A modular and cost-effective closed-system process for CAR-T cell manufacturing using the Sepax C-Pro and SMART® G-Rex 100M-CS platforms

Abstract 245

Borwyn Wang[1], Penney McWilliams[1], Victor Bass[1], Rodrigo Borrega[1], Yang Liu[1], Jessica Morris[1], Yanping Xie[1], Oxana Slessareva[1], Beatrix Ferencz[1], Ibeawuchi Oparaocha[1], Zhongyu Zhu[1], Mike Kadan[2], Andrew Worden[2], Boro Dropulić[1,2], Ying Xiong[1]

[1]Caring Cross, [2]Vector BioMed, Gaithersburg, MD, United States

INTRODUCTION

Chimeric antigen receptor (CAR) T-cell therapy has transformed treatment for relapsed and refractory hematologic malignancies, but broad patient access remains constrained by the cost, complexity, and footprint of current manufacturing platforms. Fully automated closed-system instruments reduce contamination risk relative to open processing, but they restrict users into a single vendor's equipment and reagent ecosystem, driving up per-dose costs and limiting the flexibility needed for decentralized, point-of-care production. These constraints are especially evident in low- and mid-income countries, where facilities often lack access to >Grade B GMP space and the capital required for high-cost automated platforms.

To address this gap, we developed a modular, functionally closed-system manufacturing process that decouples individual unit operations rather than consolidating them into a single closed instrument. By pairing the Cytiva Sepax C-Pro for T-cell isolation and CAR-T cell manufacturing with SMART G-Rex®100M-CS bioreactors, the process maintains a strictly closed and sterile fluid path, preserves flexibility in reagent sourcing, and supports multiple parallel patient runs. Here, we demonstrate that this modular approach yields CAR-T products with critical quality attributes comparable to standard open G-Rex 100M protocols. This process incorporates non-invasive SMART sensor-based growth monitoring and offers a scalable and cost-effective pathway toward expanding global access to CAR-T cell therapy.

METHODS

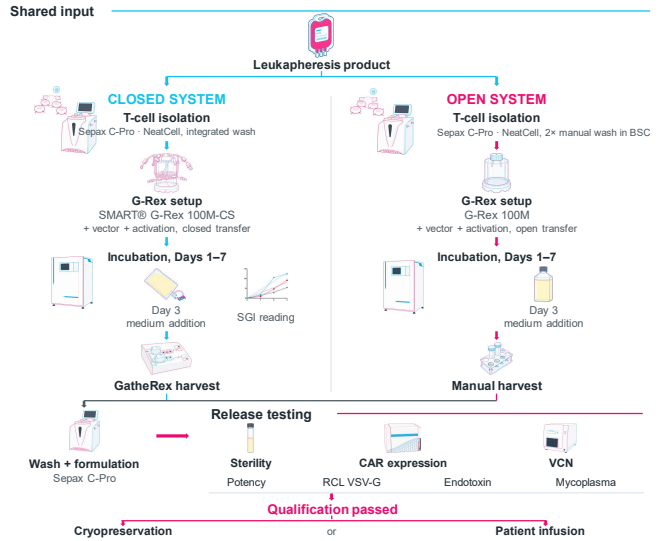


Figure 1. Modular functionally-closed and open system workflow. One leukapheresis product is the only shared input. Each arm then runs its own protocol for T-cell isolation (Sepax C-Pro NeatCell negative selection with integrated washes versus Sepax C-Pro NeatCell negative selection with two manual washes) and for vector addition and activation at G-Rex setup. All closed-system transfers use sterile-welded tubing and closed manifolds.

RESULTS

Decoupled unit operations allow staggered parallel patient runs

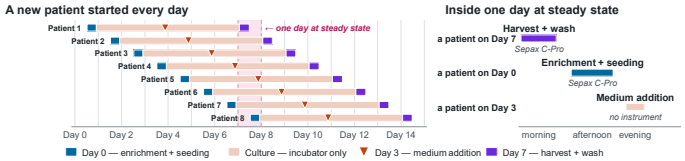


Figure 2. Steady-state schedule with one patient started per day. The leukapheresis product arrives in the afternoon, and negative selection on the Sepax C-Pro followed by G-Rex 100M-CS seeding, activation, and transduction are performed the same afternoon. Medium is added at +70–72 h by gravity fill, which requires no instrument. Harvest and wash return to the Sepax C-Pro on the morning of Day 7. Because the two instrument-bound steps sit at opposite ends of the day, one Sepax C-Pro supports a new patient every day, with seven vessels resident in the incubator at steady state.

Day 7 product matches the validated open-system benchmark

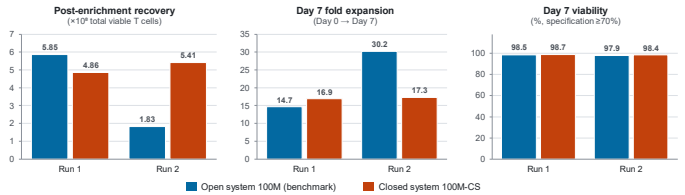


Figure 3. Two head-to-head runs, one donor split between arms in each. Post-enrichment recovery is the total viable T cells released by each enrichment: comparable between systems in Run 1 (5.85 vs 4.86 × 10⁸) and ~3× higher in the closed arm in Run 2 (1.83 vs 5.41 × 10⁸). Day 7 expansion was higher in the closed arm in Run 1 and lower in Run 2 whereas viability is equivalent and above specification.

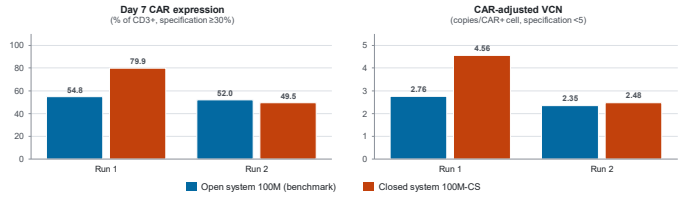


Figure 4. In Run 2, CAR expression and vector copy number are indistinguishable between systems (CAR 52.0% vs 49.5%; CAR-adjusted VCN 2.35 vs 2.48), showing that volumetric vector delivery through the closed fluid path is accurate. Run 1 received different vector volumes per arm (open 0.4 mL neat, closed 1 mL diluted), so its higher closed-arm values reflect dose, not system. CAR-adjusted VCN is total VCN divided by the CAR+ fraction.

RESULTS

QC release testing: open vs closed system across Runs 1–2

Specification	Run 1 · CAR 1		Run 2 · CAR 2	
	Open	Closed	Open	Closed
Post-enrichment T-cell purity, CD3+ (≥70%)	84.3%	85.0%	91.5%	89.2%
Post-enrichment viability (≥70%)	93.5%	89.3%	98.5%	99.5%
Day 3 total viable cells	1.38 × 10⁸	1.10 × 10⁸	1.42 × 10⁸	1.36 × 10⁸
Day 7 total viable cells	1.47 × 10⁸	1.69 × 10⁸	3.02 × 10⁸	1.73 × 10⁸
Day 7 T-cell purity (≥90%)	96.4%	96.2%	96.6%	98.8%
Day 7 cell viability (≥70%)	98.5%	98.7%	97.9%	98.4%
Day 7 CAR expression (≥30%)	54.8%	79.9%	52.0%	49.5%
Fold expansion, Day 0 → Day 7	14.7	16.9	30.2	17.3
Total VCN (<5 copies/genome)	1.51	3.64	1.22	1.23
CAR-adjusted VCN (<5 copies/CAR+)	2.76	4.56	2.35	2.48
RCL, VSV-G at Day 7 (cps/µL)	0.07	0.03	not tested	not tested

Table 1. Release testing across the two head-to-head runs, open versus closed system. Day 3 values are the total viable cells in the 50 mL culture volume before medium addition. Fold expansion is post-wash for Run 1 and pre-wash for Run 2.

Complete QC release panel: two dedicated closed-system 100M-CS runs					
Run	Potency — IFN-γ fold change relative to unstimulated control (CD19 / CD20 / CD22 · ≥5-fold)	Mycoplasma (<3 positive partitions)	Endotoxin (≤2 EU/mL)	Sterility, non-USP (no growth at 7 days)	RCL, VSV-G at Day 7 (no increase over Day 3)
Run 3 · CAR 4	67.8 / 102.8 / 285.9	Negative	0.925 EU/mL	No growth	0.05 cps/µL
Run 4 · CAR 2	49.3 / 77.6 / 92.8	Negative	0.824 EU/mL	No growth	0.39 cps/µL

Table 2. Two further closed-system 100M-CS runs for our tri-cistronic CAR-T product completed the full QC release panel, passing the four assays shown here and every specification tested in Runs 1-2.

Non-invasive SMART monitoring tracks viable-cell yield in a sealed vessel

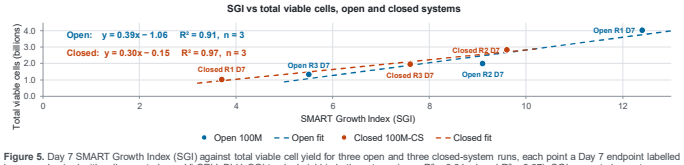


Figure 5. Day 7 SMART Growth Index (SGI) against total viable cell yield for three open and three closed-system runs, each point a Day 7 endpoint labelled by run and paired with cells counted on a Vi-CELL BLU. SGI tracked yield in both systems (open $R^2 = 0.91$; closed $R^2 = 0.97$). SGI supports harvest assessment rather than a validated release threshold.

CONCLUSIONS

- A modular, functionally closed process built from the Sepax C-Pro and the SMART G-Rex 100M-CS yields CAR-T products whose critical quality attributes match our validated, tech-transferred open-system benchmark.
- Vector delivery through the closed fluid path is accurate: total and CAR-adjusted VCN were within 3% of the open arm in the matched-dose run.
- Closed-system T cell enrichment eliminates the manual wash steps and recovered ~3× more T cells where leukapheresis input was matched.
- Non-invasive SMART sensor readings track Day 7 yield (open $R^2 = 0.91$; closed $R^2 = 0.97$; n = 3 runs per system).
- Decoupled unit operations let runs be staggered in parallel, which is a practical route to decentralized manufacturing without >Grade B space or a high-capital consolidated platform.

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Contact information: partner@caringcross.org