Crispr-Cas9 mediated gene-therapy for Wiskott-Aldrich Syndrome restores T cell functionality

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Objectives

The Wiskott-Aldrich syndrome (WAS) is an X-linked primary immune deficiency disease caused by a mutation in the WAS gene. This leads to altered or absent WAS protein (WASp) expression and function resulting in thrombocytopenia, eczema, recurrent infections, auto-immunity and an increased risk of leukemia. WAS patients suffer from a suboptimal T cell response to pathogens since the WAS protein is involved in the T cell receptor signaling pathway through actin-filament formation. Furthermore, we have shown that both the immune deficiency and auto-immunity may be explained by a deficient T cell differentiation (Laskowski et al). Yet, more research has to be done to fully elucidate the influence of WASp on the thymopoiesis. Current treatment consists of stem cell transplantation from an HLA matched donor. Because HLA matched donors may not be available and because of the severe side-effects of allogenic transplantation, alternative therapeutic approaches are needed. We have developed an efficient Crispr/Cas9 gene editing technique to knock-in wildtype WAS cDNA into patient derived T cells. The corrected T cells are subsequently tested for functionality.

Materials and Methods

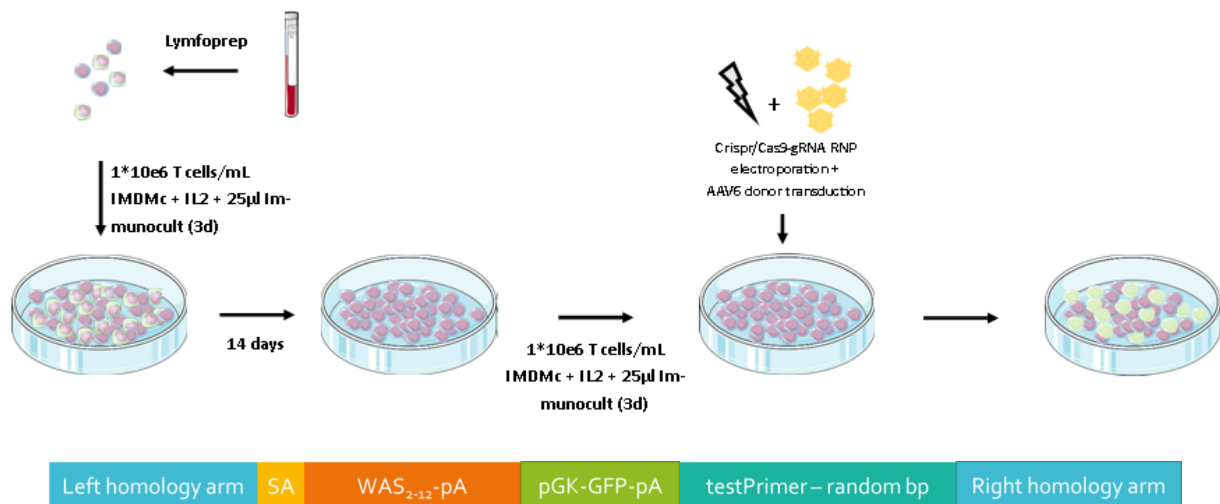

Figure 1: Transduction protocol (top) and donor construct (bottom) for homology directed repair

T cells obtained from PBMC's were stimulated with CD3+CD28 (Immunocult™) for 3 days and subsequently electroporated with preassembled Crispr/Cas9-gRNA ribonucleoprotein complex. Immediately after electroporation, the cells were transduced with wild type WAS encoding donor-template packaged in viral AAV6 particles (Fig.1). After two-rounds of feeder-expansion, eGFP$^+$-cells were sorted out. Corrected and non-corrected T cells were then assessed for WAS protein expression and subjected to functionality tests. A cytokine production, stimulation and proliferation assays were conducted (Fig.2). Corrected and non-corrected cells were stimulated on aCD3 coated plates and IFN-

γ and IL-2 production, CD69 up-regulation and CD3 down-modulation were assessed. Proliferation was assessed after 6 days by CellTrace™ Violet blue dye dilution.

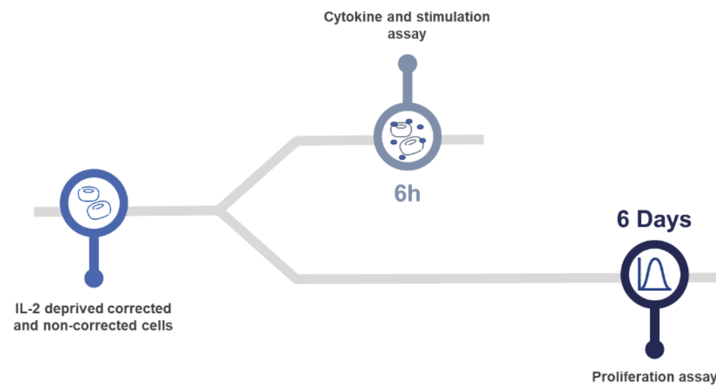

Figure 2: Functional assays timeline

Results

Transduction of T cells derived from a WAS patient was successful in generating WASp corrected cells. Flowcytometric analysis showed a restoration of the intracellular WASp level. Not only was WASp present in the T cells, the T cell function was restored. We showed an increase in IFN-γ and IL-2 production compared to the non-corrected cells. The corrected cells were also able to proliferate upon polyclonal T cell stimulation. Increased CD3 modulation and CD69 up-regulation could be observed in the corrected cells upon Tcell receptor stimulation. Finally, confocal microscopy demonstrated a restoration in protrusion formation suggesting that the actin filament polymerization defect was remediated.

Conclusion

We were able to restore the functionality of WAS^- T cells using our Crispr/Cas9 mediated gene-editing protocol. These results show promise for the future alternative therapy for WAS-patients. Furthermore, this strategy is also effective in restoring WAS functionality in hematopoietic stem cells.