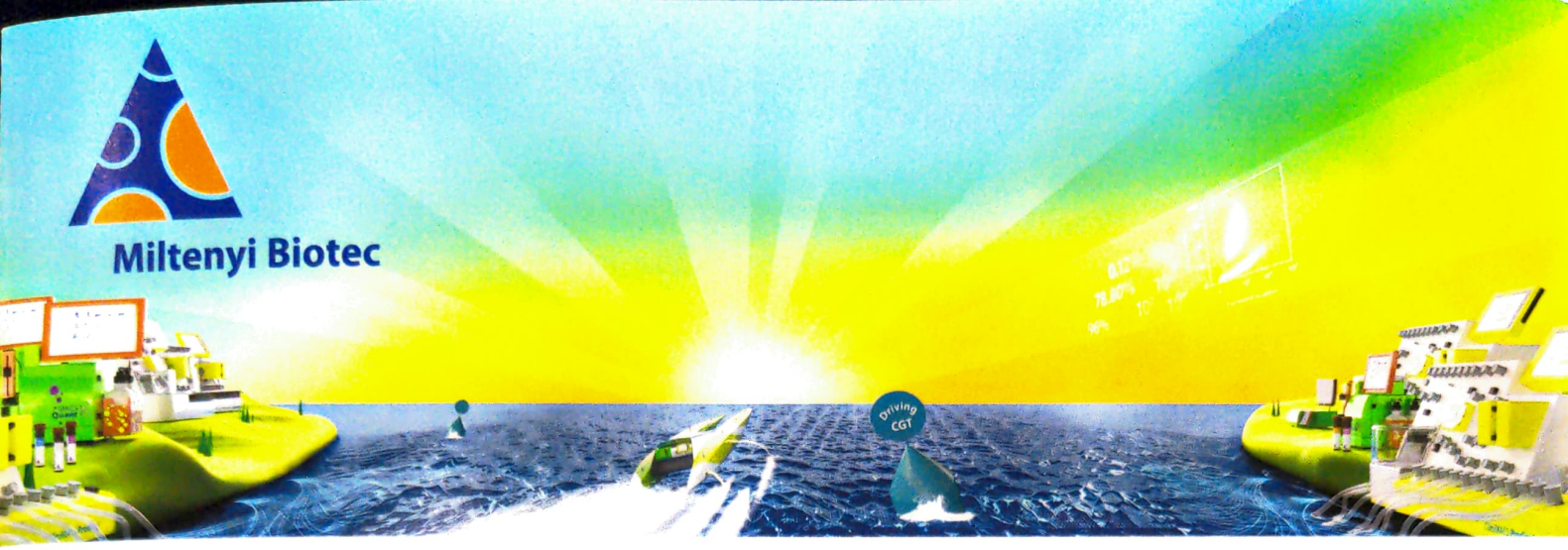

A streamlined, end-to-end solution for robust and efficient large-scale CAR T manufacturing

Background

Chimeric antigen receptor (CAR) T cell therapy has rapidly progressed over the past decade, expanding from a treatment option for refractory patients to earlier lines of care.[1-2] As the field advances, manufacturing demands continue to rise, increasing the need for safe, efficient, and scalable production systems.

Automated platforms are ideal for fast-tracking CAR T cell therapies considering the challenges of resource-intensive and unpredictable traditional open and manual processes — issues that can slow down the delivery and accessibility of these life-saving therapies.

To support continued clinical adoption, next generation manufacturing platforms must deliver streamlined, automated, and closed workflows that reduce hands-on time while safeguarding product quality.

Recent developments, as outlined in the 2026 publication Sidana *et al.*, have accelerated cell-processing steps, shortening activation, transduction, and expansion from several weeks to just a few days, without compromising functional potency, proliferative capacity, or antigen-specific activity.[3]

In this study, we evaluated four total T cell transduction (TCT) workflows using an automated manufacturing strategy designed to preserve critical quality attributes (CQAs) and enable cross-platform and cross-scale comparability. Three workflows were executed on the CliniMACS Prodigy®: the standard TCT process and two TCT Large Scale (TCT-LS) variations. The fourth workflow was performed on the CliniMACS® Plus Instrument paired with a G-Rex® bioreactor (Wilson Wolf Manufacturing) to support automated cell enrichment and expanded culture capacity.

The incorporation of auxiliary systems demonstrated compatibility with automated enrichment steps and flexible volume expansion. Pairing the CliniMACS Prodigy with a G-Rex bioreactor is also routinely performed but was not included in this study.

Across these workflows, we compared immune cell composition, viability, cell yield, activation profiles, memory phenotype distribution, and exhaustion markers. The 7-14 day TCT process on the CliniMACS Prodigy enabled efficient, scalable autologous T cell manufacturing, yielding approximately 3×10^9 cells in the core TCT workflow and up to 2×10^{10} cells in the TCT-LS configuration. This closed, GMP-compliant platform features built-in automation and auxiliary compatibility to reduce manual handling, simplify operations, and support cost-effective CAR T cell manufacturing.

Materials and methods

Study plan and design

Workflows were performed in triplicate using three independent donors to mitigate biological variability. As illustrated in figure 1, for each donor, a single leukapheresis product was divided into two parallel T cell enrichment processes: one performed on the CliniMACS Prodigy (fig. 1 A-C) and one on the CliniMACS Plus Instrument (fig. 1 D). Following enrichment, cells were seeded to generate four experimental conditions (fig. 1 A-D), which differed in starting cell numbers, activation reagents, and culture conditions.

For workflows A-C, cells enriched on the CliniMACS Prodigy were seeded into the cultivation chamber of the instrument and cultured as follows:

- **A** (TCT): 2×10^8 cells; The cells were activated with standard-scale MACS(R) GMP T Cell TransAct™ and cultured under static conditions.
- **B** (TCT-LS): 6×10^8 cells; The cells were activated with MACS GMP T Cell TransAct Large Scale and cultured under agitated conditions.
- **C** (TCT-LS): 1×10^9 cells; The cells were activated with *high-concentration MACS GMP T Cell TransAct and cultured under agitated conditions.
- **D** The cells were enriched using the CliniMACS Plus Instrument, transferred to a G-Rex 500M bioreactor, and activated under conditions comparable to C (same cell number and with high-concentration MACS GMP T Cell TransAct), then cultured under static conditions.

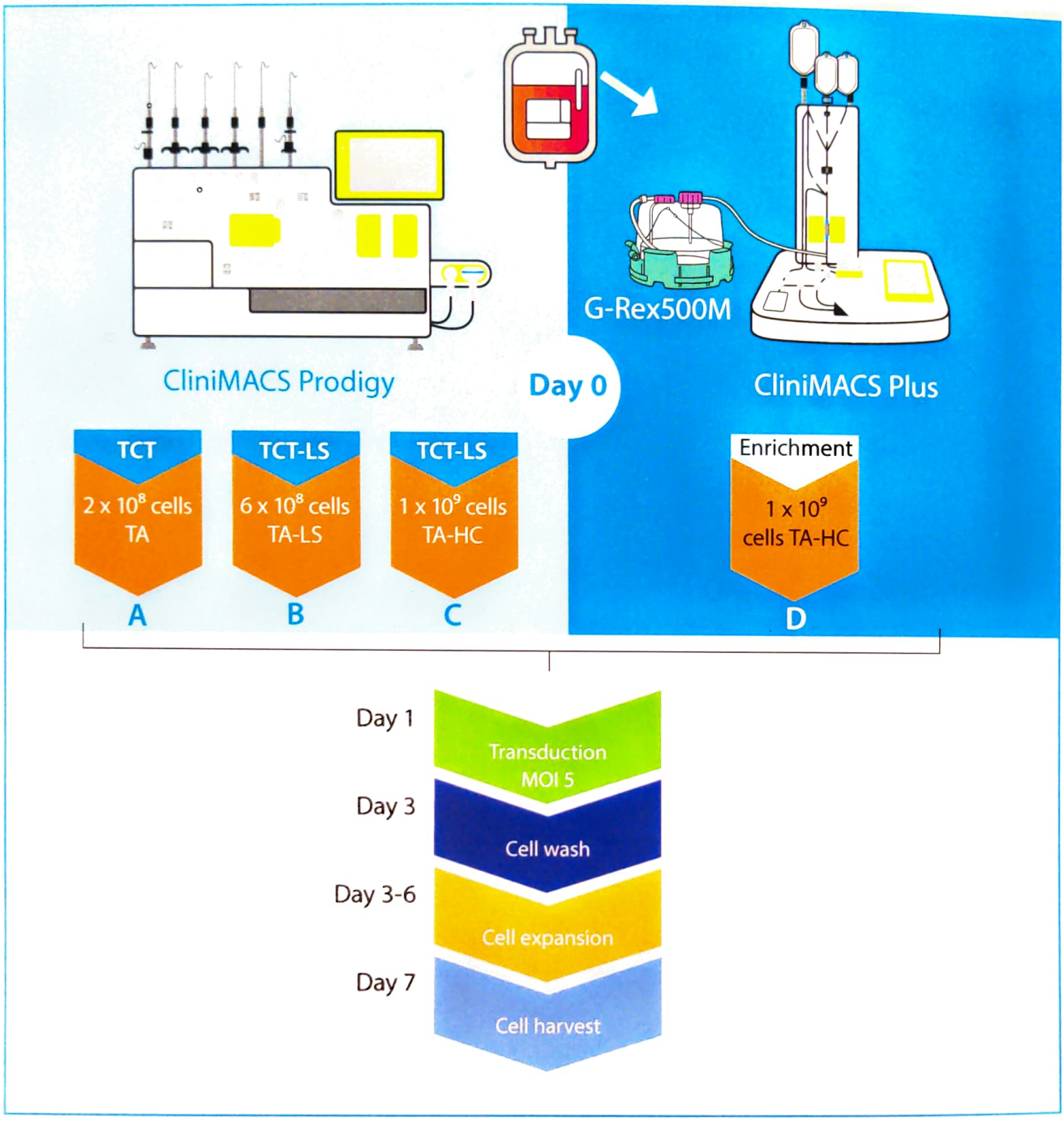

Figure 1. Process design for various capacities using flexible workflows, reagents, and equipment.

CAR T manufacturing workflow (Days 0-7)

On Day 0 (D0), cells were enriched and seeded at the specified densities. Workflows A, B, and C were processed in the CliniMACS Prodigy chamber, while D utilized the G-Rex 500M; all were activated with T Cell TransAct at the corresponding process scale. This step and subsequent steps are depicted in Figures 1 and 2.

On Day 1 (D1), 24 hours post-activation, cells from all workflows were transduced with a lentiviral vector encoding hCD19 at a multiplicity of infection (MOI) of five.

On Day 3 (D3), the cells were washed to remove the activation reagent and residual viral vector. From Day 3 to Day 7 (D7), the cells were cultured and expanded in TexMACS™ Medium supplemented with MACS GMP Recombinant Human IL-7 and MACS GMP Recombinant Human IL-15, in the presence of 3% human AB serum.

On Day 7 (D7), the cells were harvested and formulated in a cryoprotectant solution for storage. At each designated time point, cell samples were collected from all workflows and analyzed by flow cytometry using the MACSQuant® Analyzer 10 with the express mode add-on for automated acquisition and data analysis.

CD137 (4-1BB) displayed the greatest separation between conditions. Agitated workflows (B and C) showed higher Day 3 levels and mid-culture increases in CAR⁺ CD4⁺ cells, consistent with stronger costimulatory engagement. Static workflows exhibited lower, steadily decreasing expression.

Overall, agitated T Cell TransAct Large Scale workflows B and C supported more robust and sustained activation, whereas static workflows A and D produced weaker and shorter-lived responses.

Transduction efficiency

Figure 6 compares transduction efficiency and CAR T cell expansion across the four workflows (A-D), which differ in activation method and culture conditions.

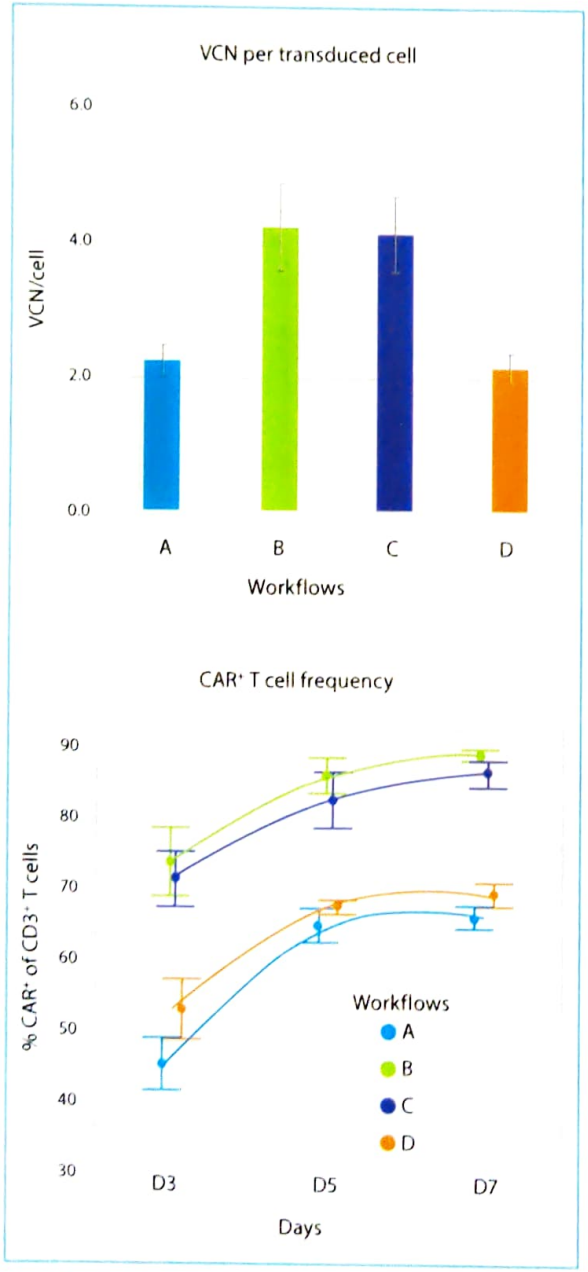

Figure 6. Comparison of vector copy number and CAR⁺ T cell frequencies across automated manufacturing workflows.

Workflows B and C, cultured with agitation, produced higher VCN values and stronger CAR⁺ T cell expansion. Workflows A and D, using static culture, show lower VCN and slower CAR⁺ T cell increases.

These results highlight the performance advantage of combining scale-appropriate T Cell TransAct format activation with agitated culture for more efficient CAR T manufacturing.

Interestingly, this evidence suggests that workflows B and C could achieve equally high transduction efficiency even at an MOI lower than five, compared with workflows A and D, potentially reducing manufacturing costs and enhancing product safety.

Memory-enriched differentiation profile and low-exhaustion CAR products

Additional data (not shown) demonstrated that across all four workflows (A-D), our process reliably yields CAR⁺ T cell products dominated by central memory and effector memory phenotypes in both CD4⁺ and CD8⁺ population, an enrichment associated with durability and long term functional performance.

At the same time, expression of key exhaustion markers (LAG-3, PD-1, TIM-3) remained uniformly low in both lineages, indicating minimal early exhaustion until Day 7. Together, these results highlight a manufacturing approach that consistently delivers memory-rich, less-differentiated CAR T cells with preserved functional potential and strong persistence.

CAR T cytotoxic potency

To demonstrate the potency of our CAR T cells, we evaluated both cytotoxicity (fig. 7) and cytokine release (data not shown) using complementary assays.

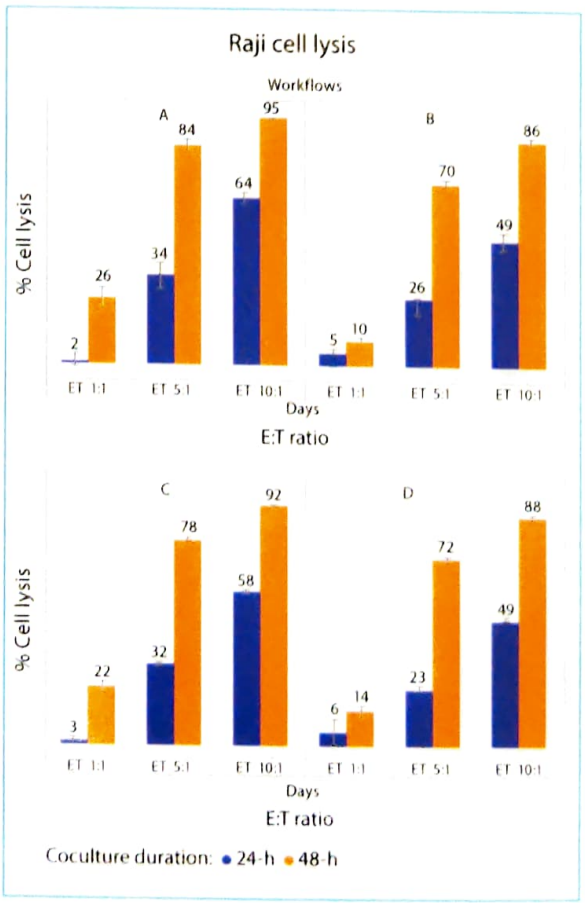

Figure 7. Robust, dose- and time-dependent CAR T cytotoxicity across the workflows.

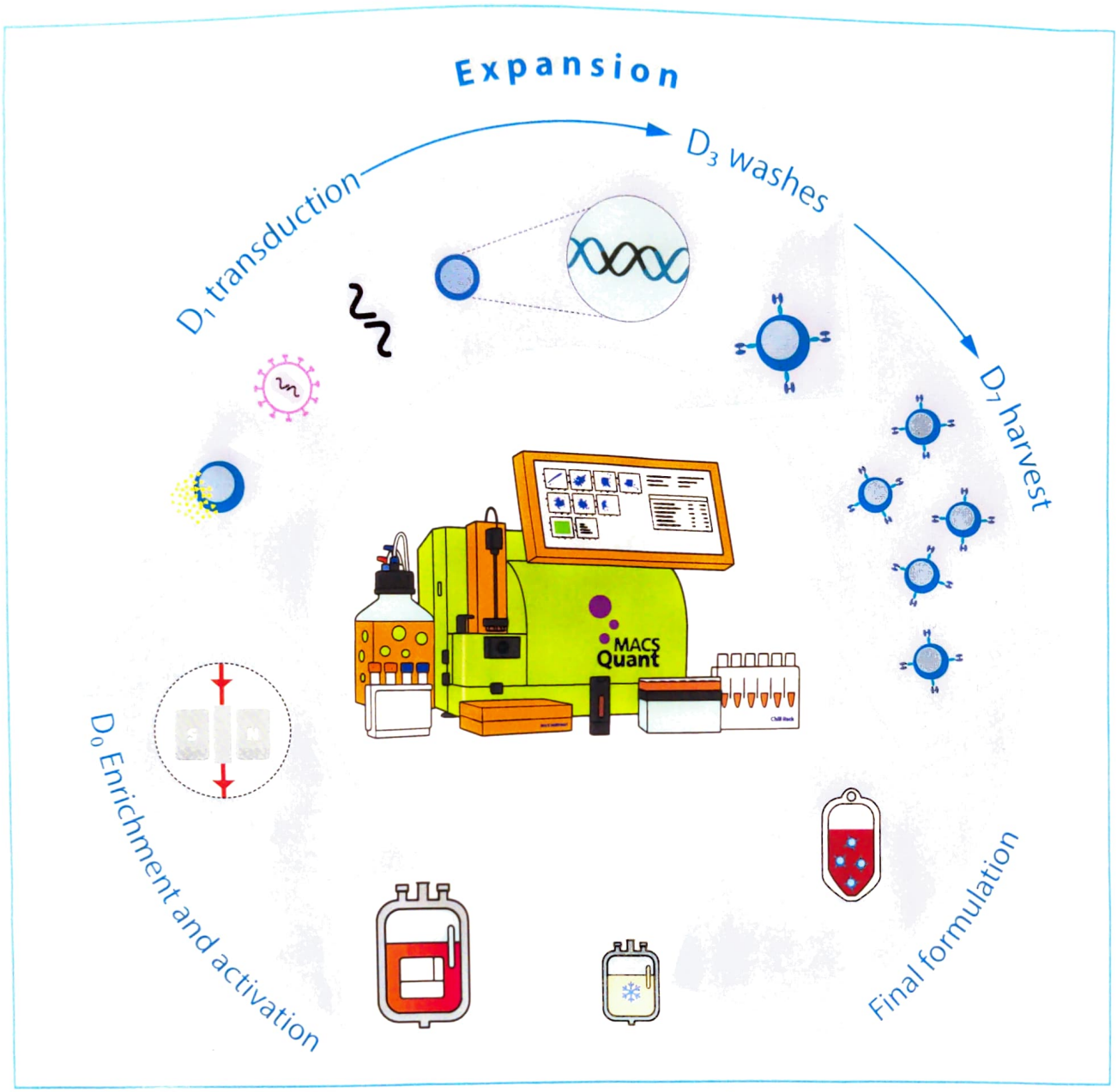

Figure 2. Process overview: At each time point, cell samples were analyzed on the MACSQuant Analyzer 10 utilizing the Express Mode add-on for automated acquisition and data analysis.

Results

Immune cell composition (Day 0 vs Day 7)
Figure 3 displays the frequency (%) of major immune cell lineages at two time points.

Day 0 (top panel) and Day 7 (bottom panel).
Each plot displays the distribution of T cells, NKT cells, monocytes, B cells, NK cells, neutrophils, and eosinophils, with individual data points.

At Day 0, the leukapheresis product exhibits a heterogeneous lineage composition. Post-enrichment, the CliniMACS Plus Instrument and CliniMACS Prodigy both generated products with markedly higher T cell frequencies and significantly reduced non-target cell populations. At Day 7, all four workflows (A-D) converge toward highly pure T cell populations, with non-T-lineages remaining at minimal or near zero levels across all conditions.

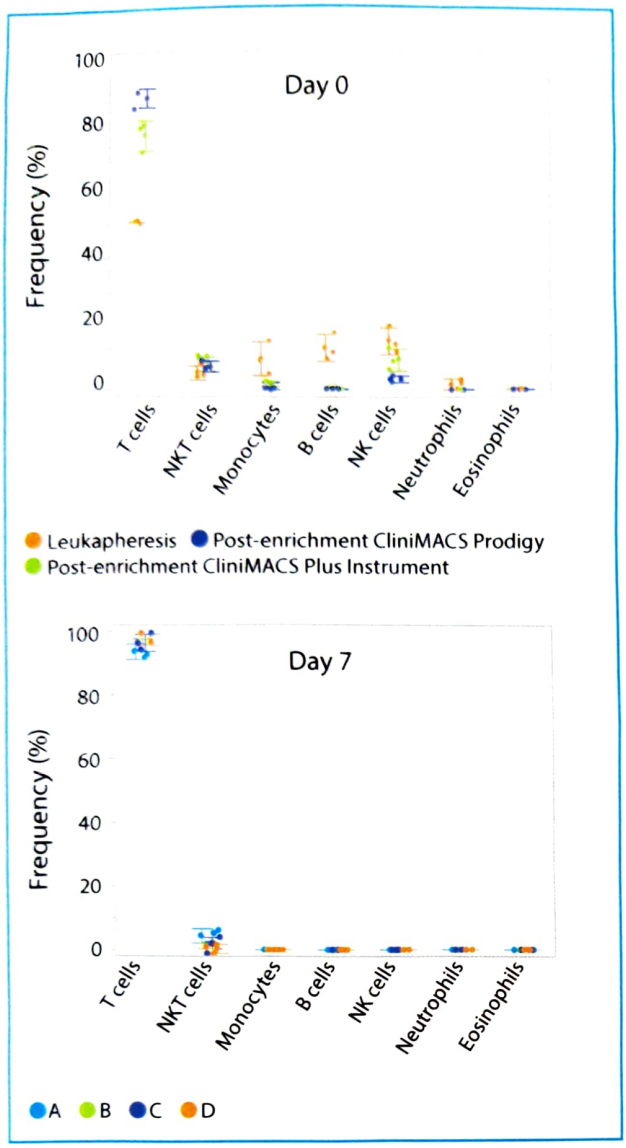

Figure 3. Comparison of immune cell lineage frequencies in leukapheresis material, post-enrichment products (Day 0), and Day 7 cultures from workflows A–D.

Viability and expansion

Cell viability at Day 7 was consistent across all workflows, ranging from 95% to 98% (data not shown). Average fold expansion from Day 0 to Day 7 was also comparable among replicates (fig. 4).

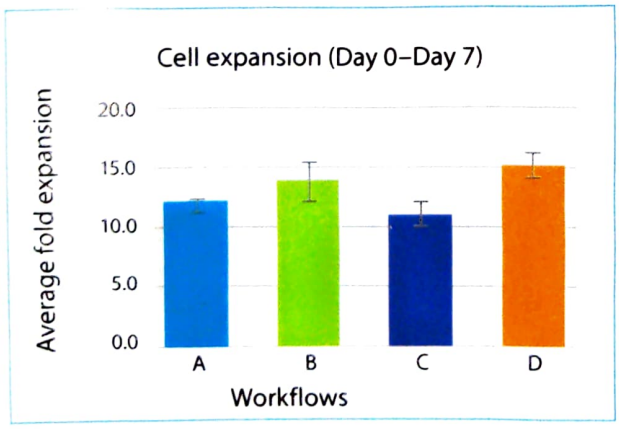

Figure 4. Cell expansion from Day 0 to Day 7 workflows A–D.

Workflows B and D yielded the highest expansion levels, demonstrating similar performance with minimal standard deviation, indicative of highly consistent, and reproducible outcomes.

Activation efficiency

Expression of the activation markers CD69, CD25, and CD137 was measured on Days 3, 5, and 7 in CAR⁺ CD4⁺ and CAR⁺ CD8⁺ T cells across four workflows (fig. 5).

As expected, the expression of all markers was highest early in culture and declined over time, with clear differences between static (A, D) and agitated (B, C) culture conditions.

As an early activation marker, CD69 peaked at Day 3 in all workflows but reached higher levels in agitated workflows B and C, both using T Cell TransAct Large Scale, indicating stronger early activation. Static workflows A and D showed lower peaks and a faster decline.

CD25, which supports IL-2-driven proliferation, was highly expressed at Day 3 across all workflows. Agitated workflows (B and C) maintained CD25 expression longer, especially in CAR⁺ CD4⁺ cells, suggesting more sustained proliferative signaling, while static workflows showed a sharper reduction.

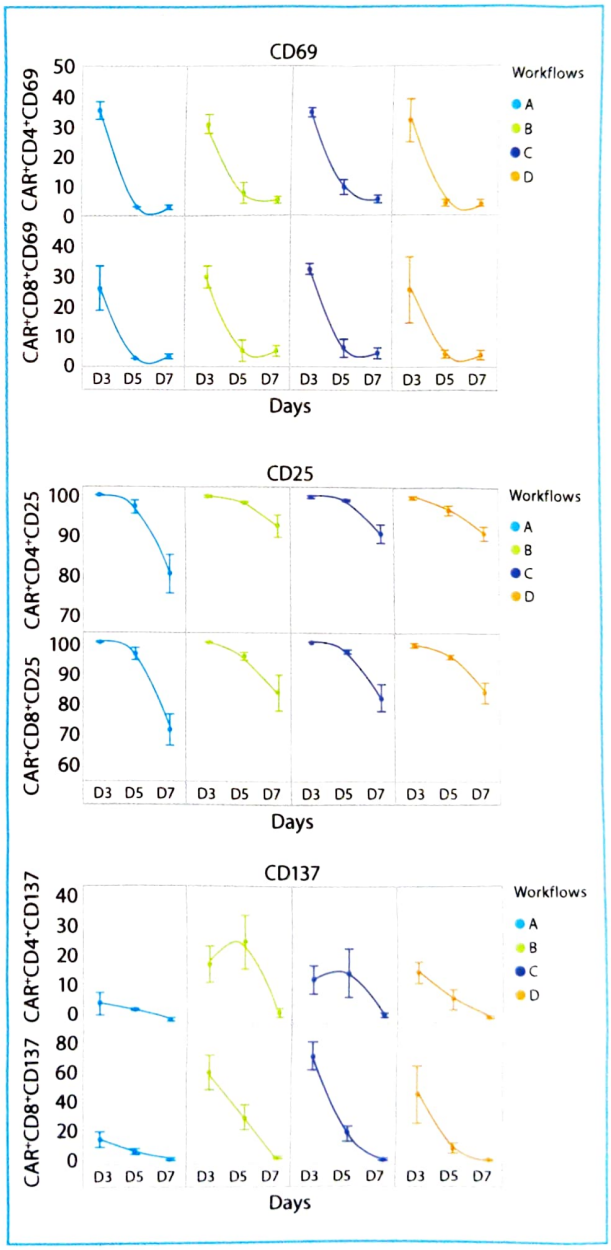

Figure 5. Time-dependent expression of activation markers on CAR⁺ T cell subsets across four workflows.

Across all four workflows, the CAR T cells showed strong, dose- and time-dependent killing of Raji target cells, with clear activity at low effector-to-target ratios and up to 80-95% lysis at higher ratios after 48 hours, highlighting robust, reliable tumor cell clearance. In parallel, cytokine profiling revealed high, targeted secretion of IFN-γ, IL-2, GM-CSF, TNF-α, and IL-9 when CAR T cells engaged with K562 control cells, while responses to Raji remained minimal.

This combination of potent cytotoxicity and clean, antigen-dependent cytokine release demonstrates a CAR T product with strong functional activity, low background activation, and the performance consistency expected for scalable manufacturing.

Conclusion

The CliniMACS Prodigy and ClinicMAC Plus Systems deliver scalable, consistent performance while maintaining highly comparable T cell quality and function across production ranges. MACS GMP T Cell TransAct reagents enable reliable and efficient activation at multiple manufacturing scales, and the CliniMACS Prodigy and CliniMACS Plus Systems provide dependable T cell enrichment for predictable downstream kinetics.

Light culture agitation can further enhance transduction efficiency, and our workflows show that equivalent transduction performance can be achieved using less viral volume, reducing costs, and improving safety. Both the CliniMACS Plus and CliniMACS Prodigy Systems integrate seamlessly with a wide range of external equipment, including bioreactors and automated systems, offering maximum flexibility to scale and configure processes as needed. Together, these technologies create a streamlined, end-to-end solution for robust, efficient CAR T manufacturing.

Products	Cat no.
CliniMACS Prodigy®	200-075-301
CliniMACS Plus Instrument	**151-01**
MACS GMP Recombinant Human IL-15	170-076-114
MACS GMP Recombinant Human IL-7	**170-076-184**
MACS GMP T Cell TransAct™ CR/GMP	200-076-202
MACS GMP T Cell TransAct™ High Concentration (HC) CR/GMP	–
MACS GMP T Cell TransAct Large Scale (LS) CR/GMP	200-076-204
MACSQuant Analyzer 10 Flow	**130-096-343**
T Cell TransAct, human	130-111-160
TexMACS GMP Medium	**170-076-306**

Table 1. Equipment and reagents used in the experiments.

References

1. Sidana, S. *et al.* (2026) Uncovering Gaps in the Integration of CAR T-Cell Therapy in Earlier Stages of Multiple Myeloma Treatment: Results from a Nationwide Survey. Transplant. Cell. Ther. 32:(2):S312. doi.org/10.1016/j.jtct.2025.12.449
2. Weltin, A.L. *et al.* (2026) Industrializing CAR-T cell therapy: impact of automation on cost and space efficiency of manufacturing facilities. Front. Bioeng. Biotechnol.13:1612248. doi.org/10.3389/fbioe.2025.1612248
3. Wang, Q. *et al.* (2025) A 3-in-1 integrated automated platform for rapid CAR-Tcell manufacturing: activation, transduction, and expansion in a hollow-fiber system Cytotherapy. 27(8):1001-1012. doi.org/10.1016/j.jcyt.2025.05.006

* MACS GMP T Cell TransAct High Concentration is not yet available for purchase.

Miltenyi Biotec

Miltenyi Biotec, Inc | 1201 Clopper Road | Gaithersburg, MD 20878, USA | Phone 800 FOR MACS | macsus@miltenyi.com | **www.miltenyibiotec.com**

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

US568