholders and employees of Editas Medicine. JLG is a shareholder of Editas Medicine. GS, VV, LN, and PG received research funding support from Editas Medicine.