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

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Introduction & Objectives

Engineered allogeneic T cells may be applied to enable "off-the-shelf" approaches to the treatment of cancer and auto-immunity. Two important engineering goals for enabling use of allogeneic T cells are:

- Elimination of TCR expression, to avoid the potential for graft vs. host disease following T cell engraftment.
- Regulation of cell proliferation and survival, to provide for physician control over the duration of therapeutic activity.

To achieve these goals, we are developing a cell engineering approach in which the TCR alpha and common cytokine receptor ɣ chain genes are disabled, while the cell is provided an exogenous drug-regulated synthetic common ɣ chain cytokine receptor to support engineered cell survival and proliferation

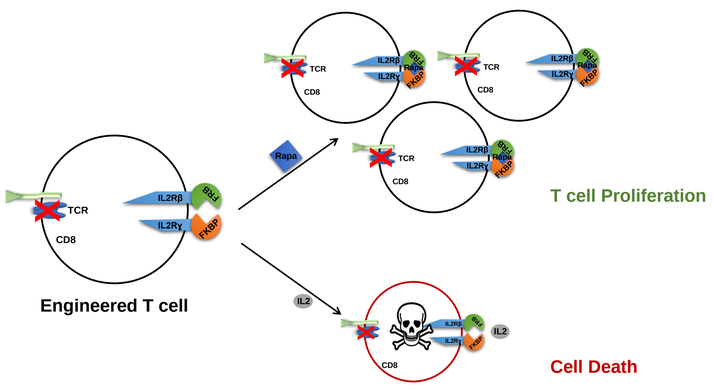

Figure 1. Properties of Casebia's engineered T cell. Schematic depicting the main features of cytolytic engineered T cells; TCR knockout and regulation of T cell proliferation through the IL2Rɣ

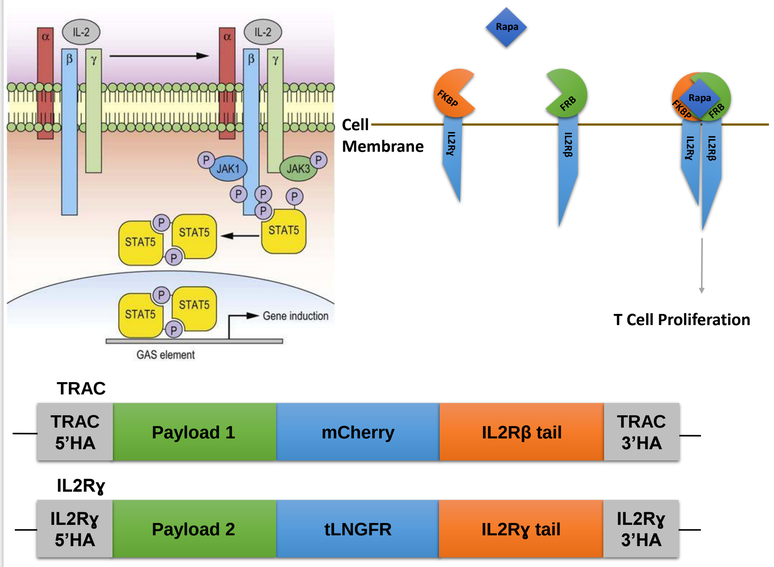

Figure 2. Schematic showing mechanism of signaling for Chemically-induced signaling complex (CISC) in edited cells. The two halves of the CISC are split between the TRAC and IL2Rɣ loci. Therefore, only multi-edited cells will be successfully enriched in culture with CISC

Conclusions

- Multi-editing was achieved with ~80% and ~65% TCR and IL2Rɣ indel efficiencies respectively, followed by ≥20% targeted integration at each loci.
- Approximately half of the single edited cells were also multi-edited, resulting in ~12% multi-edited T cells.
- Multi-edited cells were successfully expanded in the presence of rapalog, AP21967 to generate >90% purity of edited T cells.
- CISC expansion with rapalog potentially eliminates the need for an isolation/purification step during the manufacturing process for edited T cells.
- CISC signaling is sufficient for T cell proliferation following silencing of the IL2 signaling pathway.

Methods

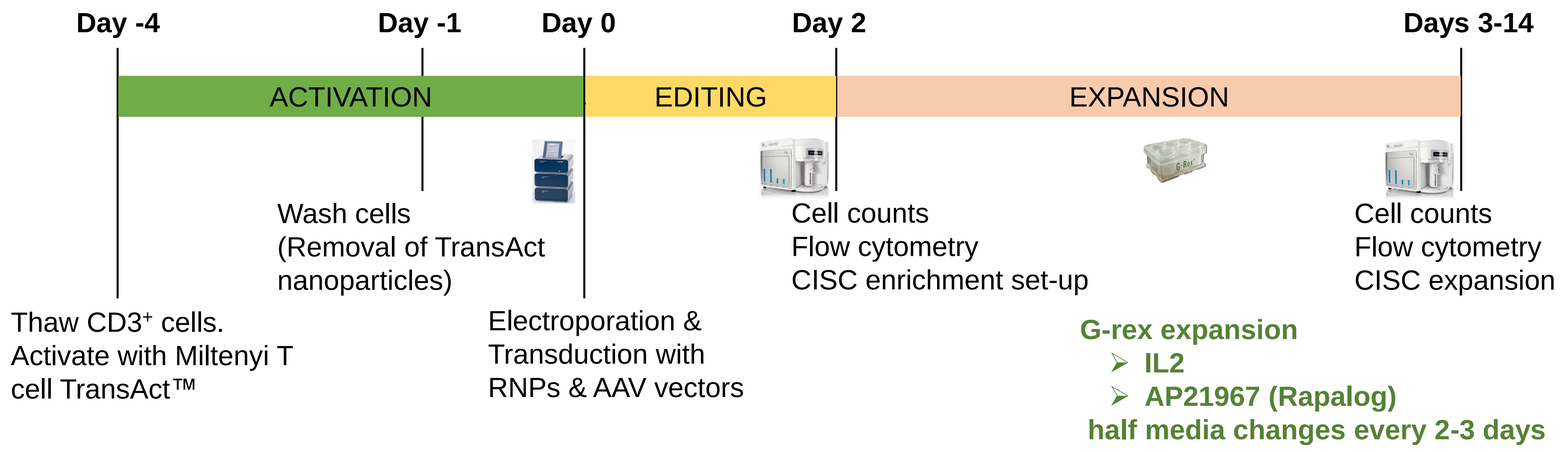

Figure 3. T cell editing protocol

Results: CRISPR/Cas9 Multi-Editing at TRAC & IL2Rɣ Loci

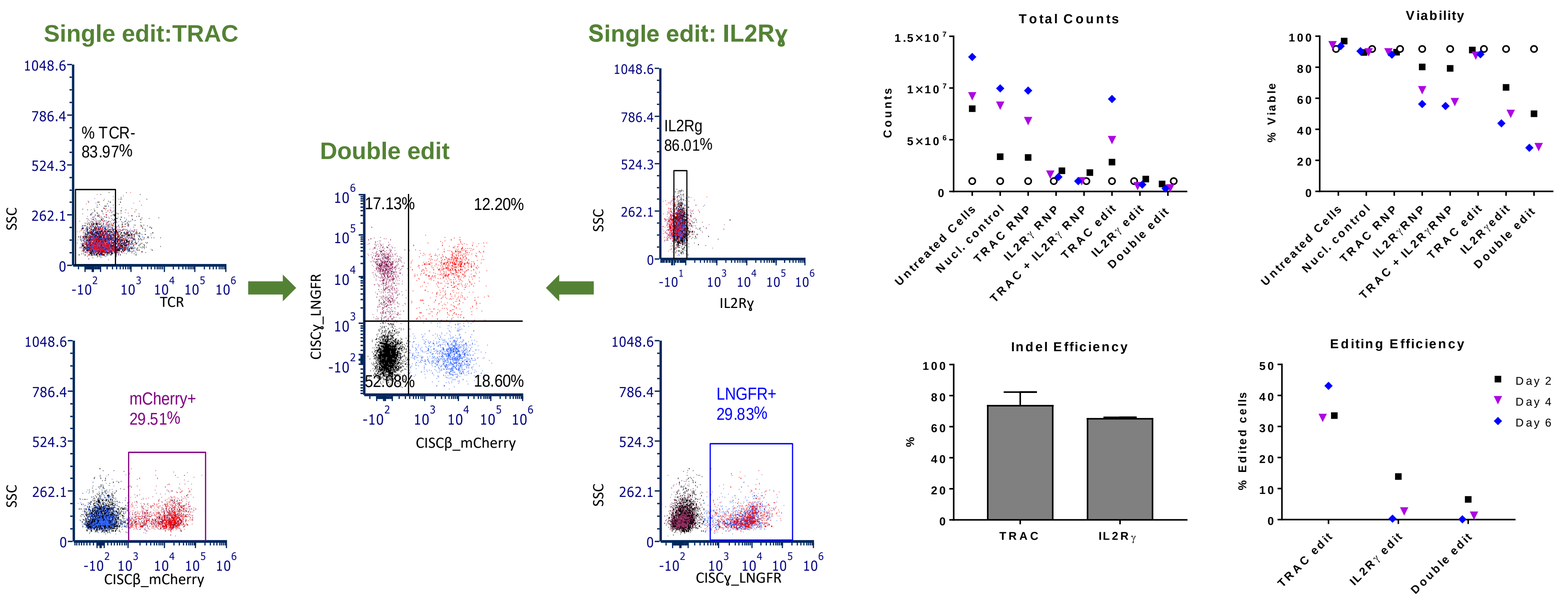

Figure 4. Multi-edited cells are unresponsive to IL2 signaling for T cell proliferation. Representative flow cytometry plots of CD3⁺ cells, on Day 2 post editing. ~12% double-edited cells was achieved through CRISPR/Cas9 RNP knockdown of TRAC & IL2Rɣ, followed by targeted integration of CISC AAV donor templates. Cell counts, viability and editing efficiency was measured over time following silencing of IL2 proliferation signal

Results: Targeted Expansion of Edited T Cells Through CISC

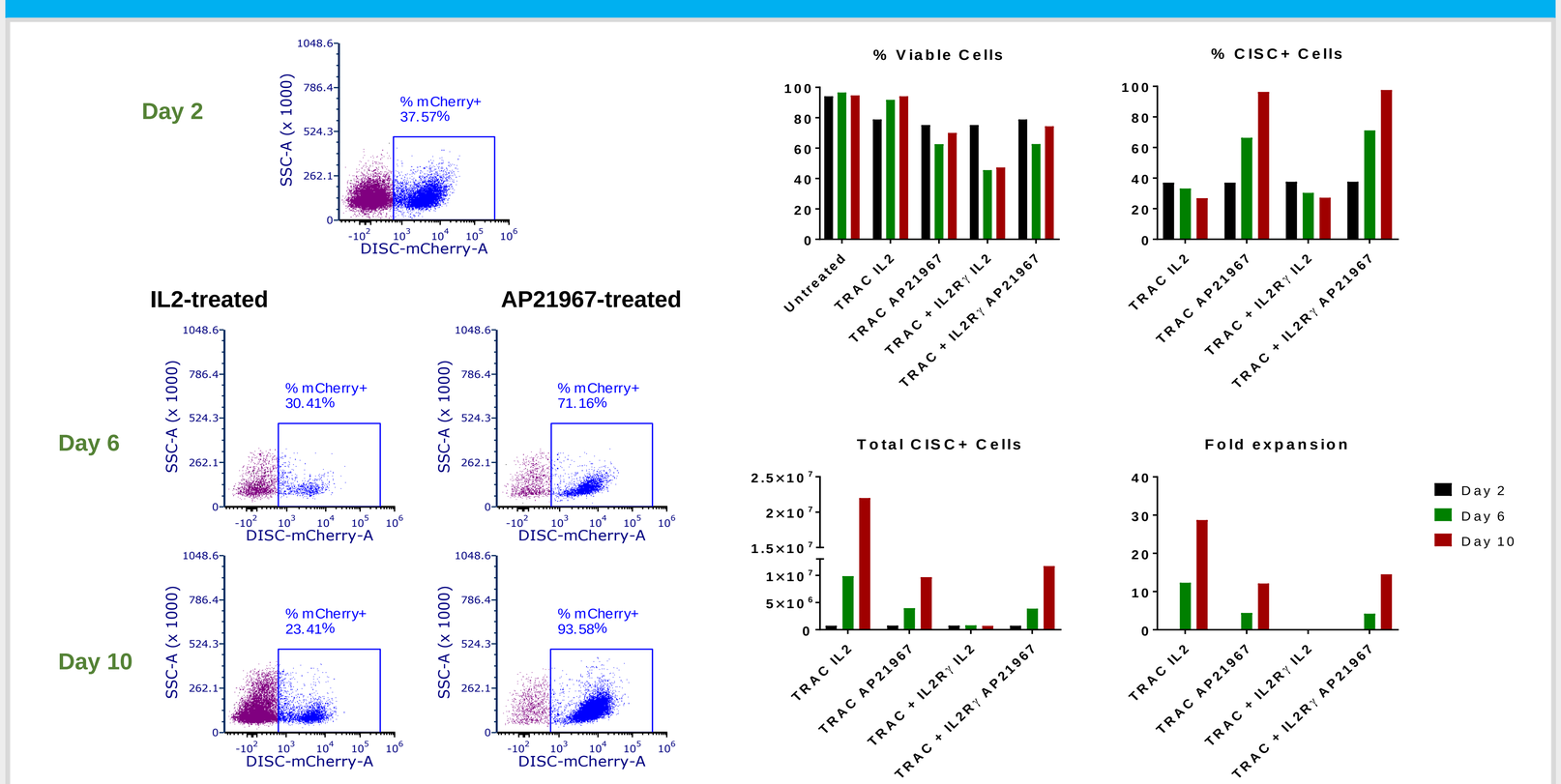

Figure 5. Regulation of edited T cell proliferation through CISC. Representative flow cytometry plots for CISC enrichment & expansion post editing. Edited cells were cultured in either IL2 or AP21967 (rapalog). Expansion of edited cells was monitored over time. CISC expansion with AP21967 allows for preferential expansion of edited cells as shown by both flow cytometry and cell counts