Generation of Selectable, Multi-edited Allogeneic CD3+ T cells

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The engineering of allogeneic “off the shelf” T-cell therapeutics would be facilitated if multiple gene edits could be created by a single manufacturing step. Towards this goal, we are developing a strategy for multi-editing of T cells at the TCR alpha (TCRA) locus and a second locus, (locus “B”) to generate non-alloreactive, engineered T cells that can be inducibly expanded both in vitro and in vivo. This approach employs a two component cytokine receptor, termed a chemically induced signaling complex (CISC) to generate an IL2-like trophic/growth signal in the presence of rapamycin. The proposed strategy involves simultaneous integration of one CISC component at the TCRA locus and a second component at the “locus B”. Using Cas9/guide RNA ribonucleoproteins (RNPs), both TCRA and a “locus B” were successfully knocked down at >90% efficiency. Targeted integration of the CISC components at the TCRA and “B” loci was accomplished using AAV donor templates with homology arms directed at each locus. Combined targeted integration rates of ~15% were achieved over multiple experiments in cells from normal donors, indicating that approximately half the single edited cells are amenable to multi-editing and would be expandable via rapamycin-mediated CISC activation.