CRISPR-Cas9 T cell editing pipeline for Finnish founder diseases

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Streamline CRISPR-Cas9 tool selection in expanded patient T cells

Background

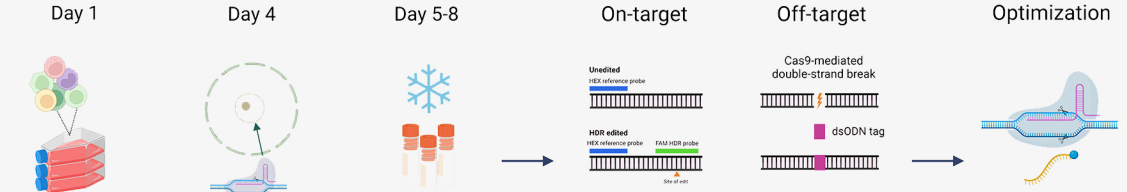
- Protocols for T cell correction & expansion from small blood volumes are useful in pediatric hematology studies and future immunotherapy development
- We used three Finnish founder diseases as models for protocol development for CRISRP-Cas9 applications:

Disease	Gene	Mutation	Mode of inheritance	Clinical phenotype
Adenosine deaminase 2 deficiency	*ADA2*	C>T p.R169Q, rs77563738	Autosomal recessive	Aplastic anemia, large granular lymphocyte leukemia, vasculitis
Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy	*AIRE*	C>T p.R257X, rs121434254	Autosomal recessive	Autoimmune polyendocrinopathy
Cartilage-Hair Hypoplasia	*RMRP*	T>C c.A70G, rs199476103	Autosomal recessive	Viral infections, immunodeficiency-associated cancers, short stature

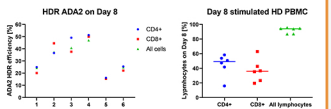
Conclusions

- Efficient T cell correction, enrichment & expansion from peripheral blood mononuclear cells (PBMC)
- Up to 50% homology-directed repair (HDR) of *ADA2*, *AIRE* and *RMRP* loci
- GUIDE-seq optimized for patient PBMC
- Pipeline applicable for other monogenic immune diseases

References:
1. Schumann et al. (2015). Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. *PNAS*
2. Tsai et al. (2015). GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat Biotechnol*
3. Renaud et al. (2016). Improved Genome Editing Efficiency and Flexibility Using Modified Oligonucleotides with TALEN and CRISPR-Cas9 Nucleases. *Cell reports*
4. Merit et al. (2021). Rapid genome editing by CRISPR-Cas9 PCLO,G fusion. *eLife*
5. Haapaniemi et al. (2018). CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nature medicine*



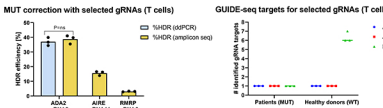
CD4+ CD8+ enrichment



What we learned

- Robust T cell correction and expansion from small PBMC quantities (≥1M initial cells with up to 24X fold exchange by Day 8)
- Cell culture consists of ~90% CD4+ and CD8+ T cells on Day 8
- Uniform editing levels across PBMC cell types
- Editing efficiency is donor-dependent[1]

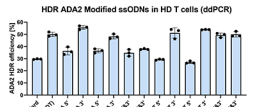
Off-target profiling by GUIDE-seq & selection of gRNAs



What we learned

- gRNAs selected by screening 7-18 gRNAs per locus
- gRNA performance correlates between primary T cells, fibroblasts and CD34+ stem cells
- Good correlation between ddPCR and amplicon seq (ADA2)
- Optimized GUIDE-seq[2] for quantifying off-target editing in patient PBMCs

HDR improvement strategies



What works

- 3' modifications improve ssODN stability and enhance HDR[3]
- HMGB1 Cas9 fusion improves editing in T cells[4]

What doesn't

- Cell cycle timer (AcrIIA2-Cdt1)[4]
- p53 inhibition[5]
- Most Cas9-fusions are cell type and locus-specific[4]

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