Simplifying Progress

Scalable Production of >100 CAR-T Doses Using a 2 L Perfusion Stirred-Tank Bioreactor with Automated Harvest

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Introduction

- Autologous CAR-T therapies have transformed treatment outcomes in hematologic malignancies but remain limited by complex, patient-specific manufacturing, long production timelines, and high per-dose costs driven by variable starting material and multi-step handling. These constraints restrict scalability, affordability, and broad patient access.
- Allogeneic, off-the-shelf CAR-T therapies aim to overcome these barriers by enabling large-batch manufacturing, immediate treatment availability, and simplified logistics using engineered healthy donor T cells. Realizing this potential, however, requires intensified, scalable, and cost-efficient manufacturing workflows capable of producing hundreds of consistent, high-quality doses per batch. Although stirred-tank bioreactors (STRs) are widely established in large-scale biologics production, their application to multi-litre CAR-T manufacturing remains limited.
- To address these gaps, a serum-free, perfusion-intensified CAR-T expansion process was evaluated in a 2 L STR with a predictive 250 mL scale-down model, in-line capacitance monitoring, and automated downstream processing.

Experimental Overview:

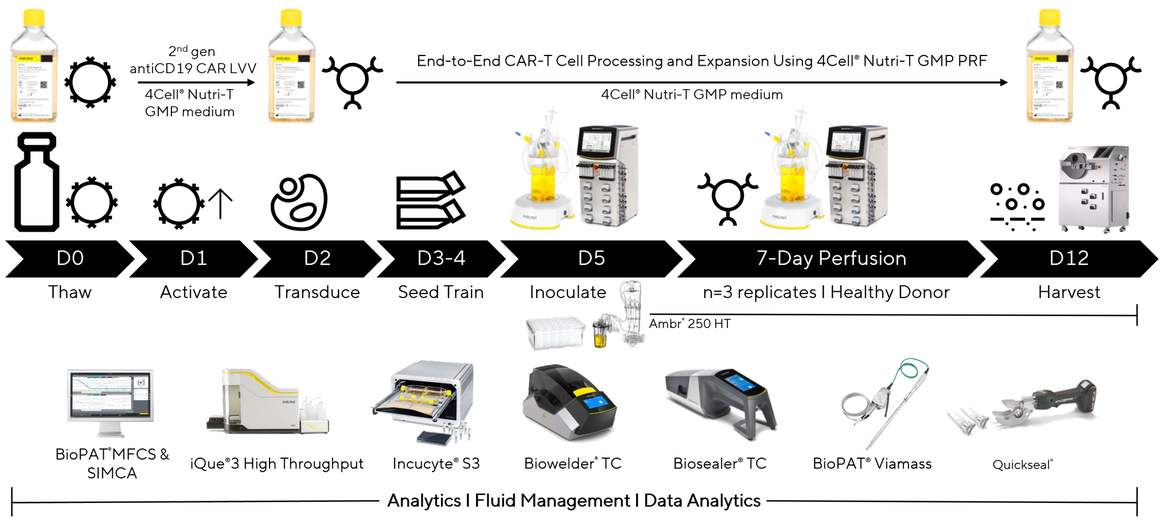

Figure 1: *Experimental overview of CAR-T cell expansion in 250 mL and 2 L stirred-tank bioreactors and static well-plates*

- Upstream & Scale-Down: CAR-T cells were expanded for 7 days under perfusion in a Univessel® 2 L single-use (SU) STR to support high cell density and dose productivity. Process scalability and predictiveness were evaluated using parallel 250 mL Ambr® 250 high-throughput (HT) runs operated at matched volumetric power input (P/V) to establish a representative scale-down model.
- Monitoring & Downstream: In-line capacitance was integrated for real-time viable cell concentration monitoring. At 2 L, automated harvest, concentration, and washing were performed using Ksep® 400 and benchmarked against manual handling to evaluate recovery and product quality.

Results:

A. Achieving Over 100 CAR-T Doses at the 2 L Scale

- Perfusion culture in 4Cell® Nutri-T xeno-free and serum-free medium within the Univessel® 2 L SU STR achieved the highest expansion and batch output across platforms.
- Total viable cell concentration and fold expansion increased significantly after day 3, while cell viability remained high and comparable across systems throughout the 7-day culture.

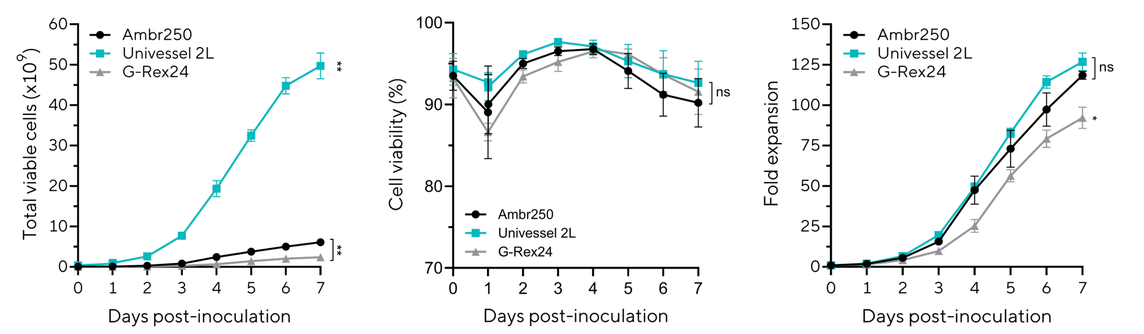

Figure 2: *Growth kinetics & expansion performance across culture platforms over 7 days. Data shown as mean ± SD (n = 3).*

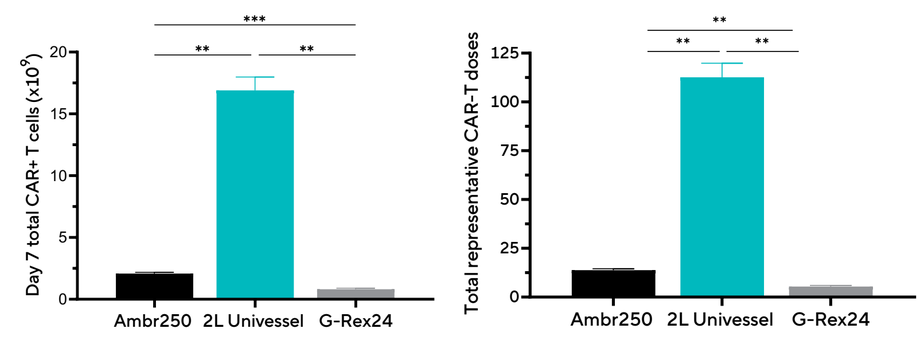

Figure 3: *Day-7 total viable cell yield and representative CAR-T dose output across platforms. Data shown as mean ± SD (n = 3).*

Parameters (Day 7)	Ambr® 250 HT	Univessel® 2 L	G-Rex® 24
Working vol.	0.21 L	1.68 L	24*8=0.19 L
Fold expansion (avg.)	118X	126X	90X
Total cells (avg.)	6.1E9	50.0E9	2.4E9
Total CAR-T doses	14	116	6

Table 1: *Summary of day-7 cellular output and representative CAR-T dose productivity across culture platforms. Dose calculations assume 35% CAR⁺ cells and 150 × 10⁶ CAR-T cells per dose.*

B. Consistent CAR-T Quality Attributes Across Bioreactor Scales & Culture Platforms

- **CAR-T phenotype and function were maintained across platforms:** CAR-T cells expanded in the Ambr® 250 HT, Univessel® 2 L STR, and G-Rex® 24 showed comparable CD4/CD8 composition, similar naïve/central-memory enrichment (>90%), low exhaustion marker co-expression (PD-1/LAG-3/TIM-3), and consistent antigen-specific TNF-α and IFN-γ secretion across systems. Cells from all platforms showed equivalent NALM-6 target-cell killing kinetics, confirming scale- and platform-independent cytotoxic activity.

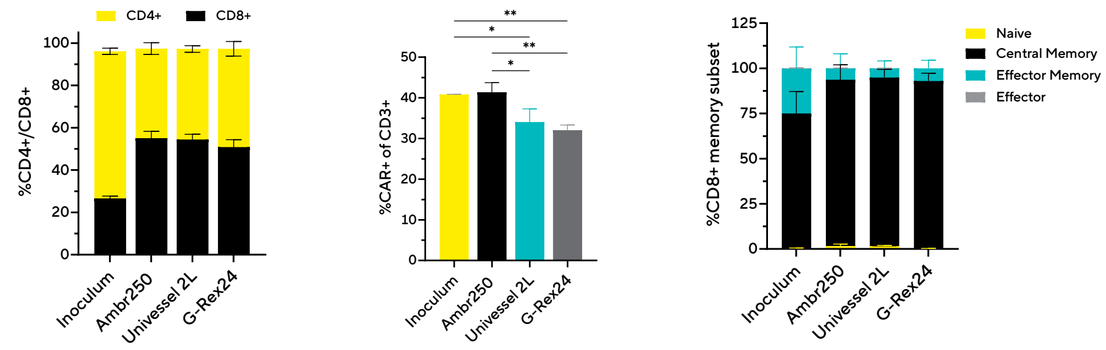

Figure 4: *CAR-T cell phenotype and exhaustion markers are consistent across bioreactor scales and culture platforms.*

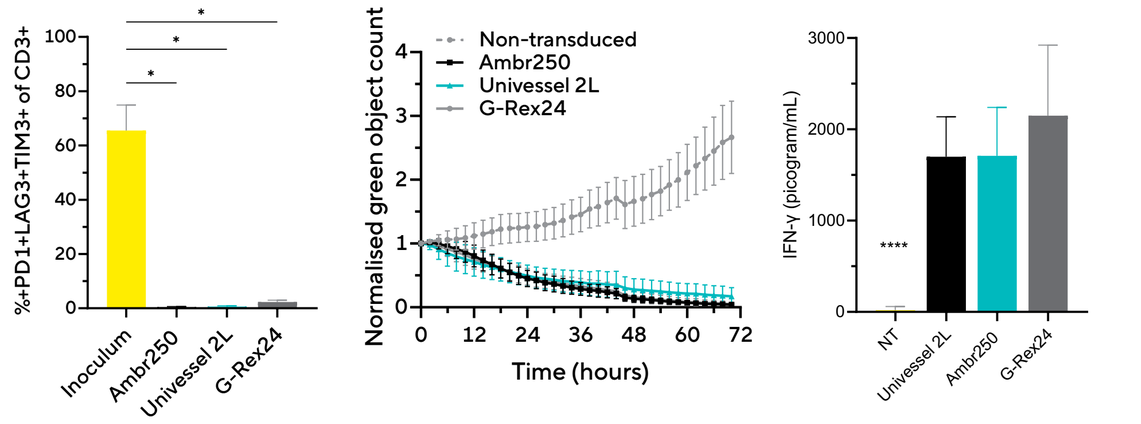

Figure 5: *Comparable cytotoxic activity and antigen-specific cytokine release of CAR-T cells across expansion platforms.*

C. Automated Harvest Preserves CAR-T Cell Quality and Cytotoxic Function

- Integration of the Ksep® 400 enabled closed, automated harvest (>90% recovery), washing, and concentration, with CAR-T cell viability, phenotype, cytotoxicity, and cytokine response comparable to the manual process, supporting rapid, closed and scalable downstream processing (n = 3).

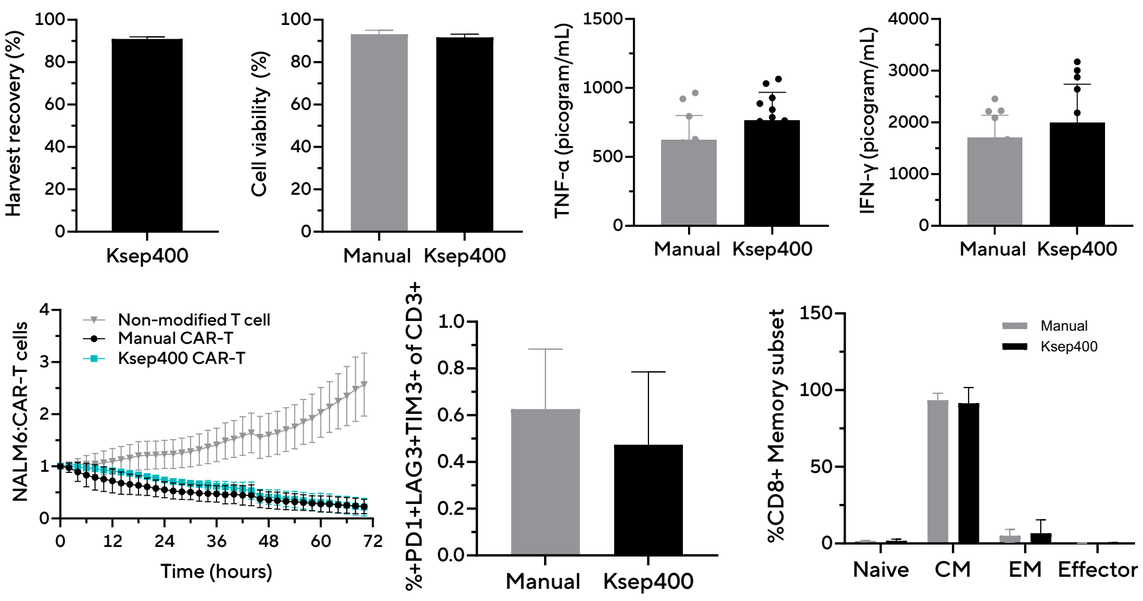

Figure 6: *Scalable automated CAR-T harvest using the Ksep® 400 preserves product quality*

Conclusion

A fully integrated, end-to-end workflow supporting scalable CAR-T manufacturing

- **Scalable, high-yield CAR-T expansion:** Perfusion-enabled CAR-T expansion was successfully scaled to 2 L in SU STR using 4Cell® Nutri-T serum-free medium, producing >100 CAR-T doses per batch within a 7-day expansion while maintaining cytotoxic function, low exhaustion, and central memory–enriched phenotypes.
- **Predictive scale-down model:** Comparable CAR-T expansion at 250 mL and 2 L (1:1) validated the Ambr® 250 HT as a representative scale-down model for high-throughput process development.
- **Automated downstream processing:** Automated harvest, washing, and concentration at 2 L using the Ksep® 400 preserved CAR-T critical quality attributes.