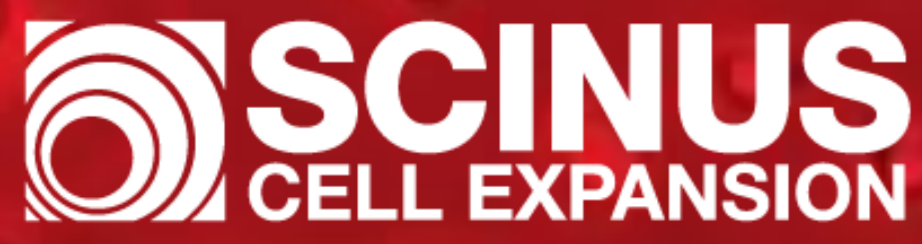


Mitigating complexity in T-cell manufacturing through a fully integrated end-to-end bioreactor workflow



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INTRODUCTION

Adoptive T-cell therapies show strong potential but are limited by complex, multi-device manufacturing. We present a simplified, fully closed workflow performed entirely in the Osilaris™ bioreactor (Figure 1). T cells are isolated directly in-bioreactor using Akadeum's buoyant microbubble-based negative selection (BACS™) technology, followed by activation and >140-fold expansion from an initial 55 million cells.



Figure 1A: The Osilaris™ bioreactor

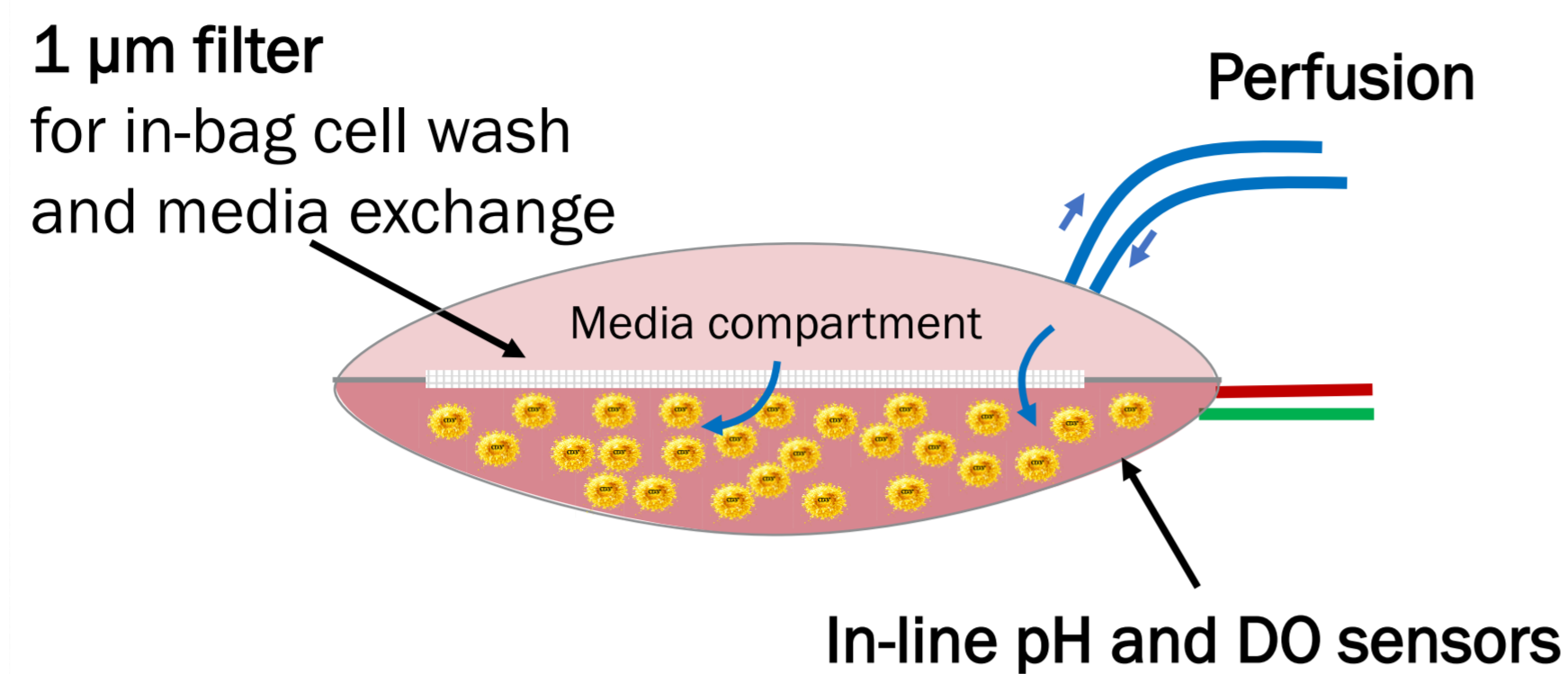


Figure 1B: Cross-section view of the Osilaris™ suspension culture bag.

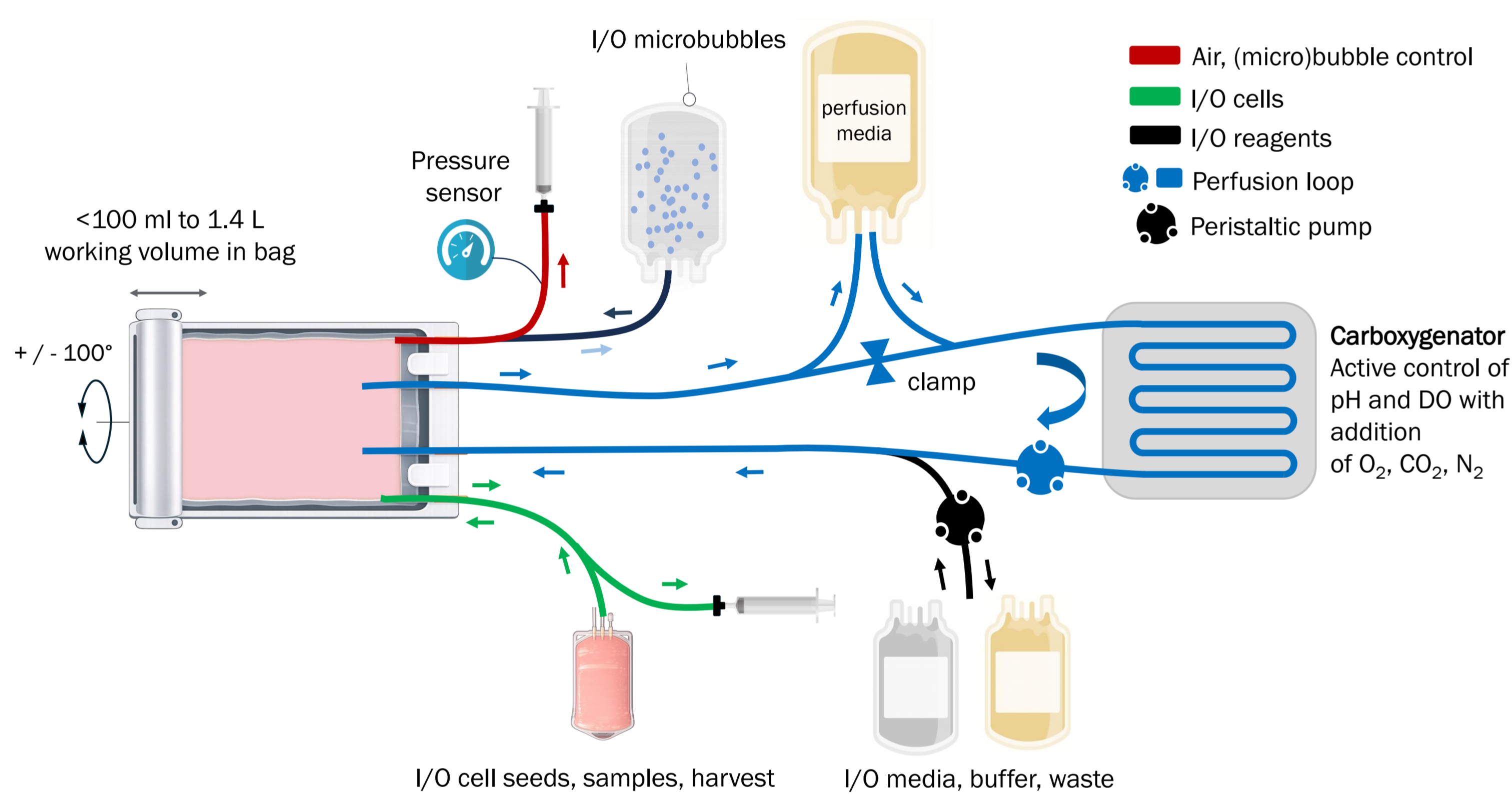


Figure 1C: Schematic of the fluidic pathway in the Osilaris™ platform

MATERIALS AND METHODS

T-cell selection using the Osilaris™ bioreactor and Akadeum® Human T Cell Leukopak Isolation Kit:

Thawed leukopak fractions (n = 2), were incubated with a biotin-conjugated antibody cocktail in the Osilaris™ suspension bag, followed by the addition of streptavidin-conjugated microbubbles to perform buoyancy-activated negative CD3 isolation for 30 min via an adjustable rocking platform (Figure 2).

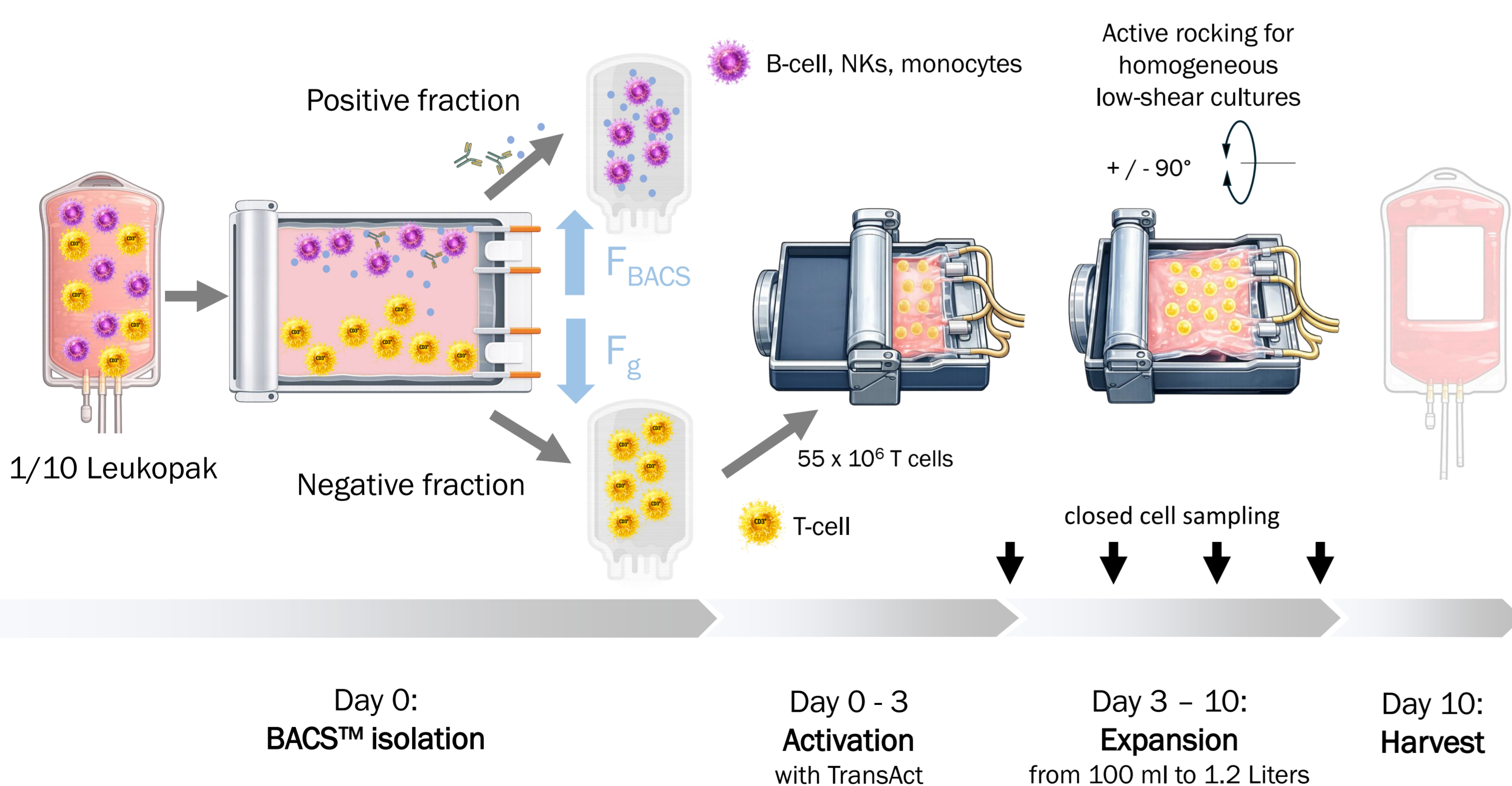


Figure 2: Process overview of T-cell selection, activation and expansion in the Osilaris™ bioreactor.

T cell activation and expansion

From the post-selection yield, 55×10^6 T cells were cultured in 100 mL TumorPlus263 medium supplemented with 100 IU/mL IL-2 in an Osilaris™ suspension bag. Cells were activated with TransAct (1:100) under static conditions with direct gas control (Figure 2). After 3 days, cultures entered a 7-day expansion phase where volume was gradually increased to maintain 2×10^5 cells/mL.

RESULTS

T-cell selection

Upstream processing using the BACS selection system integrated into the Osilaris™ bioreactor yielded $3-5 \times 10^8$ CD3⁺ cells from one-tenth-scale leukopaks. CD3⁺ T-cell purities of 92-94% were achieved (Figure 3), with viability of ~98%.

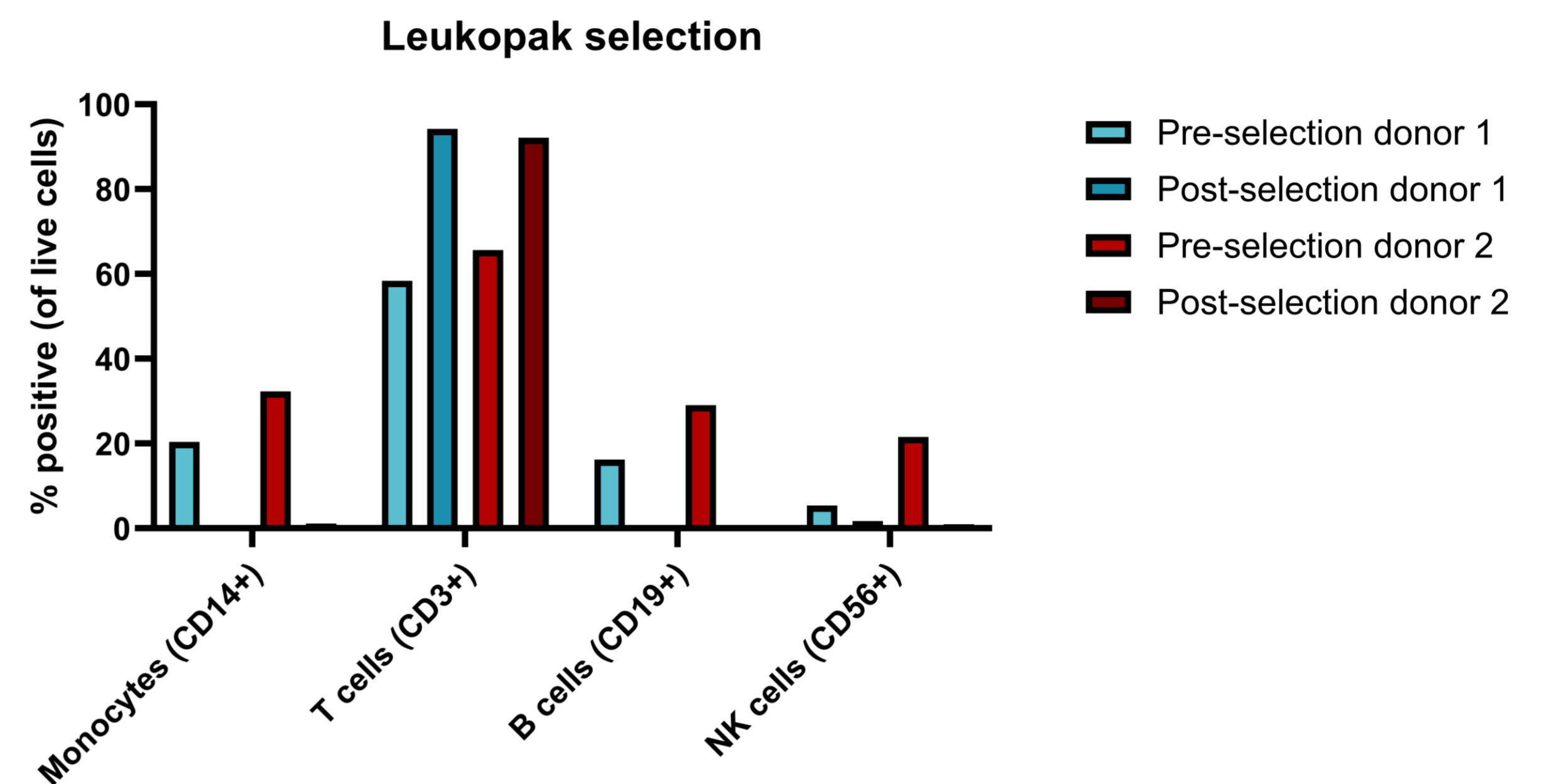


Figure 3: Frequency of lymphocyte cellular subsets pre and post negative selection.

Cell yield and characterization

Both healthy donors yielded approximately 8×10^9 fully TransAct-activated, expanded T-cells at the end of culture, corresponding to >140-fold expansion (Figure 4), while maintaining stable T-cell subset distributions (in terms of CD4/CD8 ratios, Figure 5).

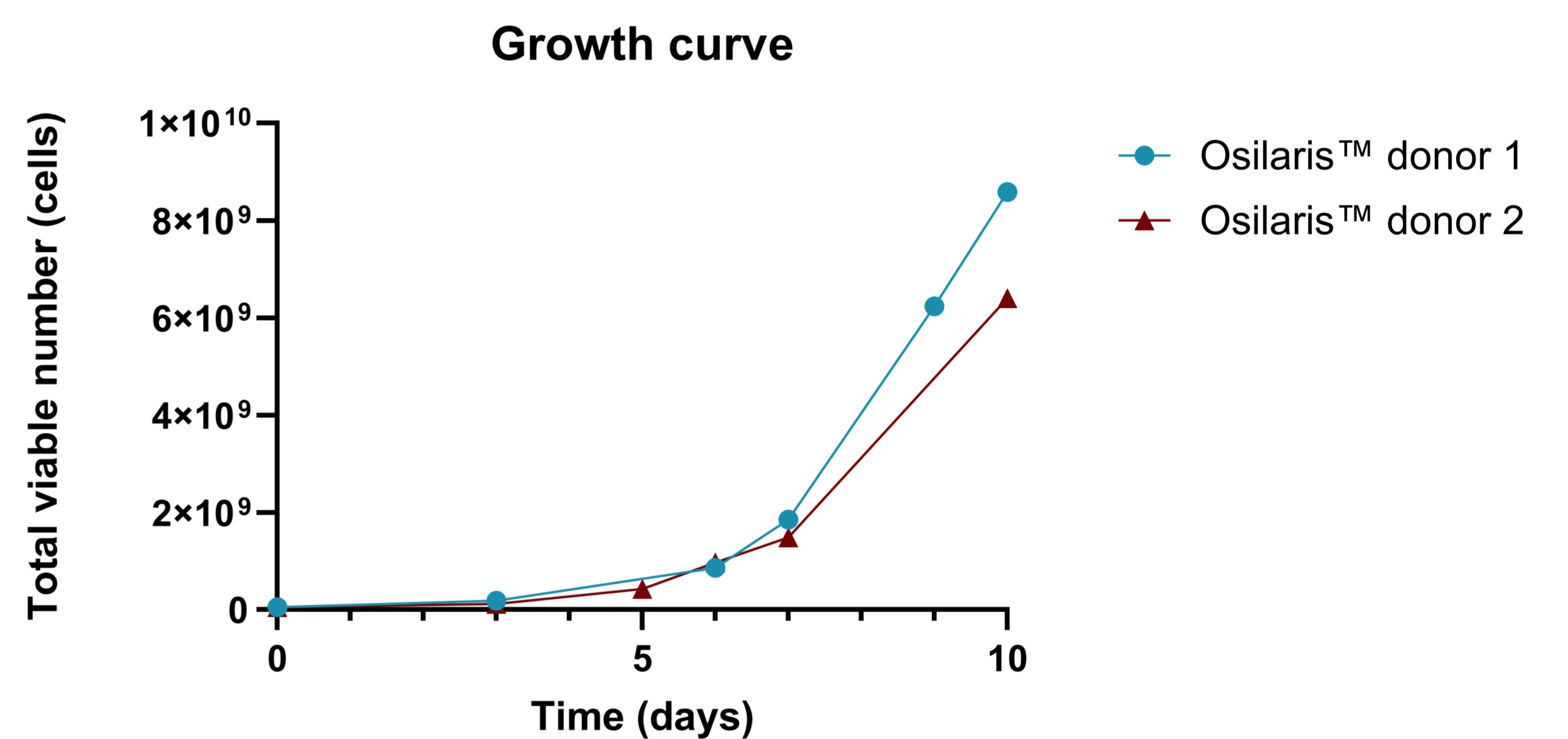


Figure 4: Expansion of activated T cells in the Osilaris™ bioreactor.

T cell subpopulation

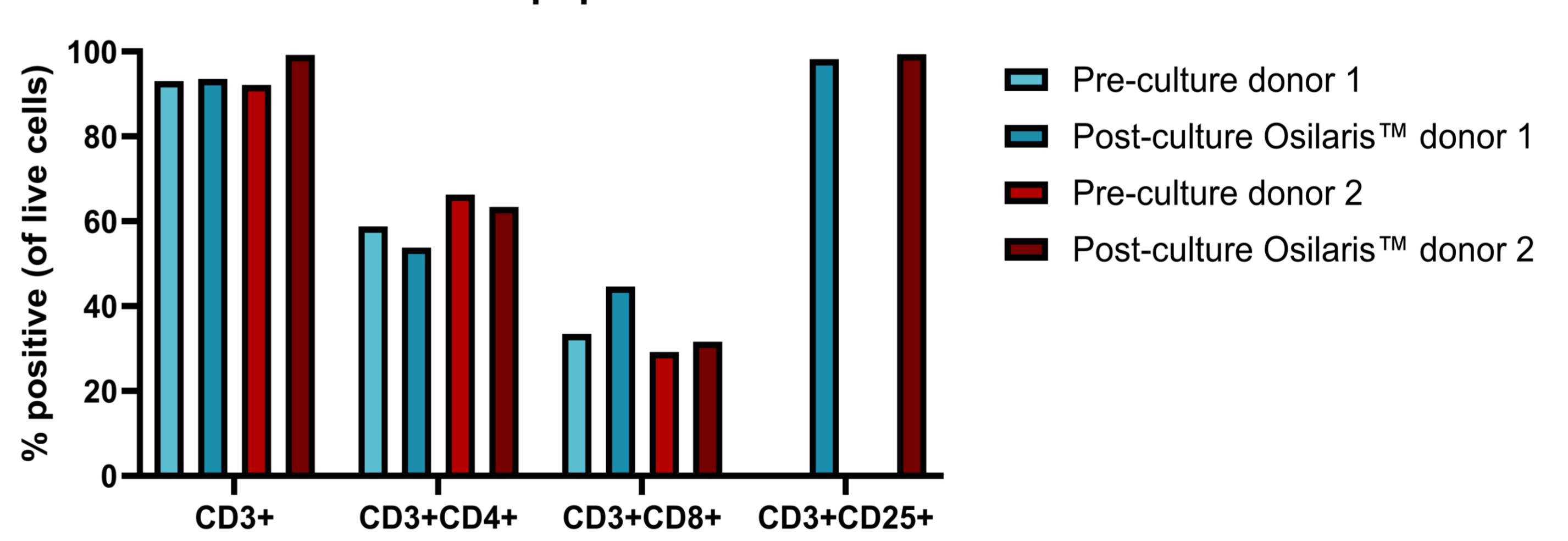


Figure 5: Phenotypic Analysis of Activated T Cells

CONCLUDING REMARKS

The workflow described in this study, positions the Osilaris™ bioreactor as a unique platform enabling translation of T-cell therapies via:

- **Efficient T-cell selection:** Easily integrated BACS process with a flexible bag and rotating rocker design yielding ultra low-shear rapid cell purifications of $300-500 \times 10^6$ cells from one-tenth of a leukopak.
- **Robust expansion performance:** Starting from 55×10^6 total viable cells, $6-8 \times 10^9$ fully activated, viable T cells were reproducibly generated from two independent donors.
- **Manufacturing efficiency:** Sustained culture densities exceeding 7×10^6 cells/mL were achieved, supporting efficient production at clinically relevant scales.
- **GMP-aligned, closed workflow:** A fully closed, single-use bag system enables streamlined, end-to-end T-cell manufacturing compatible with GMP-compliant clinical productions demanding flexible and controllable culture parameters.