Efficient and sustained *FOXP3* locus editing in hematopoietic stem cells as a therapeutic approach for IPEX syndrome.

Abstract Topics:

1. (B) Gene Targeting and Gene Correction

2. (G) Stem Cell Engineering and Therapies

Key Words: IPEX, FOXP3, RNP, CRISPR, gene editing, HSC, AAV

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Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare monogenic primary immunodeficiency, characterized by multi-organ autoimmunity. IPEX syndrome is caused by defective regulatory T (Treg) cell generation and function due to mutations in transcription factor FOXP3. The dysregulation of Treg cells is the main pathogenic event leading to early disease onset and fatality typically within the first few years of life. Supportive therapies combined with pharmacological immunosuppression are currently used for controlling the symptoms of the disease. Allogeneic hematopoietic stem cell transplantation leads to best outcomes provided a matched donor is available. Gene therapy by transduction of $CD4^+$ T cells with lentiviral vectors constitutively expressing FOXP3 can convert effector T cells into Treg cells, although long term survival of transduced cells in vivo is still under investigation. Replacement of the defective FOXP3 gene with a functional cDNA in hematopoietic stem cells is likely to provide an effective and sustained long-term cure as this will allow locus specific expression of FOXP3 regardless of the downstream mutation. To this end, we tested multiple guide RNAs for their ability to cleave at the *FOXP3* locus and achieved up to 80% cleavage in mobilized $CD34^+$ peripheral blood stem cells (PBSC) from multiple GM-CSF-mobilized donors with the top performing guide RNA. We then utilized the CRISPR/Cas9 (delivered as RNPs) to introduce a double strand break and provided donor templates with homology arms flanking a GFP expression cassette via an AAV6 donor vector to achieve homology directed repair (HDR). HDR rates ranging from 40-60% were achieved in PBSC from multiple healthy donors. Next, we assessed the long-term engraftment of edited (GFP-expressing) PBSC in NOD.Cg-$Kit^{W\text{-}41J}$ $Tyr^{+}Prkdc^{scid}$ $Il2rg^{tm1Wjl}$ recipient mice. Experimental mice were analyzed 12-16 weeks post-transfer of edited PBSC for engraftment of $hCD45^+$ cells in the bone marrow. Up to 80% of the cells in the bone marrow were $hCD45^+$ with ~5% of those cells expressing GFP, indicating the ability of edited cells to engraft long term. All recipients harbored edited cells in the myeloid, granulocyte, and B cell populations and these lineages were present at ratios equivalent to recipients of mock-edited human $CD34^+$ cells, suggesting that the differentiation capacity is not compromised by editing the *FOXP3* locus. Finally, we edited the *FOXP3* locus with AAV6 donors designed to introduce a FOXP3 cDNA to enable expression from the endogenous promoter upon targeted integration. Using this approach, as determined by droplet digital PCR, we achieved efficient locus editing with rates comparable to our GFP donor constructs. Overall, these findings demonstrate efficient editing of the *FOXP3* locus in human hematopoietic stem cells and sustained engraftment of FOXP3 edited cells at levels predicted to provide clinical benefit. Taken together, these finding support pursuit of this approach as a potential long-term therapy for IPEX patients.