Manufacturing of gene-edited, regulatory-like, T cells (edTreg) for treatment of IPEX and other autoimmune disorders

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IPEX (immunedysregulation, polyendocrinopathy, enteropathy, X-linked) is a severe congenital autoimmune disorder resulting from hemizygous inheritance in males of a mutant *FOXP3* allele. *FOXP3* encodes a transcription factor that governs the development, maintenance, and function of regulatory T cells (Treg). Tregs are a distinct T cell lineage that restrict autoimmune responses in healthy subjects by suppressing self-reactive effector T cell activation and proliferation. To date, more than 70 *FOXP3* mutations have been identified in IPEX patients. IPEX syndrome manifests as early and severe onset of multi-organ autoimmunity, including severe enteropathy, T1D, thyroiditis and dermatitis. Without stem cell transplant or aggressive immunosuppression, most of affected subjects will die within the first few years of life. Even if a stem cell transplant is available, achieving sufficient clinical stability to undergo the transplant procedure is challenging. Here, we aim to develop an autologous regulatory T cell therapy for IPEX and other severe autoimmune disorders using a modification of a previously reported gene-editing approach. In our previous proof-of-concept gene editing design we co-delivered TALEN nucleases and AAV6 donor template designed to introduce a constitutively active MND promoter into the *FOXP3* locus via homology-derived repair (HDR). This approach permitted us to bypass endogenous *FOXP3* promoter silencing and enforce FOXP3 expression in CD4 effector T cells (Teff). The resultant high level and stable expression of endogenous FOXP3 converted Teff to Treg-like cells with immunosuppressive activity. In the current study, to permit therapy for IPEX, we modified our gene editing of *FOXP3* to utilize co-delivery of CRISPR/Cas9 RNPs and an AAV6-delivered donor template designed to integrate an expression cassette containing the MND promoter driving expression of two coding sequences separated by a ribosome skip peptide: functional FOXP3 cDNA and a surface LNGFR tag. On-target donor template integration will provide both stable FOXP3 and selection marker expression for use in manufacturing and also simultaneously knock-down expression of the mutant *FOXP3* allele in IPEX. In parallel, we established a clinical cell manufacturing protocol enabling the transition to CMC process development. Using the current protocol, we obtained efficient HDR rates across multiple healthy donors. Edited cells were consistently enriched to >95% purity by a magnetic LNGFR antibody selection and expanded 50-fold in a week. Expression of FOXP3 cDNA in edited cells was sufficient to enforce Treg-like phenotypes including the up-regulation of Treg-associated markers (CD25, CTLA-4, and ICOS), and down-regulation of CD127 and inflammatory cytokines (IL2, IFNgamma, TNFalpha). Importantly, we demonstrate sustained *in vivo* suppressive activity of these edited Treg-like cells (edTreg) in a xeno-GvHD mouse model. edTreg (as well as expanded natural Treg) limited effector T cell expansion and tissue infiltration and significantly protected mice from xeno-GvHD induced by co-transferred autologous effector T cells. Along with preliminary data showing successful editing in CD4 T cells from IPEX patients, our data provide key pre-clinical proof-of-concept and safety data supporting use of edTreg in a clinical trial for IPEX and, potentially, for use other autoimmune diseases.