



Application Note

Rapid Cocoon® Platform CAR-T: Integrated T-cell selection using CTS™ Detachable Dynabeads™ CD4 and CD8 to enable rapid manufacturing

To enable rapid manufacturing (RM) of CAR-T cells, an upstream T cell selection step is often integrated to ensure generation of a high-purity product within a short manufacturing time.

CTS™ Detachable Dynabeads™ CD4 and CD8 can be used to bulk-select total T cells or to sequentially separate each subset to achieve desired ratios of CD4+ and CD8+ T cells in the input material. Immediately, following cell selection, beads are detached from the cells and removed from the culture. The positively selected, bead-free T cells are subsequently activated and transduced.

Automation in the Cocoon® Platform simplifies production, reduces labor and improves manufacturing reliability. The use of CTS™ Detachable Dynabead™ CD4 and CD8 reagents for T cell selection may also allow for increased flexibility in manufacturing including integrating a T cell activation reagent of choice.

In this application note, we describe a three-day rapid CAR-T cell manufacturing process with integrated CD4+ and CD8+ T cell selection in the Cocoon® Platform that consolidates T cell enrichment, activation, transduction, and final product harvest into a single-use, functionally closed cassette (Figure 1).

Rapid CAR-T manufacturing with Integrated Selection on the Cocoon® Platform

- Integrated CD4+ and CD8+ T cell selection directly from apheresis material
- Selection of up to 4 billion T cells with the ability to cryopreserve excess selected cells for future manufacturing runs
- Flexibility to activate T cells with a soluble reagent of choice
- Automated magnetic bead removal and product harvest in the Cocoon® Platform
- RM products have a robust stem cell memory profile and strong anti-tumor responses

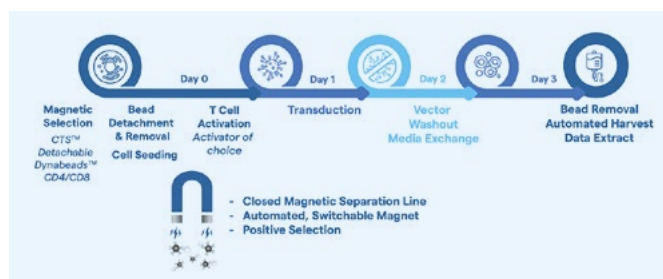


Figure 1. Rapid manufacturing of CAR-T cells with integrated CD4 and CD8 T-cell selection in the Cocoon® Platform

Efficient CD4+ and CD8+ T cell selection and manufacture of RM CAR-T cells using the Cocoon® Platform

For this application, three cryopreserved healthy donor apheresis samples (containing 45-53% T cells) were thawed and directly incubated with CTS™ Detachable Dynabeads™ CD4 and CD8, achieving greater than 84% purity post-selection bead release (Figures 2A, B). As expected, CD4+ and CD8+ T cell subsets were both enriched during the selection process (Figure 2C, D). Notably, platelets and RBC were reduced respectively to a mean of 2.7% and 4.0% of total live events after CD4 and CD8 positive selection in the Cocoon® Platform (Figure 2A). This workflow also allows for processing larger apheresis volumes and preserves excess selected CD4+ and CD8+ T cells for later use.

Selected T cells were subsequently activated and transduced using a lentiviral vector delivering a CD19-CAR. Following virus washout, cells were cultured in TheraPEAK® X-VIVO® 15 media containing 5% human serum with IL-2 for one additional day before product harvest. The 3-day manufacturing process results in maintenance of viable T-cells with greater than 80% T cell recovery and robust Dynabead™ removal (Figure 2E-G). By carrying out satellite cultures of a small proportion of the harvested product to day 7, we observed CAR-T cell expansion and stability of CAR expression (Figure 3G).

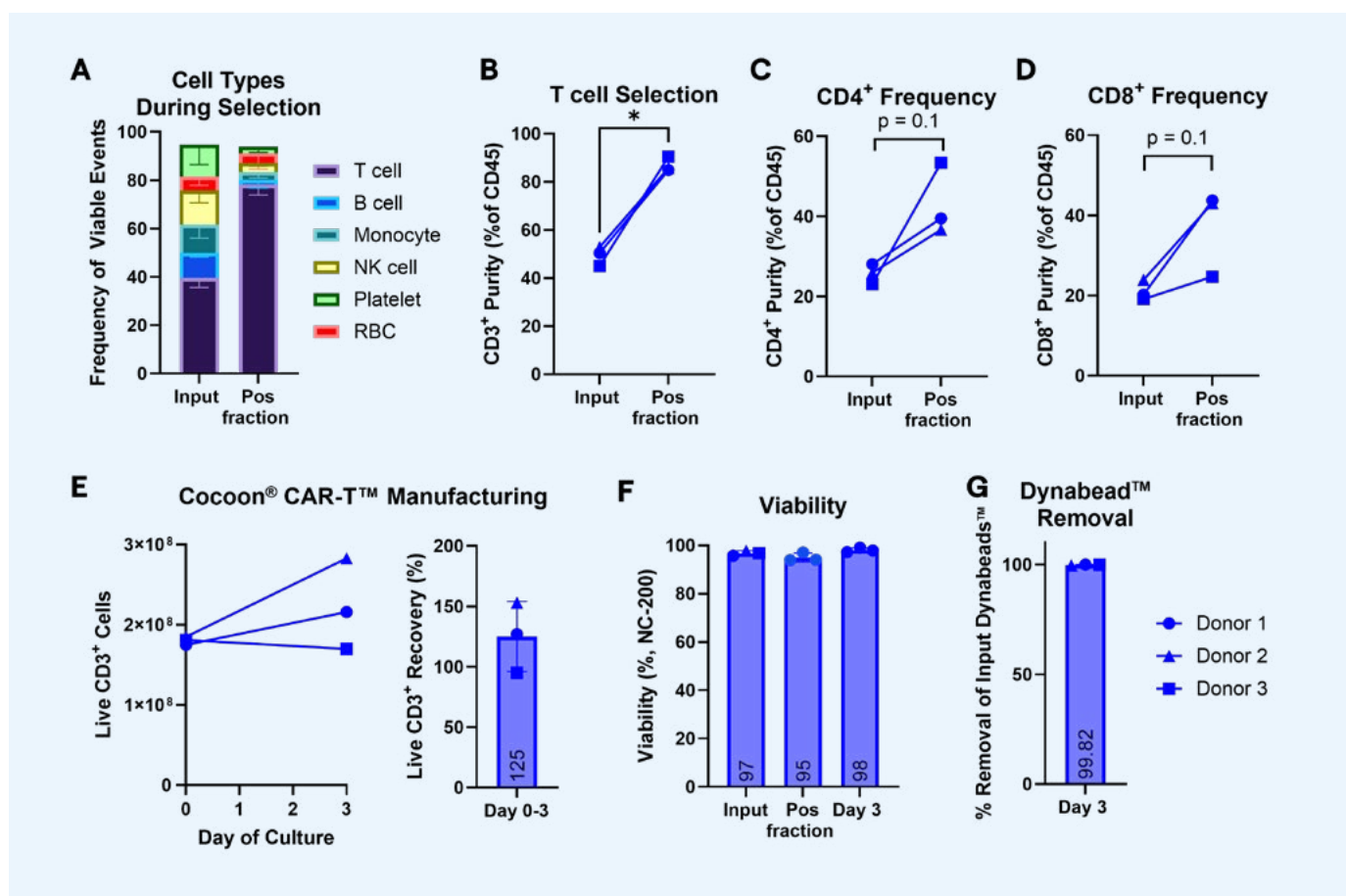


Figure 2. CD4+ and CD8+ T cell selection and Rapid Manufacturing on the Cocoon® Platform:

A) Proportion of cell types as a fraction of total live flow cytometry events in the input material and in the positively selected Cocoon® Platform product on day 0. T cell, CD3+; B cell, CD19+; monocyte, CD14+; NK cell, CD56+CD3-; platelet, CD45-CD41+, red blood cell (RBC), CD45-CD235a+. Error bars represent standard deviation of n=3 donors.

B-D) Robust enrichment of CD3+, CD4+ and CD8+ T cells in the positively selected Cocoon® Platform product on day 0. Frequency is reported as % of live CD45+ cells. * p<0.05; (paired t-test).

E) Left graph shows the number of live CD3+ T cells seeded on day 0 and the total CD3+ cells harvest on day 3. Right graph shows the percentage recovery of live CD3+ as a proportion of the input. For two of the donors, notable cell expansion was observed.

F) Cell viability remains high throughout the process: Input apheresis material (prior to selection), post-selection positive fraction on day 0, and in the final drug product on day 3.

G) Automated removal of CTS™ Detachable Dynabeads™. Nearly 100% of the beads are removed through the automated bead-removal steps integrated into the Cocoon® Platform protocol.

CD4+ and CD8+ selected Cocoon® Platform CAR-T cells have a strong memory phenotype and show robust CAR-T mediated cytotoxicity

The Cocoon® Platform RM protocol reproducibly yielded CAR-T cell products that maintain robust memory characteristics, activation profile, and proliferative potential (Figure 3D-H). Rapid Cocoon® Platform CAR-T products showed excellent cryostability (post-thaw recovery, viability and proliferative potential), when cryopreserved

in a controlled-rate freezer (Figure 3 I,J). Functionally, RM Cocoon® Platform CAR-T products exhibited strong CAR-mediated cytotoxicity and cytokine release when activated in co-culture with NALM6 tumor cells over two successive 4-day challenges (Figure 4A-E).

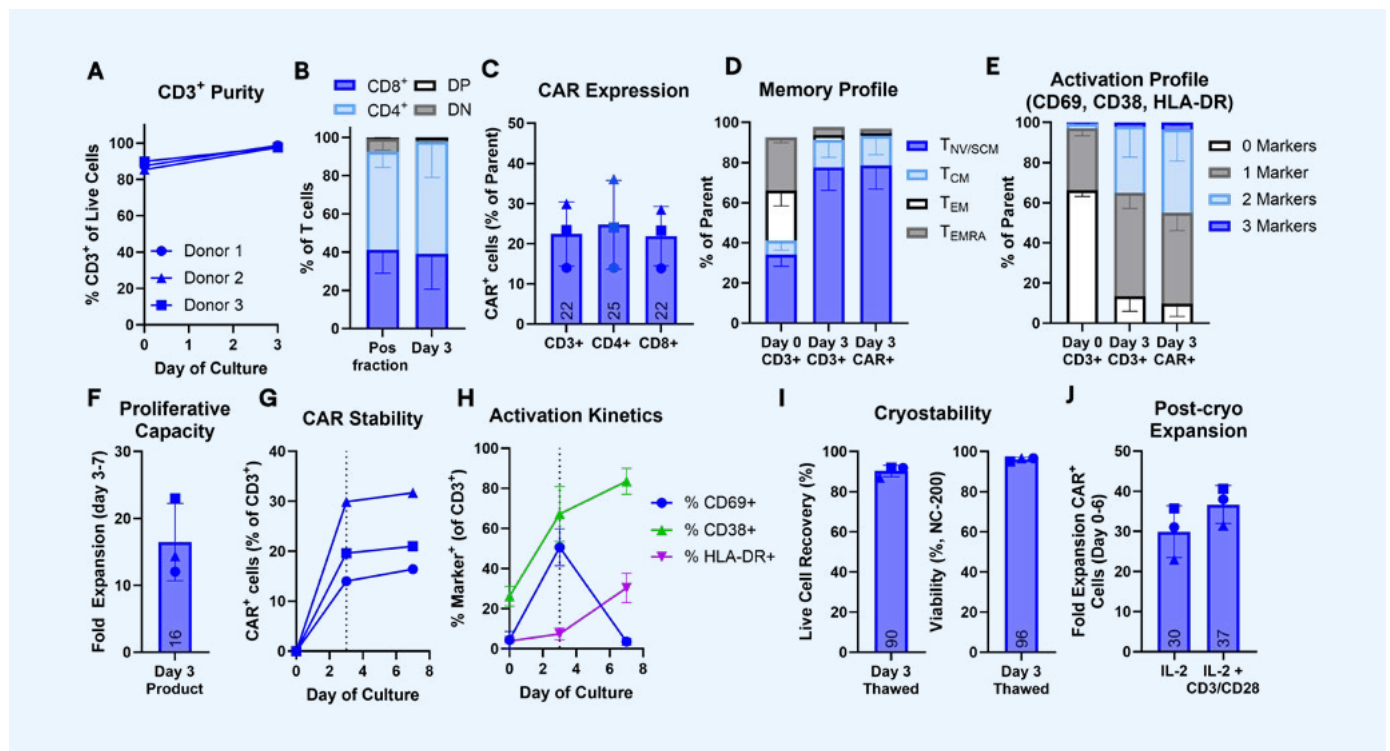


Figure 3. Characterization of Rapid CAR-T Products:

A) Cocoon® Platform CAR-T product phenotype was assessed by flow cytometry for n=3 donors. The CD3+ frequency (of live cells) in the positive fraction on day 0 was further enriched to greater than 95% in the Cocoon® Platform Products on day 3.

B) Consistent T cell phenotype is maintained during manufacturing. Stacked bar graphs show the proportion of CD3+ T cells in the positive fraction on day 0 and in the Cocoon® Platform products on day 3 that are CD8+, CD4+, CD4+CD8+ (Double Positive; DP) and CD4-CD8- (Double Negative; DN).

C) The proportion of CD19-CAR+ transduced cells among total CD3+ T cells, CD4+ T cells, and CD8+ T cells in the Cocoon® Platform products on day 3, MOI = 0.5. Numbers in bar graphs indicate the mean value. Error bars represent standard deviation.

D) Stacked bar graphs show the proportion of CD3+ T cells on day 0 and day 3 that are naïve or stem cell memory ($T_{NV/SCM}$: CD45RA+CCR7+), central memory (T_{CM} : CD45RA-CCR7+), effector memory (T_{EM} : CD45RA-CCR7-) and effector memory re-expressing CD45RA (T_{EMRA} : CD45RA+CCR7-). Greater than 80% expression of $T_{NV/SCM}$ and T_{CM} subsets in both the CD3+ and CAR+ subsets.

E) Stacked bar graphs show the proportion of T cells on day 0 and day 3 and CAR+ T cells on day 3 that are expressing 0, 1, 2 or 3 of the activation markers CD69, CD38 and HLA-DR.

F) Products were cultured in TheraPEAK® X-VIVO® 15 media containing 5% human serum with 100 IU/mL IL-2 until day 7 in small-scale satellite cultures. Graph shows robust proliferative capacity of RM Cocoon® Platform CAR-T.

G) The frequency of CD19-CAR+ transduced T cells from day 0 to 3 in the Cocoon® Platform, and from day 3 to 7 in satellite cultures were monitored. Day 7 CAR-T cell frequency is stable within 5-10% of the day 3 product when transduced at an MOI of 0.5.

H) Stacked bar graph shows CD3+ T cell expression of activation markers CD69, CD38 and HLA-DR from day 0-3 in the Cocoon® Platform, and, upon extended culture to day 7 in small-scale (after dotted line).

I) Graphs show the live cell recovery and viability of cryopreserved day 3 RM Cocoon® Platform products upon cell thaw.

J) Cryopreserved Cocoon® Platform CAR-T products were thawed and stability of cells cultured for six days in TheraPEAK® X-VIVO® 15 media containing 5% human serum with 100 IU/mL IL-2, or, with 100 IU/mL IL-2 plus soluble CD3/CD28 activation was monitored.

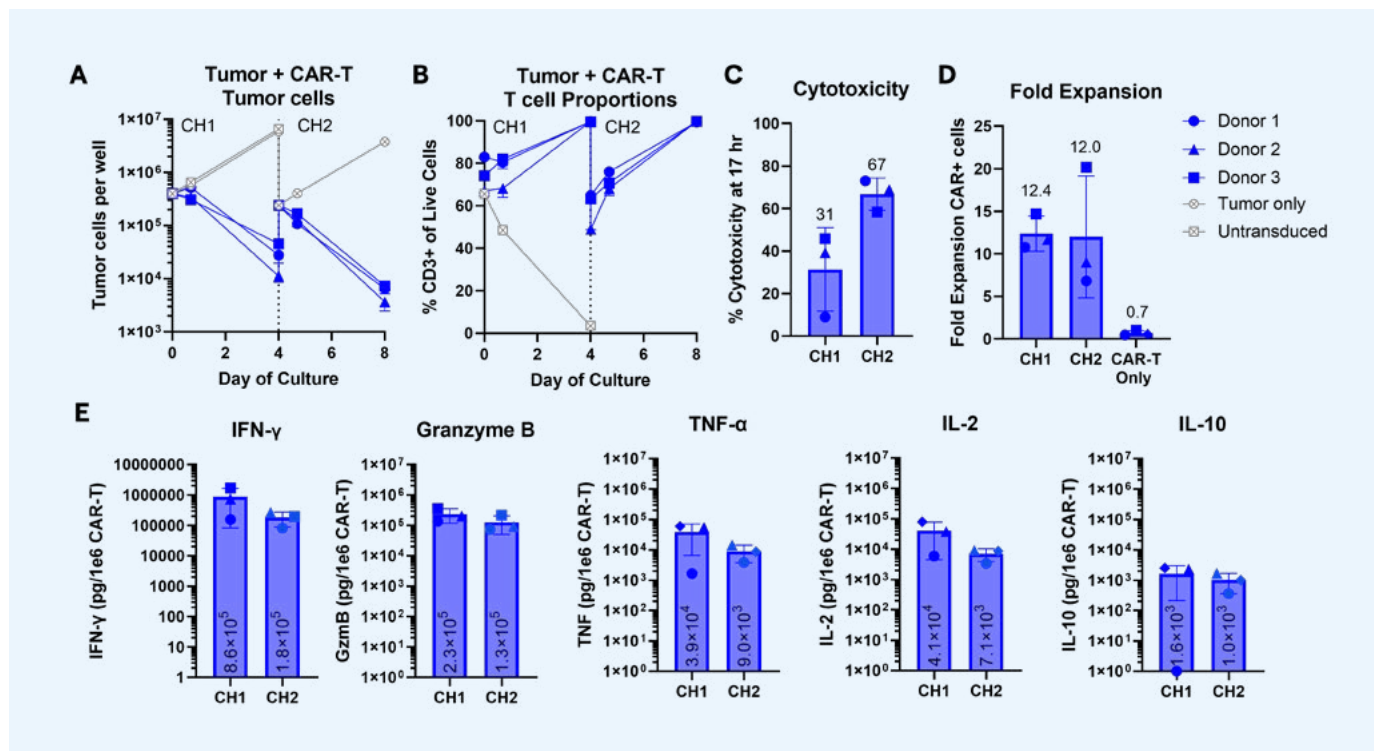


Figure 4. Rapid CAR-T cells, Efficiently Kill Tumor Cells:

A-C) CAR-T cells were co-cultured with NALM6 tumor cells at a 1:2 CAR-T:tumor cell ratio for four days. Cells were sampled after 17 hours of culture. At the end of four days (CH1; challenge 1), the process was repeated by addition of fresh NALM6 to the CAR-T cell cultures (CH2; challenge 2). Graphs show the number of live tumor cells (A), and the proportion of CD3+ T cells (B) in each well during CH1 and CH2. Co-cultures with untransduced Cocoon® Platform T cells, and cultures with only NALM6 tumor cells are also shown. (C) Graph shows the percentage cytotoxicity of each Cocoon® Platform CAR-T product at the 17 hour timepoint for CH1 and CH2. Each symbol represents a distinct donor. Numbers on the bar graphs indicate

the mean value, error bars represent standard deviation.

D) Graph shows the fold expansion of CAR-T cells over four days in CH1 and CH2. Robust 12-fold expansion is observed for both challenges. In comparison, no expansion occurs when Cocoon® Platform CAR-T are cultured without tumor cell addition (CAR-T only).

E) Cytokines in the supernatant of Cocoon® Platform CAR-T and tumor cell co-cultures were analyzed by LEGENDplex™ Multiplex Assay. Graphs show levels of the indicated cytokines (picograms per one million CAR+ T cells) in CH1 and CH2. High levels of IFN-γ and Granzyme B were produced in response to tumor cells.

Contributors

Kathleen Zohorsky, Beth Macleod, Khalil Dayekh, Aman Mehrotra, Janet J. Sei, Kelly Purpura, Tamara Laskowski

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