

Consumable-enabled transduction of resting T cells for CAR-T manufacturing



Lopamudra Sadhu, Aisling McCrudden, Kirti R. Mahadik, Jude Kevin Lim, Aya Nashat, Riona Downes, Lauren Sullivan, Aine O'Sullivan, Emer Hackett, Louise Gallagher, Darren Martin, Lisa O'Flynn
Avectas Ltd, Dublin, Ireland

Introduction

Transduction of resting T cells represents an attractive strategy for preserving favourable cellular attributes during CAR-T manufacturing. In this study, we evaluated the feasibility of CAR transduction of resting T cells within a closed, consumable-based manufacturing process. A consumable-only workflow integrating lentiviral transduction, expansion, and harvest was assessed using a streamlined 3-4 day CAR-T process. Resting CD3⁺ T cells were directly transduced within the consumable on Day 0 using lentiviral vectors at multiplicities of infection (MOI) ranging from 1.5 to 3. Cell viability, CAR expression, T cell phenotype, and functional activity were assessed at harvest.

Direct transduction of resting T cells within the consumable achieved >90% viability by Day 3, with >95% of cells retaining a resting CD3⁺ following CAR delivery. CAR expression exceeding 50% was achieved across multiple donors under resting transduction conditions. Phenotypic analysis demonstrated enrichment of less-differentiated, stem-like T cell populations with low activation markers. These results indicate that the Solupore® Integrated consumable-based manufacturing process enables efficient CAR transduction of resting T cells while maintaining favourable phenotypic and functional attributes. This approach provides a flexible foundation for the development of next-generation CAR-T products, with the potential to simplify manufacturing workflows and enhance cell product quality.

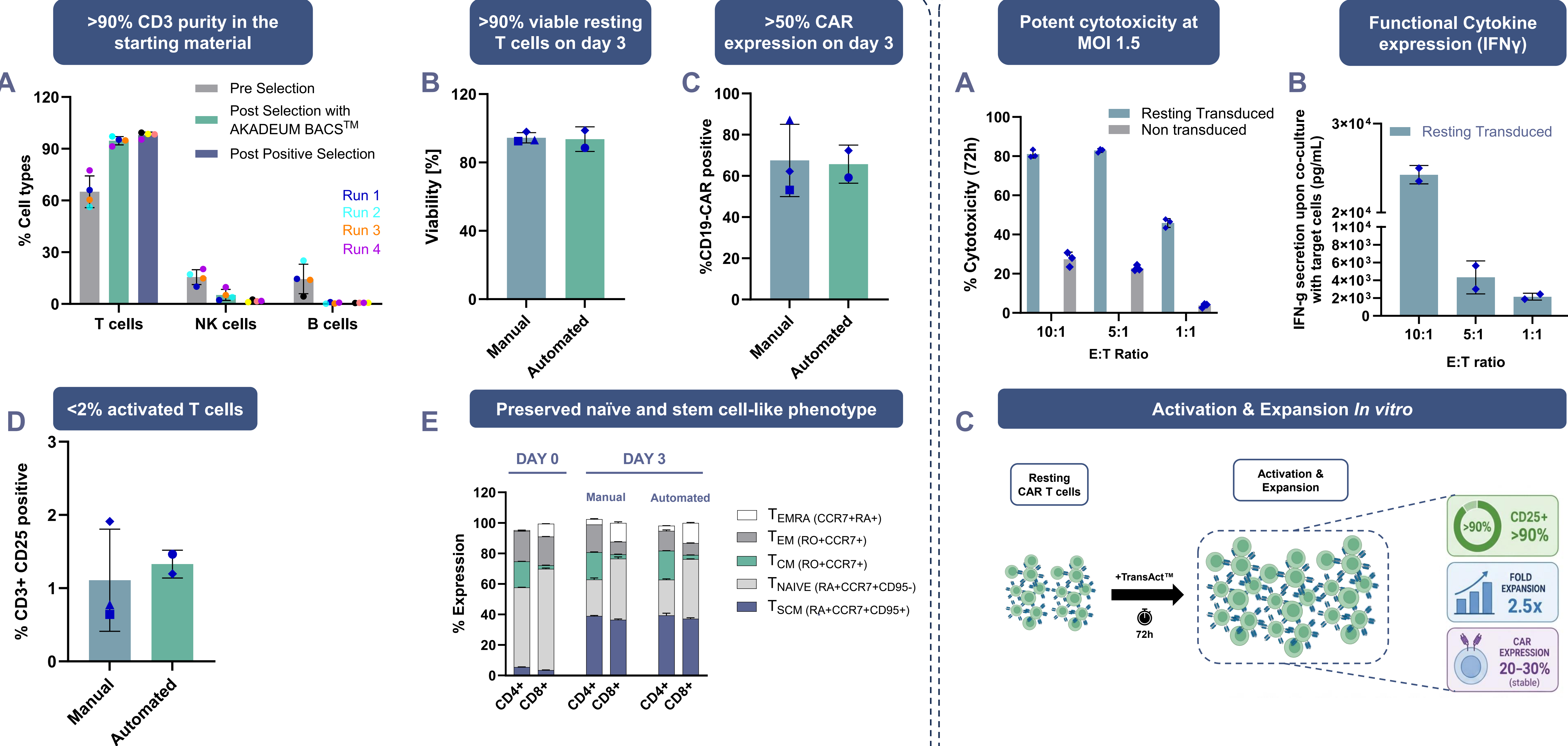
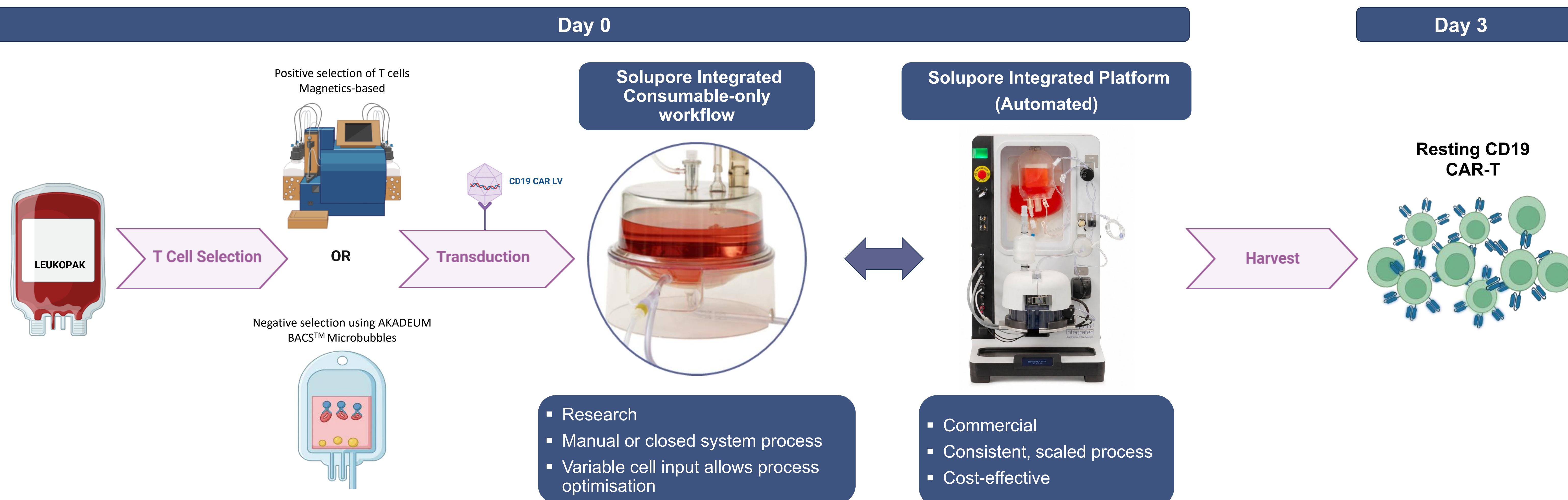


Figure 1: T cells selected either positively with magnetic sorting or using AKADEUM'S BACS microbubbles, were seeded in the Solupore consumable in TCM media + IL-7/15 immediately post selection on day 0. The consumable was used either on its own (referred to as 'manual') or as part of Solupore Integrated platform in an automated manner. On Day 0, the cells were transduced with a second generation CD19-CAR lentiviral vector (MOI 1.5-3), followed by a media feed on Day 1 and harvest on Day 3. **(A)** Representative percent of total T cells, B cells and NK cells in the starting donor material were evaluated for all donors selected with positive or negative T cell selection approaches on day 0 using TBNK staining via flow cytometry. **(B)** Representative percent viability on harvest at day 3, across 3 donors tested in Solupore consumable (manual) and 2 donors tested in Solupore Integrated platform (automated). **(C)** Consistent CD-19 CAR expression was observed on harvest at day 3 across both manual and automated process, yielding >50% CD19-CAR+ efficiency in all the donors tested. **(D)** Representative percent CD25 positive cells were assessed using flow cytometry by gating on CD25 positive cells within the CD3 positive population. Each symbol represents an independent donor. **(E)** Stem cell memory phenotyping on day 0 input material and on harvest at day 3 was performed by flow cytometry based on the gating strategy defined in figure 1E for each T cell subset. The representative percent expression data is from one donor which was processed with positive T cells selection.

Figure 2: T cells selected positively with magnetic sorting, were seeded to the Solupore consumable in TCM media + IL-7/15 immediately post selection on day 0. The consumable was used on its own (referred to as 'manual'). On Day 0, the cells were transduced with a second generation CD19-CAR lentiviral vector (MOI 1.5), followed by a media feed on Day 1, harvest on Day 3 & cryopreservation at 10E6/mL. **(A-B)** Functional capacity of cryopreserved product was assessed via co-culture with CD19+ Nalm-6 cells. Cytotoxicity was assessed via flow cytometry (A) while IFN- γ production was measured by ELISA (B). For cytotoxicity assessment upon 72h of co-culture, target (Nalm-6) cell killing defined by double positive Tag-it Violet® & Sytox Red cells were measured by flow cytometry. For IFN- γ ELISA, IFN- γ release was measured upon co-culturing for 24h with target Nalm-6 cells using the Human IFN-g ELISA kit. **(C)** Schematics shows the activation and expansion potential of the day 3 harvested resting CAR T cells *in vitro* upon stimulation with TransAct™ for 72h, yielding highly activated and expanded T cells with possible stably integrated CAR.

Summary

- The Solupore Integrated consumable supports CAR-T manufacturing across a range of scales (cell inputs), enabling direct processing of resting T cells and bridging early-stage development through to commercial manufacture.
- Reproducible performance across donors, enabled by the consumable and automated platform supports a cost-effective and scalable approach to CAR-T cell therapy development and manufacture.
- CD19 CAR-T cells generated via direct transduction of resting T cells using Solupore Integrated platform retain early memory/stem-like T cell subsets and exhibit high cytotoxic potency along with robust CAR expression.

Discussion

This study demonstrates how the Solupore Integrated enables efficient CAR-T manufacturing through direct transduction of resting T cells within a scalable, automated and GMP- aligned workflow. By eliminating the need for pre-activation steps this approach supports preservation of less-differentiated T cell states, contributing to favourable phenotypic and functional attribute in the final product.

This process reduces complexity and process time while supporting consistent and reproducible manufacturing. These features position the platform as a promising approach for accelerating clinical translation and improving the economic feasibility of CAR-T therapies.

solupore
integrated
Engineered by Avectas