Robust, Reproducible and Rapid CAR T-cell Manufacturing for an Agile Phase I Platform Trial

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INTRODUCTION

Chimeric antigen receptor (CAR) T-cells directed against the B-cell antigen CD19 are a standard of care for relapsed or refractory large B-cell lymphomas. We are conducting phase 1 and 2 trials (ENABLE-1, NCT04049513; ENABLE-2, NCT06486051) of autologous CD19-directed third-generation CAR T-cells incorporating CD28 and TLR2 intracellular co-stimulatory domains.

Automation of CAR T-cell manufacturing can result in cost and operator time savings, while shortening the period of *ex vivo* CAR T-cell expansion limits facility utilisation, and may result in a CAR T-cell product exhibiting a less terminally differentiated immunophenotype and enhanced function. However, changes to manufacturing processes, including cell expansion time, reagent selection and platform may influence CAR T-cell phenotype and function.

During the ENABLE-1 trial, products were manufactured using an 11-day manual process using standard tissue culture vessels, before transitioning to a proprietary automated process using the Lonza Cocoon® Platform within a dose expansion cohort. To assess the impact of implementing CAR T-cell manufacturing processes across different culture platforms, we sought to compare CAR T-cell yield and phenotype between the 11-day process conducted using standard tissue culture vessels, a proprietary automated Cocoon® protocol, and in G-Rex® devices, equipped with gas permeable membranes.

Next, we sought to abbreviate the culture period from 11 to 7 days using the G-Rex platform, and assessed CAR T-cell yield, transduction efficacy, function and immunophenotype across four human donors.

3RD GENERATION CAR T-CELLS WITH TLR2 DOMAIN

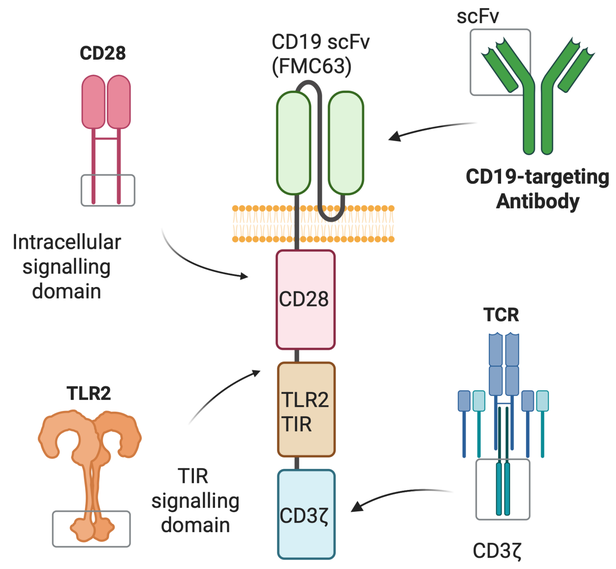

Figure 1. CAR1928T2z is a third generation, anti-CD19 CAR. The extracellular antigen recognition domain is an anti-FMC63 antibody single chain variable fragment. The construct contains two intracellular co-stimulatory domains; CD28 and toll-like receptor 2 (TLR2) TIR and a CD3ζ signaling domain.

11-DAY CAR T-CELL MANUFACTURING

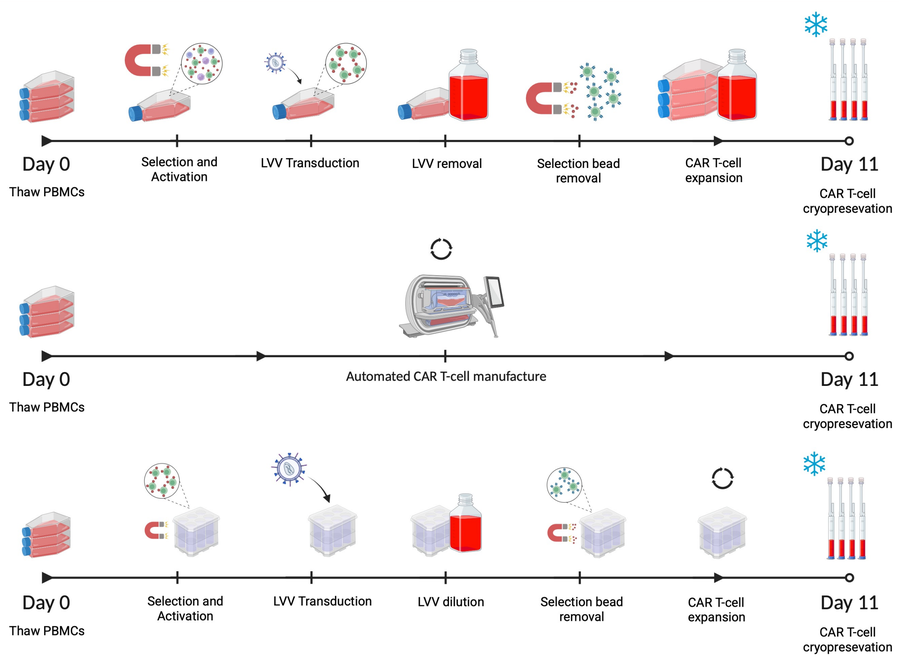

Figure 2. Manufacturing schema for 11-day CAR T-cell manufacturing. T-cells were isolated from peripheral blood mononuclear cells (PBMCs). They were then selected, activated and transduced with a third-generation lentiviral vector (LVV) encoding the 1928T2z third-generation anti-CD19 CAR. The CAR T-cells were expanded for a total of 11 days. This protocol was initially conducted manually using standard tissue culture vessels, before the adoption of a proprietary automated Cocoon® protocol. We adapted the original manual standard tissue culture vessel manufacture to the G-Rex cell culture system.

RESULTS

11-day manual, automated and G-Rex CAR T-cell manufacture

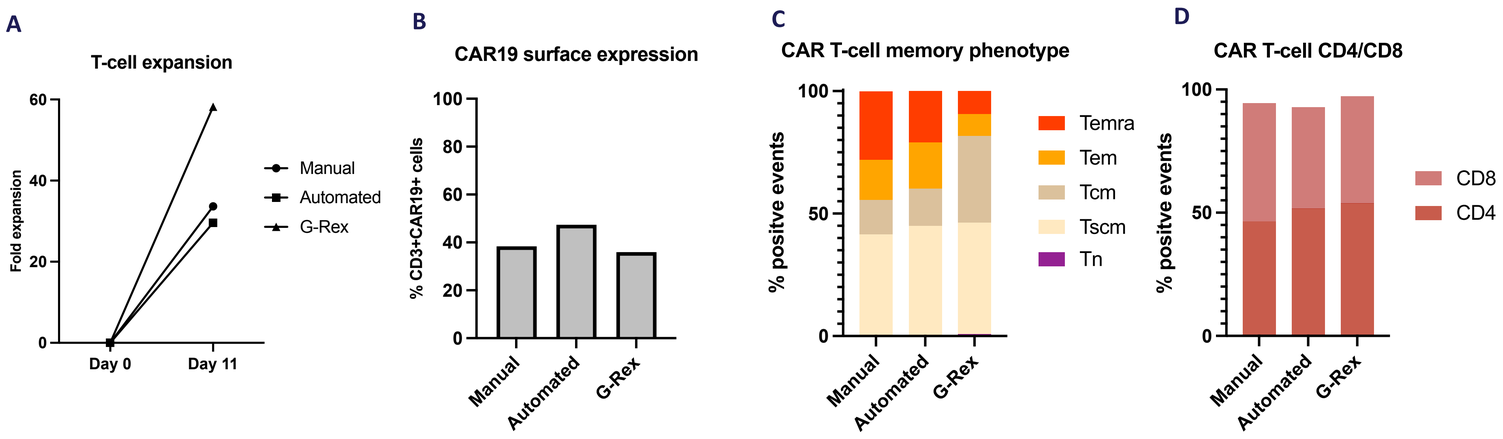

Figure 3. 11-day CAR T-cells were produced using standard tissue culture flasks ('Manual'), a proprietary Cocoon® method ('Automated') and G-Rex from PBMCs of Donor 1. A. Over 30—fold expansion was achieved on Day 11 using all three methods. **B.** CAR19 surface expression was determined by flow cytometry and was consistent across the three platforms. **C.** Day 11 CAR T-cells were phenotyped for memory/effector subsets using CD45RA, CD62L and CD95. $CD45RA^+CD62L^+CD95^+$ (T_{scm}) and $CD45RA^-CD62L^+$ (T_{cm}) memory T-cell populations were enriched in all manufacturing conditions. **D.** The CAR T-cell CD4 vs CD8 ratio remained consistent across all three platforms for this donor.

7-day CAR T-cell manufacture using the G-Rex platform

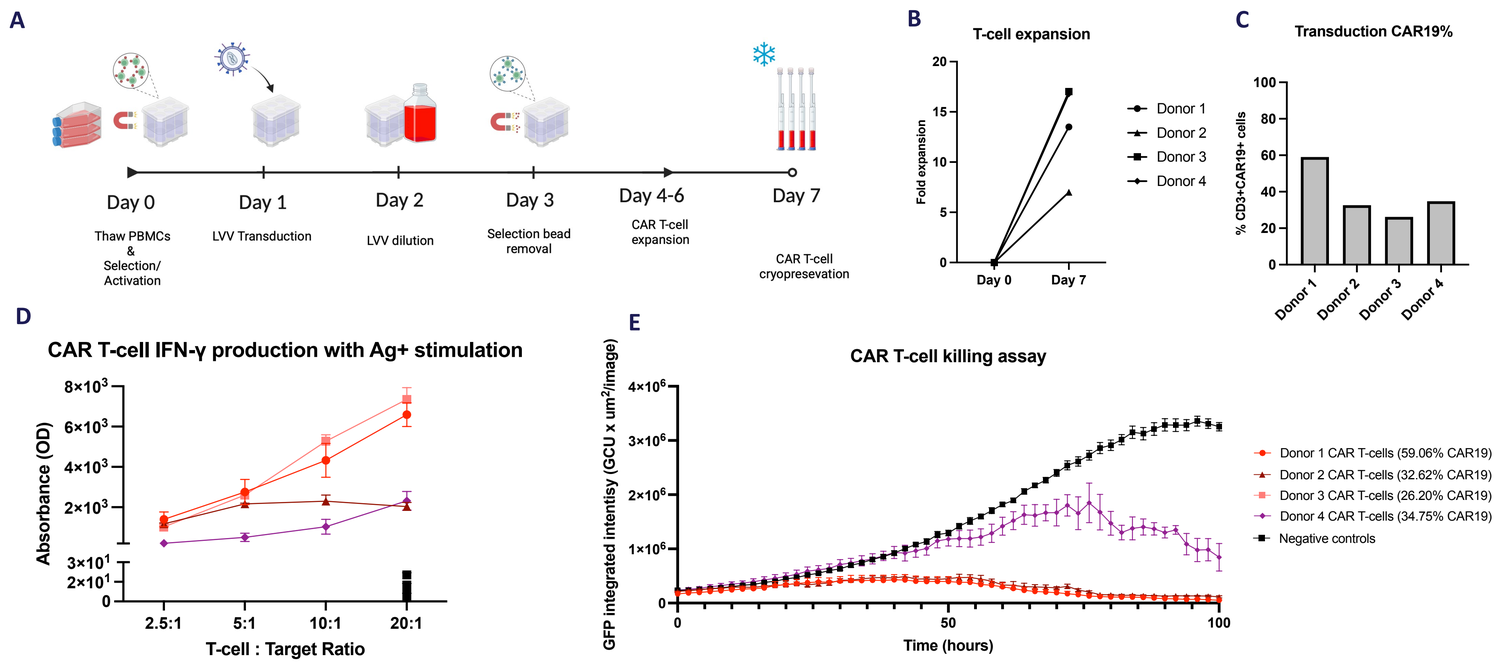

Figure 4. 7-day CAR T-cell manufacture using the G-Rex 2cm² plates using four different patient donor starting material. A. Schema of the 7-day manufacturing process, with condensed Day 0 manufacturing steps. **B.** Fold expansion of viable patient T-cells across 7-day culture. **C.** CAR19 expression of four donor CAR T-cells on Day 7 of manufacturing. **D.** Interferon gamma (IFN-γ) production detected by ELISA. CAR T-cells were co-cultured in a serial dilution with K562 Ag+ cells. ELISA was conducted in triplicate and SEMs are shown. Legend shown in Figure E. **E.** CAR T-cells co-cultured with GFP expressing K562 Ag+ cells at a T-cell : Target ratio of 2:1 were captured on the IncuCyte Live imaging system for 4-days. Samples were done in triplicate and SEMs are shown. Note: cytotoxicity data not available for donor 3.

7-day CAR T-cells exhibit memory phenotypes and balanced CD4/CD8 ratios across four patient donors

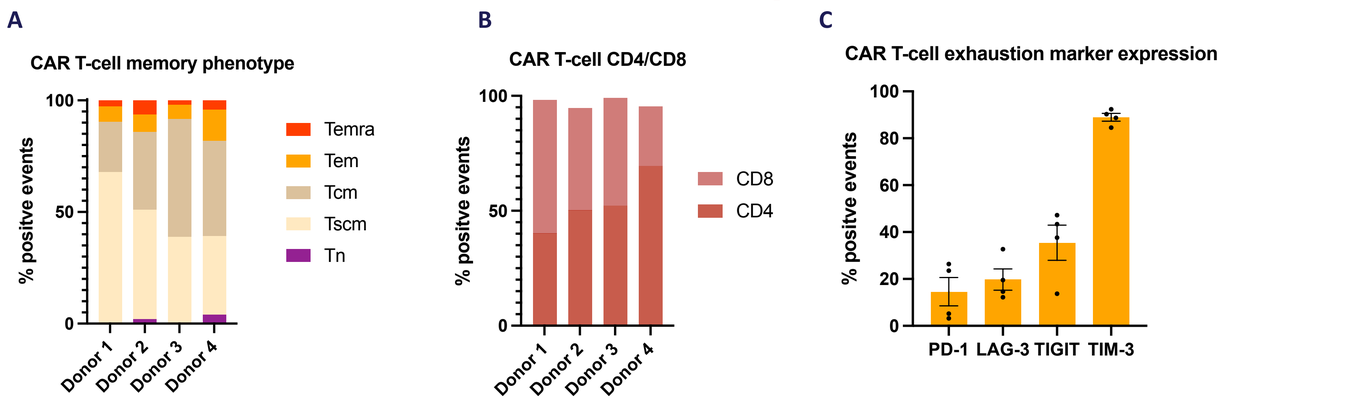

Figure 5. 7-day CAR T-cells show high proportions of resting memory phenotypes and limited exhaustion marker expression. **A.** Memory phenotypes of 7-day CAR T-cells were determined by flow cytometry. A high proportion of less-differentiated T cell subsets, were observed with enriched populations of $CD45RA^+CD62L^+CD95^+$ (T_{scm}) and $CD45RA^-CD62L^+$ (T_{cm}) T-cells. Further differentiated CAR T-cell phenotypes including $CD45RA^+CD62L^-$ (T_{emra}) and $CD45RA^-CD62L^-$ (T_{em}) were found at lower proportions. **B.** There was a balance between the CD4+ and CD8+ CAR T-cell subsets across 4 donors. **C.** Expression of common activation/exhaustion markers; PD-1 (CD279), LAG-3 (CD223), TIGIT and TIM-3 (CD366) were observed using flow cytometry. Means (bar height) and SEMs are shown.

CONCLUSIONS/FUTURE DIRECTIONS

At research scale, we were able to transfer a manual standard tissue culture vessel 11-day manufacturing process to the G-Rex platform, resulting in a satisfactory yield of CAR T-cells exhibiting similar phenotype and function. Shortening the manufacturing process from 11 to 7 days resulted in CAR T-cell products exhibiting high proportions of memory T-cells and a balanced CD4/CD8 ratio, with robust anti-tumour activity observed.

Further work will include assessment of serum-free culture media, alternative T-cell isolation and activation methods, and process scale-up with comparability studies.

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