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Development of a Semi-automated Closed CAR-T Manufacturing Process

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Introduction

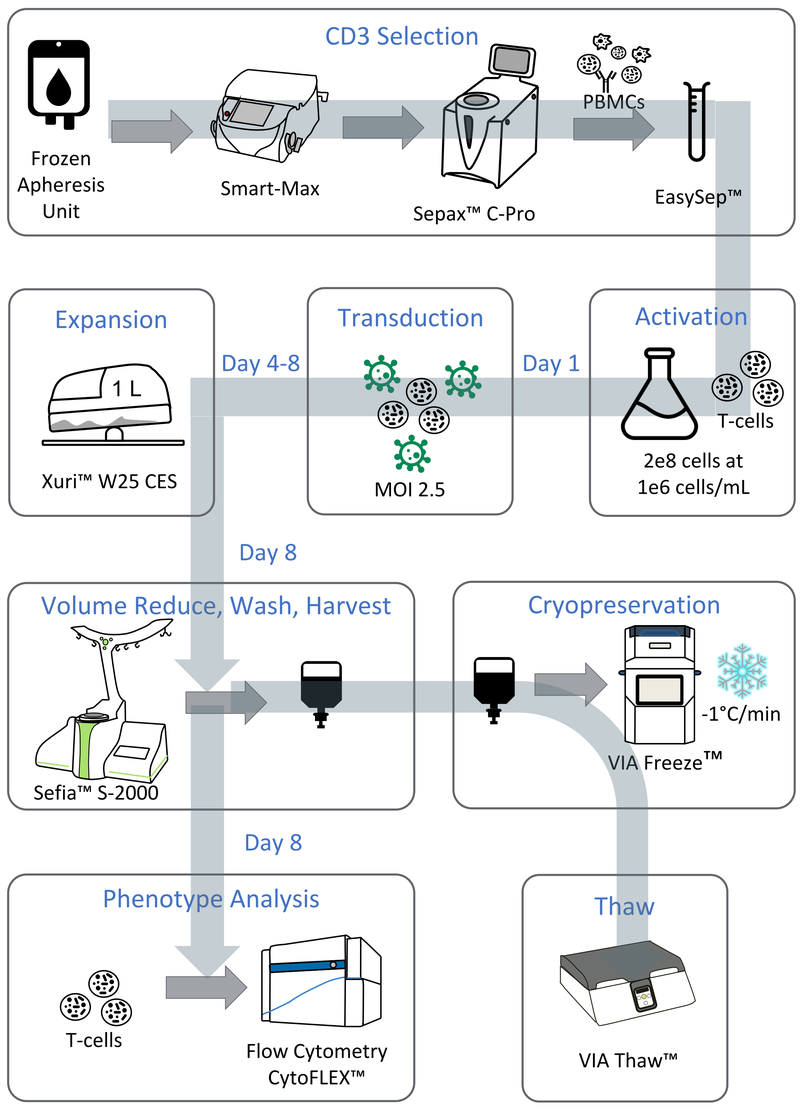
Immunotherapy has generated excitement as what many are calling the "fifth pillar" of cancer treatment. Among the several types of therapies, CAR-T cells have shown impressive therapeutic efficacy, especially in advanced blood cancers. With the recent approval of Kymriah™ and Yescarta™, CAR-T cells continue to be the focus of a growing clinical trial pipeline. However, there are remain challenges associated with routinely offering these products as treatment alternatives, including a costly manufacturing process that relies on a lengthy and complex open workflow with manual manipulations that lead to product variability. To address these challenges, we have investigated individual CAR-T unit operations to identify commercially available reagents and modular equipment to drive process closure and automation that will improve workflow efficiency and product consistency.

Objectives

Systematically build a robust semi-automated closed CAR-T manufacturing process that can be widely adopted by the T-cell immunotherapy industry:

- Identify and apply commercially available reagents that enhance process efficiency
- Integrate automation strategies to reduce manual and open operations
- Maintain flexibility to concurrently manufacture CAR-T products

Methods



Results

T-cell Selection and Enrichment

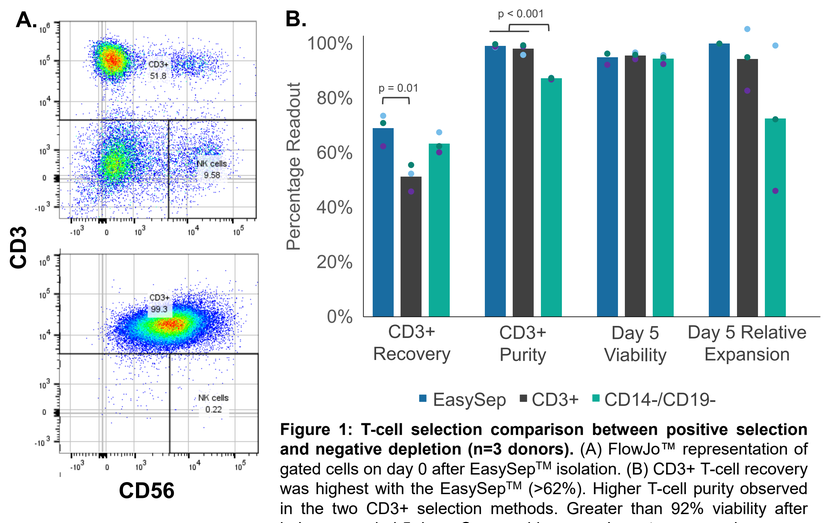


Figure 1: T-cell selection comparison between positive selection and negative depletion (n=3 donors). (A) FlowJo™ representation of gated cells on day 0 after EasySep™ isolation. (B) CD3+ T-cell recovery was highest with the EasySep™ (>62%). Higher T-cell purity observed in the two CD3+ selection methods. Greater than 92% viability after being expanded 5 days. Comparable expansion rates across donors.

T-cell Modification

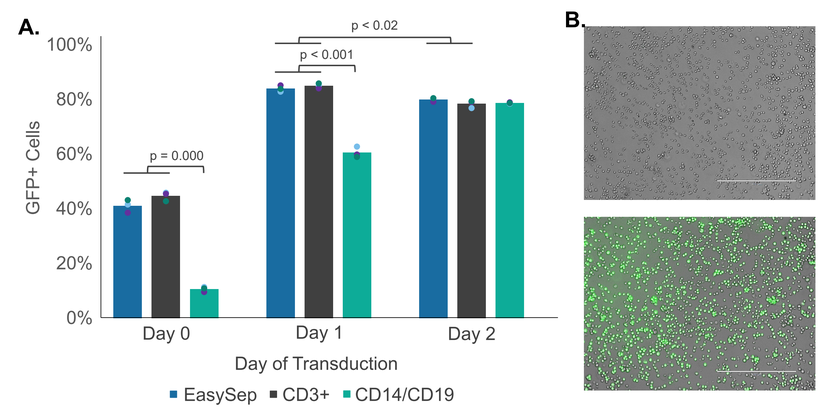


Figure 2: Effect of T-cell selection and day of infection on transduction efficiency (n=3 donors). (A) Higher transduction efficiency in CD3+ selected cells. Day 1 infection generated highest transduction efficiency. (B) Day 7 T-cells transduced with GFP.

Identifying an Early Closed Culture Alternative

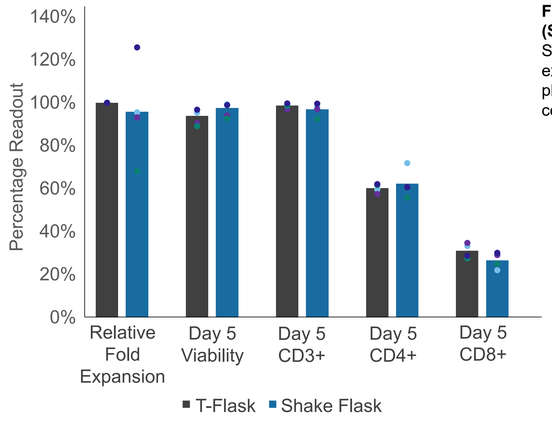


Figure 3: Comparing shake flasks (SF) to T-Flasks (n=3 donors). Shake flask platform offers similar expansion profile to T-Flasks. T-cell phenotype and viability not compromised in SF.

Evaluation of an Automated Closed Transduction (ACTd) Method

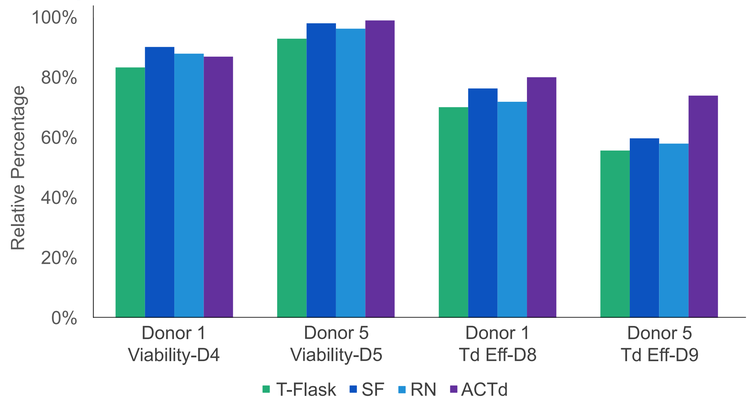
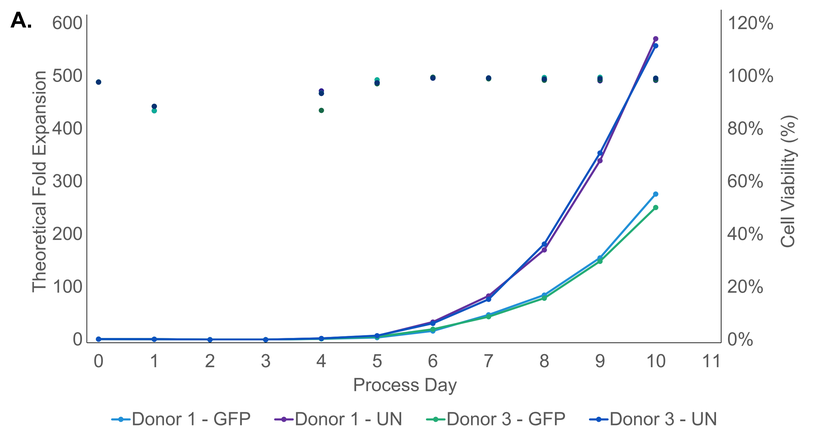


Figure 4: Comparing ACTd to T-Flasks, SF, and retronectin (RN) coated plates (n=2 donors). Highest transduction efficiencies achieved using ACTd method in two donors (>74% transduction efficiency using ACTd) with >87% viability maintained post-ACTd.

Simulated CAR-T Manufacturing Runs



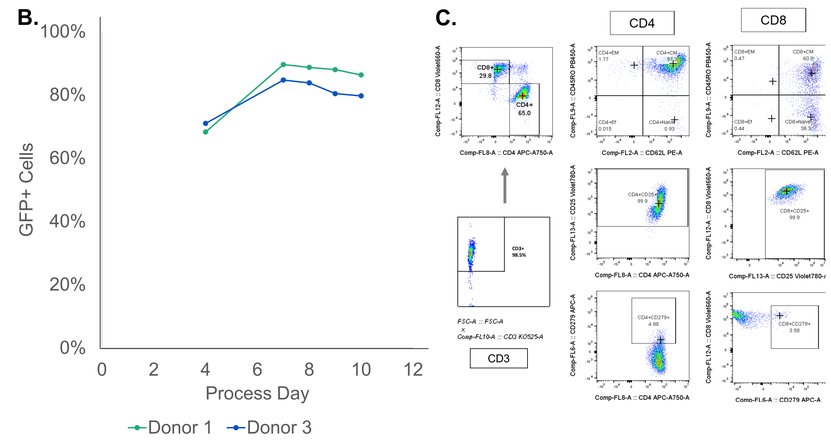


Figure 5: Simulated CAR-T transduction and expansion using GFP (n=2 donors). (A) T-cell expansion and viability. ~1E10 transduced T-cells on Day 8 (starting with 2E8 T-cells). (B) T-cell transduction efficiency. >80% transduced T-cells on Day 8 of process. (C) FlowJo™ representation of gated cells on Day 8.

Conclusions

Developed a semi-automated closed CAR-T manufacturing process that can be widely adopted by the T-cell immunotherapy industry:

- 1E10 GFP transduced T-cells in an 8 day process, n=2 donors
- Identified EasySep™ as a commercially available reagent to enhance process efficiency
- Integrated the the Smart-Max, Sepax™ C-Pro, Xuri™ W25 bioreactor, Sefia™ and VIA Freeze™ as automation strategies to reduce manual and open operations

Future Work

- Development of an isolation process with closed a EasySep™ platform
- CAR-T manufacturing runs with transduction, expansion and harvest
- CAR-T manufacturing runs with patient material
- Further optimization of T-cell bioreactor expansion
- Integration of GMP-grade reagents currently in development

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