



Application note

Cocoon® CAR-T™: manufacturing cell therapies in the Cocoon® Platform

The manufacture of Chimeric Antigen Receptor (CAR)-engineered T cells requires a series of critical steps to enable activation, CAR-delivery, and expansion of genetically-modified T cells. This process is inherently complex and labor-intensive, often requiring dedicated operator time to execute each function. Automating CAR-T cell manufacturing offers multiple advantages, including reducing operator touchpoints, improving process efficiency and robustness, and increasing throughput.

In this application note, we describe a 9-day process for functionally-closed and automated manufacture of CD19-CAR-T cells using the Cocoon® Platform. In this process, we combine multiple unit operations into a single instrument, streamlining manufacture and reducing the burdensome labor requirements. Starting with PBMCs, the Cocoon® Platform supports robust activation, T cell transduction, and expansion of CAR-T cells. An integrated automated harvest step enables efficient formulation and collection of the final drug product.

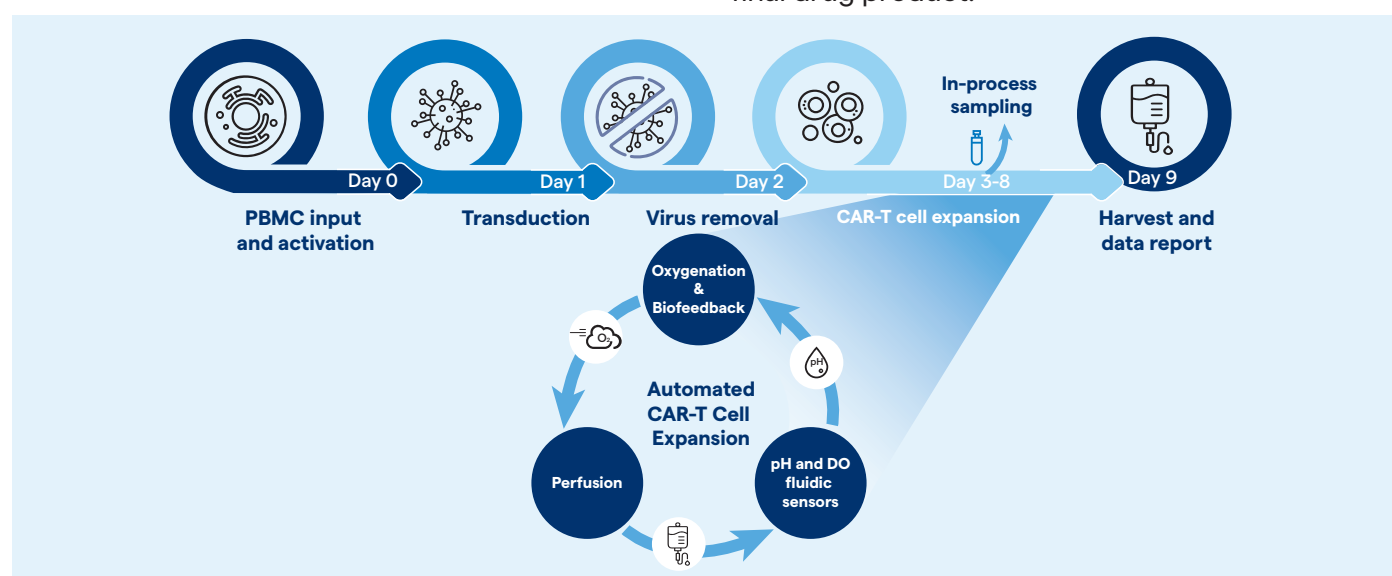


Figure 1:
Automated Cocoon® CAR-T™ manufacturing process overview.
Applicable input material includes pre-processed PBMC or selected T cells. Upon seeding, cells are activated and subsequently transduced in pre-programmed steps that comprise optimal media volumes and incubation duration.

Viral vector is removed in an automated step that completely replaces the transduction media with fresh expansion media. Optimally scheduled medium recirculation and media exchanges continue to support cell proliferation and expansion. On day 9, an automated harvest protocol is initiated and product is washed, formulated in harvest buffer, and collected.

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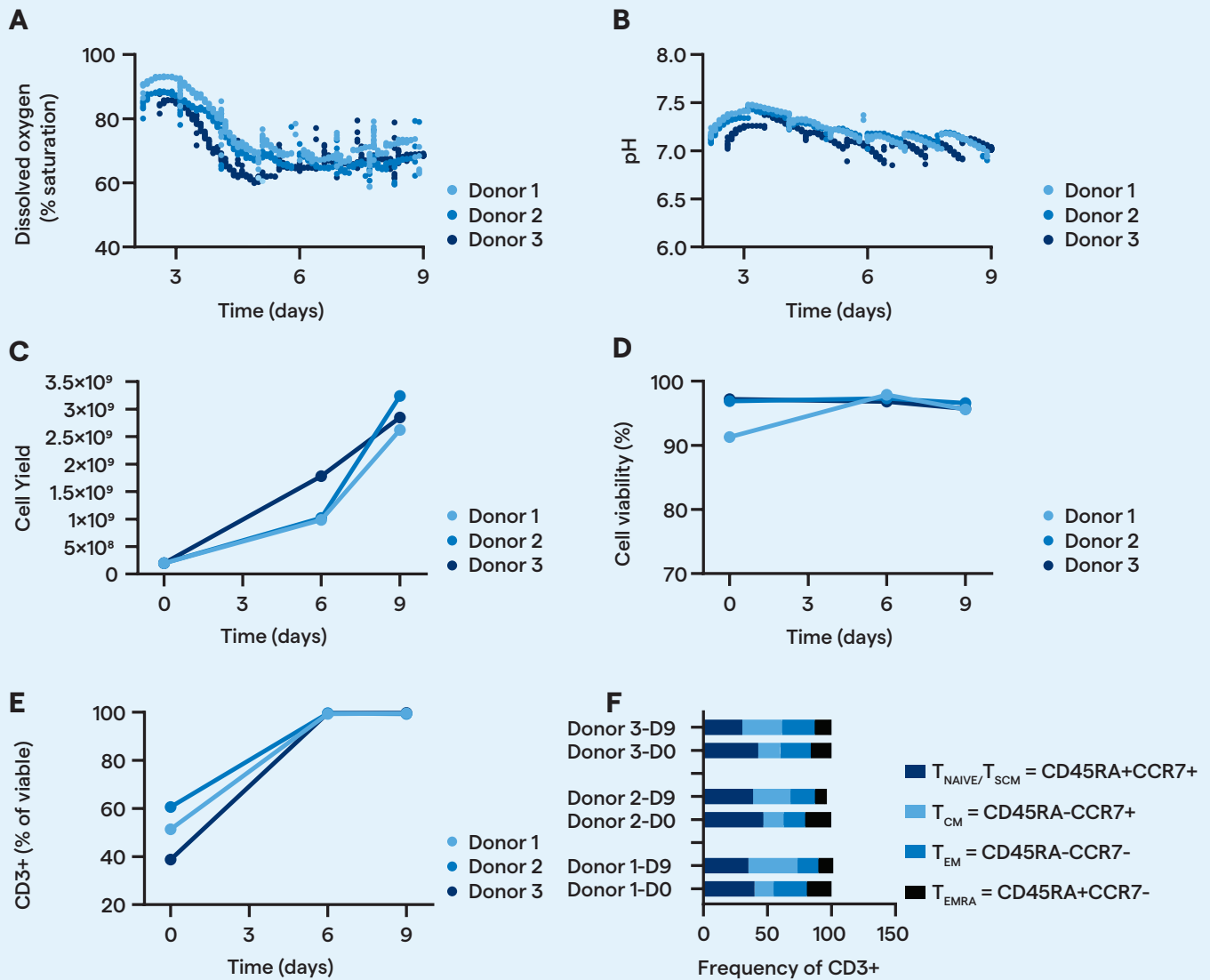


Figure 2:

T-cell expansion in Cocoon® Platform. The Cocoon® performs real-time monitoring of temperature, environmental %CO₂, pH, and culture dissolved oxygen to enable the establishment of optimal culture conditions for T cell activation and expansion.

A, B. Data extracts for dissolved oxygen and pH measurements captured continually at 10 minute intervals for the duration of the manufacturing process. Three healthy donor Cocoon® CAR-T™ manufacturing processes are shown.

C. Total T cell yields achieved for each manufacture are measured at mid-process (day 6) and end of process (day 9). Each Cocoon® run was initiated with 2e8 PBMCs (day 0).

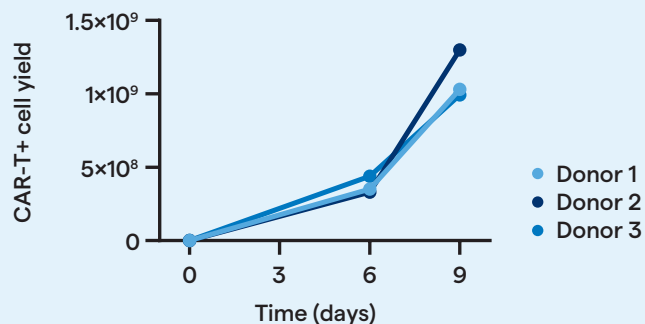
D. Cell viabilities were assessed at the start of process (day 0), mid-process (day 6), and at harvest (day 9) demonstrating maintenance of highly viable, healthy T cells throughout manufacturing.

E. Preferential expansion of T cells is achieved in the Cocoon® CAR-T™ manufacturing process for each donor PBMC input.

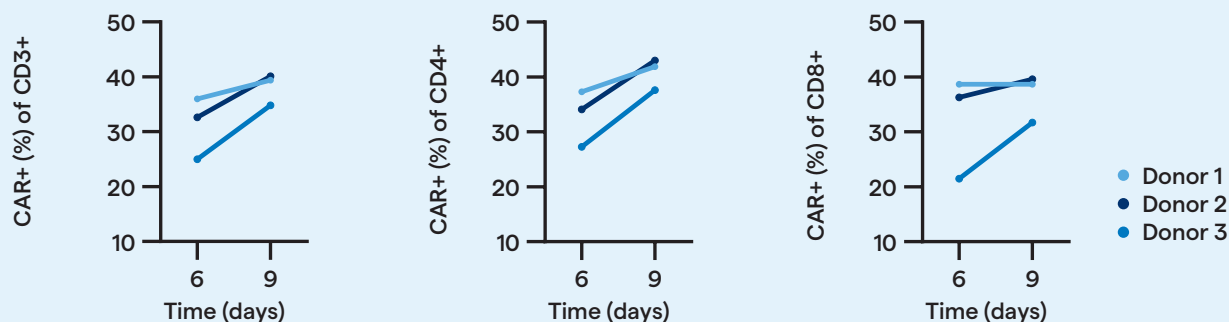
F. Memory and effector phenotype analysis of total T cells in culture for each donor, at the start of manufacture (day 0) and in the harvested product (day 9) showing maintenance of memory T cell subsets.

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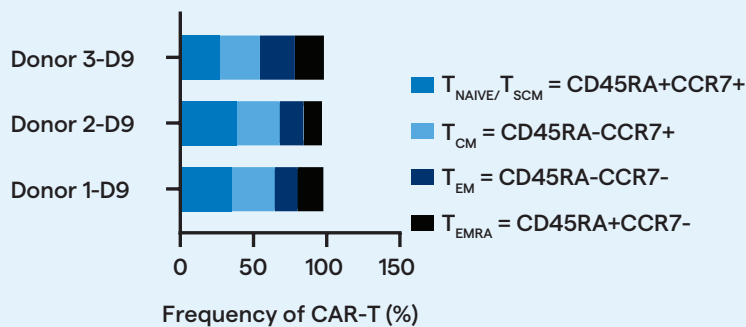
A



B



C



D

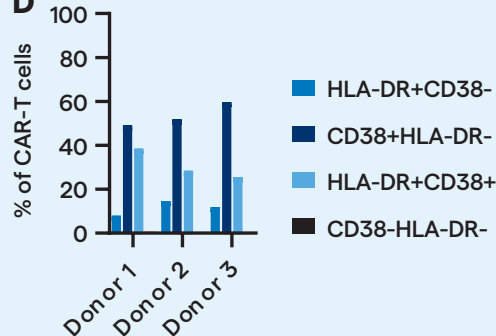


Figure 3:

Cocoon® CAR-T™ cell phenotypic characterization.

A. CAR-T cells expand well in the Cocoon® Platform, exhibiting similar kinetics to non-engineered T cells, showing consistent expansion trends from days 6-9 of manufacture, irrespective of donor-to-donor variability.

B. Distinct CD4 and CD8 T cell subsets are transduced and maintain robust growth profile throughout the process.

C. Memory phenotype analysis by flow cytometry delineates the different memory T cell subsets comprising the final Cocoon® CAR-T™ product. Notably, a large proportion of stem central memory and central memory T cells ($T_{N/SCM}$ and T_{CM} respectively) is observed.

D. Flow cytometry analysis of Cocoon® CAR-T™ cells shows robust activation profile for all day 9 donor products.

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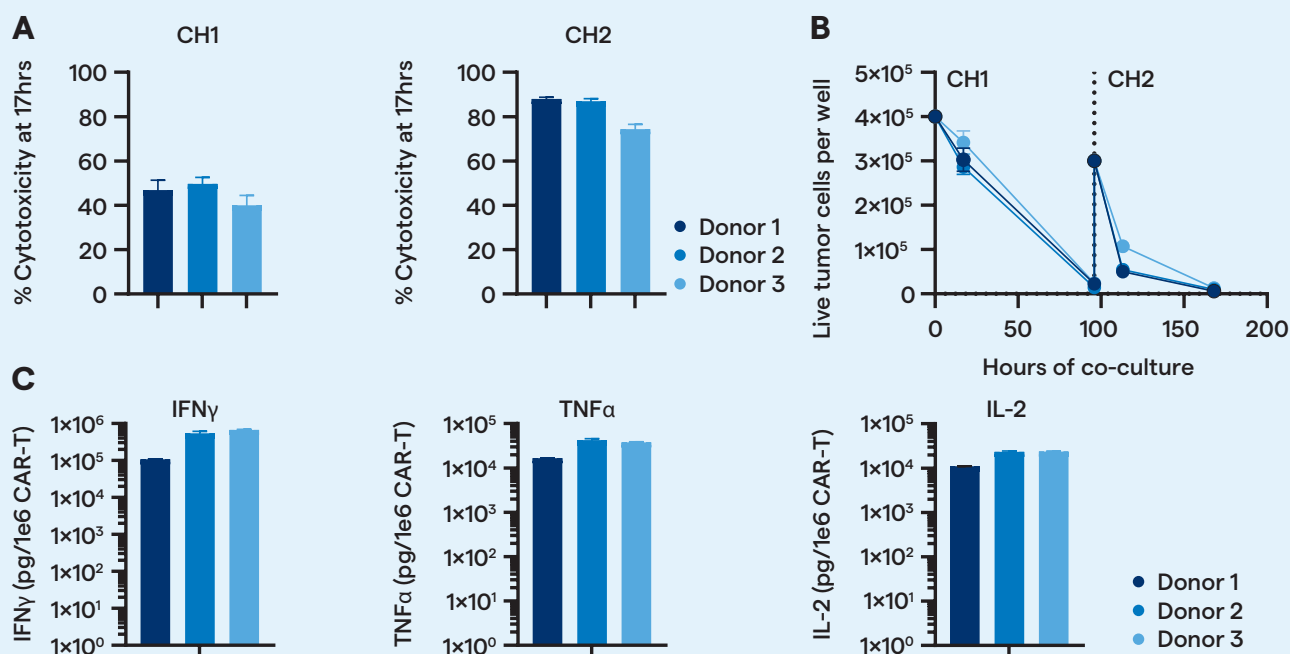


Figure 4: Cocoon® CAR-T™ cell functional characterization.

A. Potency and persistence assessments in co-culture of Cocoon® CAR-T™ products with NALM-6 tumor cells. Cocoon® CAR-T™ products exhibited potent CAR-mediated anti-tumor response upon first tumor challenge, and an even more robust response when presented with tumor cells in a sequential second challenge (CH1 and CH2 indicate first and second tumor cell challenge, respectively). Ability to potently kill tumor cells upon re-encounter indicates robust recall response and persistence.

B. Kinetics of Cocoon® CAR-T™ cell-driven anti-tumor response. Tumor clearance over time is shown for each donor CAR-T product in the two sequential tumor challenges.

C. Cocoon® CAR-T™ products exhibit strong cytokine production profile in response to tumor challenge. Data from captured cytokines produced within initial 17hr post co-culture with tumor cells show evidence of activation (IL-2 release) and strong cytotoxic response (IFNγ and TNFα release) upon engagement with tumor targets.

Flexible process design

The Cocoon® CAR-T™ application is also compatible with selected T cell input material through direct in- Cocoon® Platform selection of T cells using CD3/CD28 or CD4/CD8 magnetic beads. Upstream module integration for further T cell subset selection is also adaptable to the Cocoon® CAR-T™ application.

Contributors

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