End-to-End Production of T Cells on the Cocoon® Platform, Using Buoyancy-Activated Cell Sorting (BACS) for Direct Isolation from Leukopaks

Lonza
Specialized Modalities

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Introduction

High-purity, viable T cell isolation is essential for consistent upstream processing of CAR-T cell manufacturing. Traditional magnetic cell separation uses positive or negative selection; the latter preserves cell integrity by avoiding activation and receptor modification. Akadeum® Buoyancy-Activated Cell Sorting (BACS), uses microbubbles and buoyant forces to gently isolate target cells, offering an alternative to magnetic based cell isolation, without the need for preprocessing the apheresis material.

A challenge in cell therapy is achieving scalable and consistent cell viability and function, while maximizing cell expansion and potency. Automated bioreactors, such as the Lonza Cocoon® Platform (Figure 1), offer controlled environments that support consistent cell growth while minimizing contamination risks.

Figure 1. Lonza Cocoon® Platform and Cocoon® Cassette. Functionally closed, highly customizable and scalable integrated cell manufacturing platform. Whether it is a centralized or decentralized cell manufacturing model, the Cocoon® Platform approach reduces costs and supports manufacture of robust cell therapy products.

This system integrates various unit operations—including cell separation, activation, transduction, expansion, and harvesting—into a functionally-closed system, reducing manual interventions and associated variability, as compared to manual process. By automating these procedures, the Lonza Cocoon® Platform facilitates reproducible, commercial-scale production of therapeutic cells.

In this work we present an automated 9-day T cell isolation and manufacturing process on the Lonza Cocoon® Platform using the Akadeum® Human T Cell Leukopak Isolation Kit – GMP Grade, providing high purity, recovery, and yielding a T cell product with desirable phenotypes for CAR-T therapy applications (Figure 2).

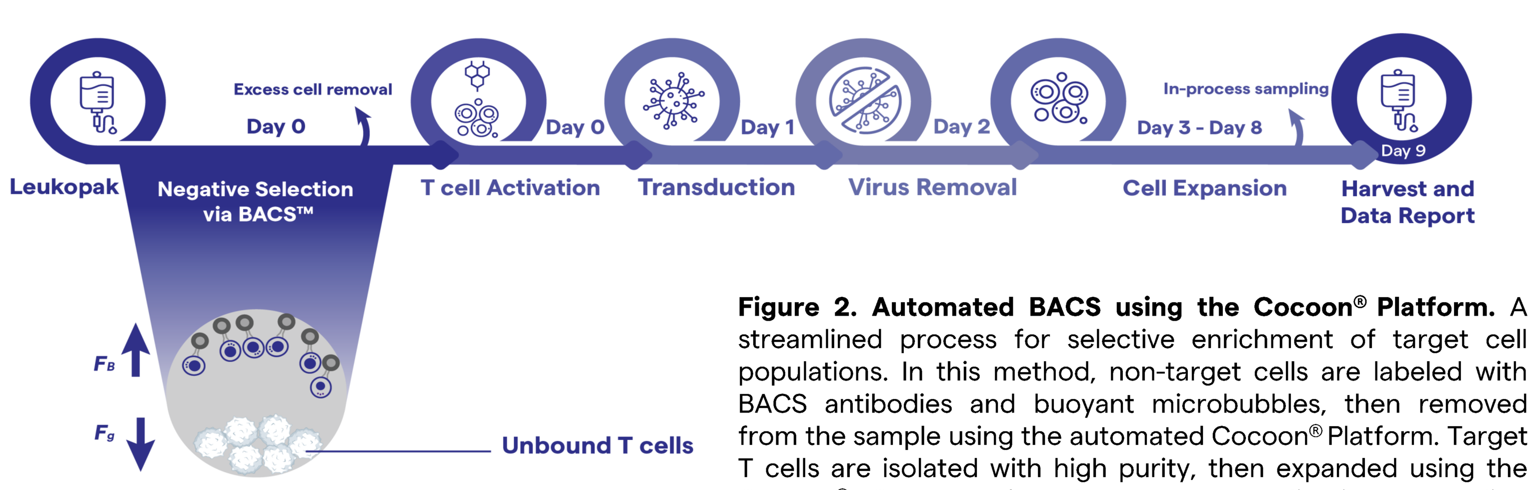

Figure 2. Automated BACS using the Cocoon® Platform. A streamlined process for selective enrichment of target cell populations. In this method, non-target cells are labeled with BACS antibodies and buoyant microbubbles, then removed from the sample using the automated Cocoon® Platform. Target T cells are isolated with high purity, then expanded using the Cocoon® Cassette for downstream applications, enabling efficient and reproducible cell therapy manufacturing.

Methods

T cell isolation using the Cocoon® Platform and the Akadeum® Human T Cell Leukopak Isolation Kit – GMP Grade:

Akadeum® biotin-conjugated antibody cocktail was added to thawed, cryopreserved leukopaks from healthy donors (full-size ≥9×10⁹ cells, n=3; quarter-size ≥2.5×10⁹ cells, n=6), followed by streptavidin-conjugated microbubbles. After 30 min, T cells were automatically isolated using the Cocoon® Platform (Figure 2).

T cell seeding and activation using the Cocoon® Platform:

Post-isolation, 1×10⁸ T cells were seeded in the Cocoon® Cassette with T cell culture media (X-VIVO® 15, Lonza), 5% Human AB serum (BioIVT), 100 IU/mL Recombinant IL-2 (Proleukin®) and 1:100 T Cell TransAct™ (Miltenyi).

Mock transduction and T cell expansion using the Cocoon® Platform:

Mock transduction was performed on day 1, with daily media exchanges (50-75%) from days 4 to 8. Cell harvest occurred on day 9 of the automated protocol.

Results

Upstream processing using the BACS selection system enabled by the Cocoon® Platform recovered 78.9±15.8% of CD3+ cells from full-size leukopaks (Full LP) and 63.6±12.7% from quarter-size leukopaks (¼ LP) (Figure 3A). We observed purities of 93.7±3.3% and 91.4±3.4% (Figure 3B), and viabilities of 95.4±3.6% and 97.9±0.8% (Figure 5B), respectively. These results demonstrate the ability to efficiently perform negative selection in the Cocoon® Platform and achieve high T cell purity in the final product. Moreover, the automation of cell selection streamlines upstream processing, reducing variability and enabling reproducible and scalable workflows for both research and clinical applications.

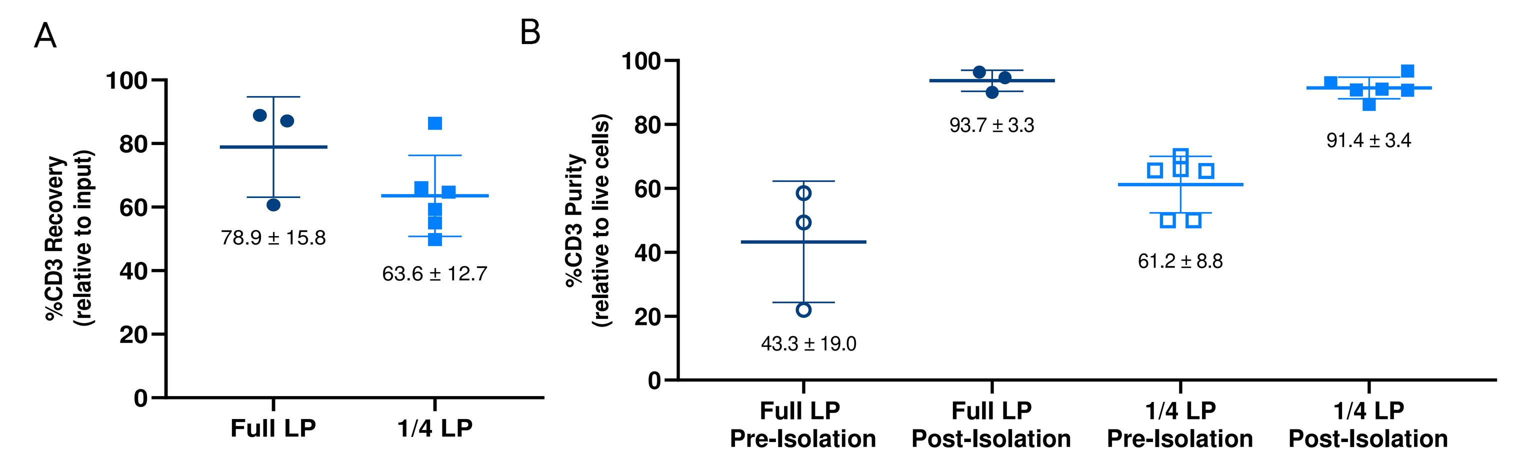

Figure 3. Automated T cell isolation achieved high CD3+ cell recovery and purity. T cells were isolated directly from cryopreserved leukopaks using the Cocoon® Platform and BACS technology. (A) CD3+ cell recovery was determined by calculating the number of CD3+ cells recovered relative to the initial input. Data show the percentage of recovered CD3+ cells ± standard deviation (SD), n=3 (Full LP) and n=6 (¼ LP). (B) CD3+ cell purity represents the proportion of CD3+ cells relative to the total CD45+ cell population after isolation and was assessed by flow cytometry. Data show the percentage of CD3+ purity ± SD, n=3 (Full LP) and n=6 (¼ LP).

Flow cytometry analysis was performed on Day 0 on both pre- and post-isolation full-sized LP (n=3) cell suspensions to evaluate the presence of monocytes, natural killer (NK) cells, B cells and red blood cells (RBCs). Day 0 post-isolation samples from full-sized LP achieved averages of 0.3% of monocytes (average reduction of 99.0% compared to pre-isolated), 1.1% of NK cells (average reduction of 79.9% compared to pre-isolation), 0.4% of B cells (average reduction of 97.7% compared to pre-isolation) and 1.1% of RBCs (average reduction of 94.0% compared to pre-isolation) (Figure 4). Reduction of these cells enhances the purity and functionality of the target T cells, providing an optimal starting population for therapy manufacturing.

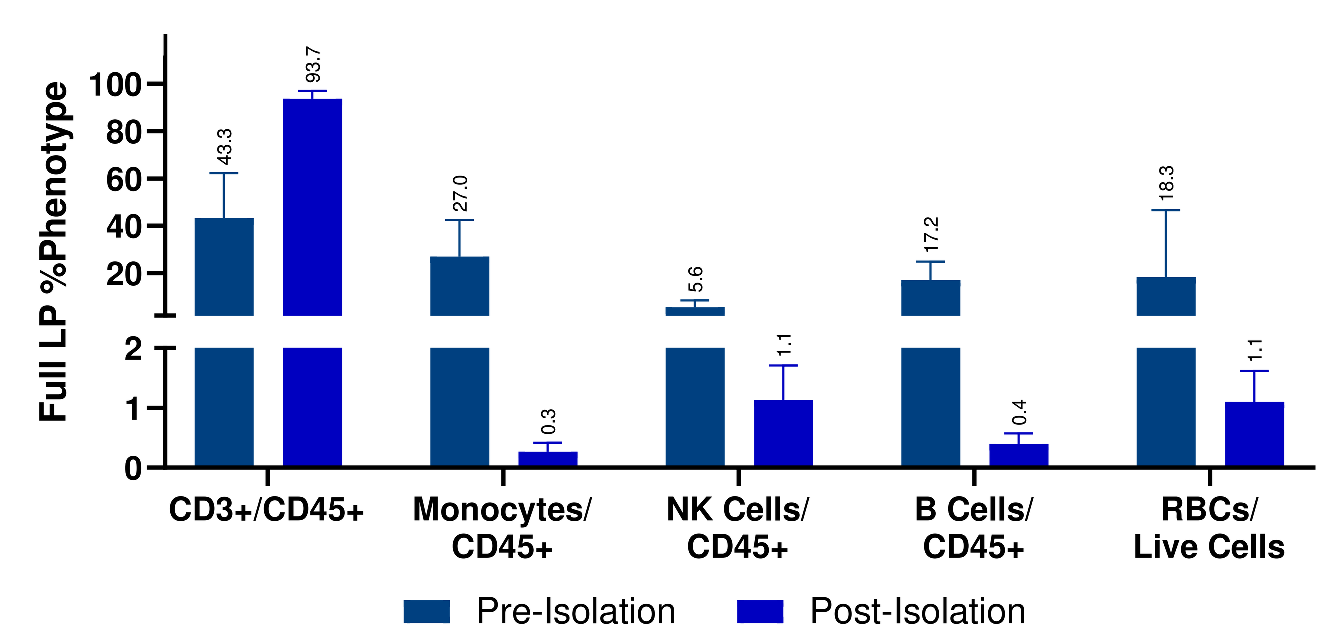

Figure 4. Frequency of cellular subsets pre and post negative selection in the Cocoon® Platform on day 0. A significant reduction in the percentage of non-target cells was observed, with a reduction of 99.0% of Monocytes, 79.9% of NK Cells, 97.7% of B cells and 94.0% of RBCs from pre-isolation to post-isolation, demonstrating the use of the automated negative selection process in enriching the target cell population. Data show the average cell type frequencies ± SD, n=3 (Full LP).

After seeding 1×10⁸ cells in the Cocoon® Cassette, T cells expanded to a total 2.9±0.5×10⁹ cells (Full LP) and 2.7±0.6×10⁹ cells (¼ LP) in the 9-day CAR-T manufacturing process in the Cocoon® Platform (Figure 5A), with cell viabilities of 97.8±0.4% for Full LP and 95.2±4.1% for ¼ LP (Figure 5B) at the end of the automated 9-day CAR-T Cocoon® Platform process. The process supported high cell viability and robust T cell proliferation, achieving an average of 28-fold expansion within a controlled environment.

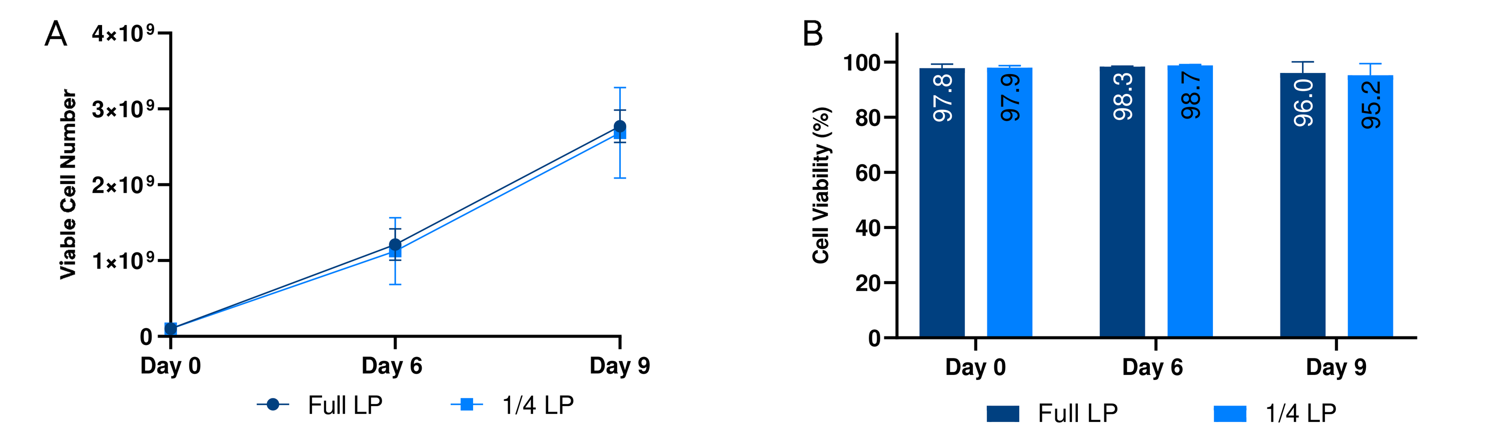

Figure 5. Robust T cell expansion maintaining high viability in the Cocoon® Platform. T cells were expanded in the Cocoon® Cassette using the standard CAR-T manufacturing process. In-process samples were collected on days 6 and 9, and cell count and viability assessments were performed using the Nucleocounter® NC-200 system. Full LP (n=3) achieved 2.9±0.5×10⁹ cells with 97.8±0.4% viability, while ¼ LP reached 2.7±0.6×10⁹ cells with 95.2±4.1% viability. Data show the total viable cell numbers ± SD (A) and cell viability (%) ± SD (B). Automation on the Cocoon® Platform demonstrated consistent, reliable cell isolation and expansion, enhancing reproducibility and efficiency of CAR-T cell manufacturing.

Flow cytometry analysis was performed to evaluate the memory phenotype of T cells expanded from ¼ LP (n=6). Results revealed high frequency of stem cell-like memory T cells (Tscm) on Day 9 (55.7%) as compared to Day 0 (11.2%) (Figure 6). The presence of Tscm subtype is advantageous for cell therapy, as these T cells possess strong self-renewal and proliferative capacity, potentially leading to sustained expansion and long-term effectiveness in CAR-T therapies. Additionally, the proportion of naïve T cells (Tn) was reduced from 35.8% to 0.1% indicating successful activation and early differentiation process, indicating a successful activation and differentiation process (Figure 6).

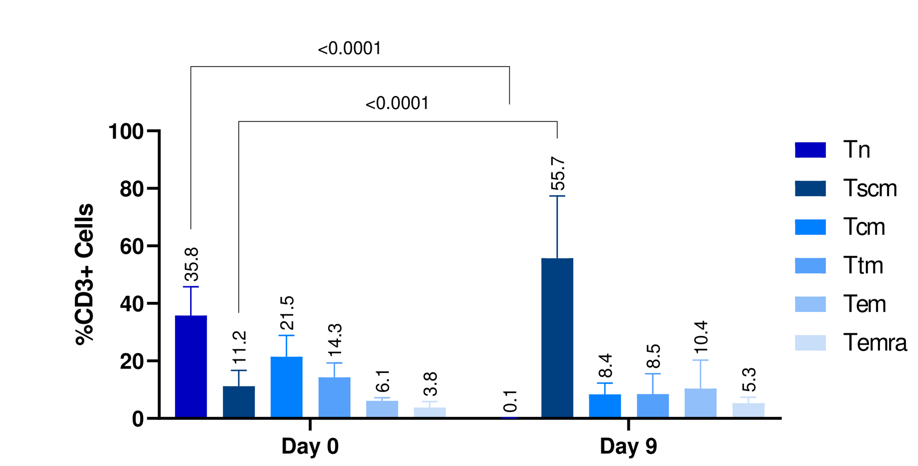

Figure 6. T cell subtype memory analysis from day 0 and day 9. Memory subtype analysis using flow cytometry revealed a high percentage of stem cell-like memory T cells (Tscm) on Day 9 of the expansion process (55.7±19.8%). Additionally, the percentage of naïve T cell (Tn) cells decreased from 35.8±9.2% to 0.1±01%. Central Memory T cells (Tcm) and Tissue-resident Memory T cells (Ttm) were 8.4% on Day 9. Effector Memory T cells (Tem) was 10.4% on Day 9 and Terminally Differentiated Effector Memory T cells (Temra) was 5.3% on Day 9. Data show the mean ± SD of each T cell subtype frequency relative to CD3+ cells. Statistical significance was determined by 2-way ANOVA with Sidak's multiple comparison test (n=6 per day).

Conclusion

The Cocoon® Platform offers flexibility to adapt manufacturing processes to specific user requirements. This work demonstrates the feasibility of integrating Akadeum® BACS-driven negative selection into a fully-automated end-to-end CAR-T manufacturing workflow. The process achieved T cell purities of 93.7±3.3% (n=3) for full-size LP and 91.4±3.4% (n=6) for quarter-size LP, and recoveries of 78.9±15.8% (n=3) for full-size LP and 63.6±12.7% (n=6) for quarter-size LP. Following activation, cells exhibited robust expansion, achieving an average of 28-fold increase in cell numbers, with 5-fold enrichment of memory T cells in the final drug product (data for full-size LP, n=3); this phenotype is associated with long-term persistence, therapeutic efficacy and maintenance of anti-tumor activity. These results demonstrate the robustness, reliability and scalability of the Cocoon® Platform, supporting its use in T cell manufacturing for both research and clinical applications.