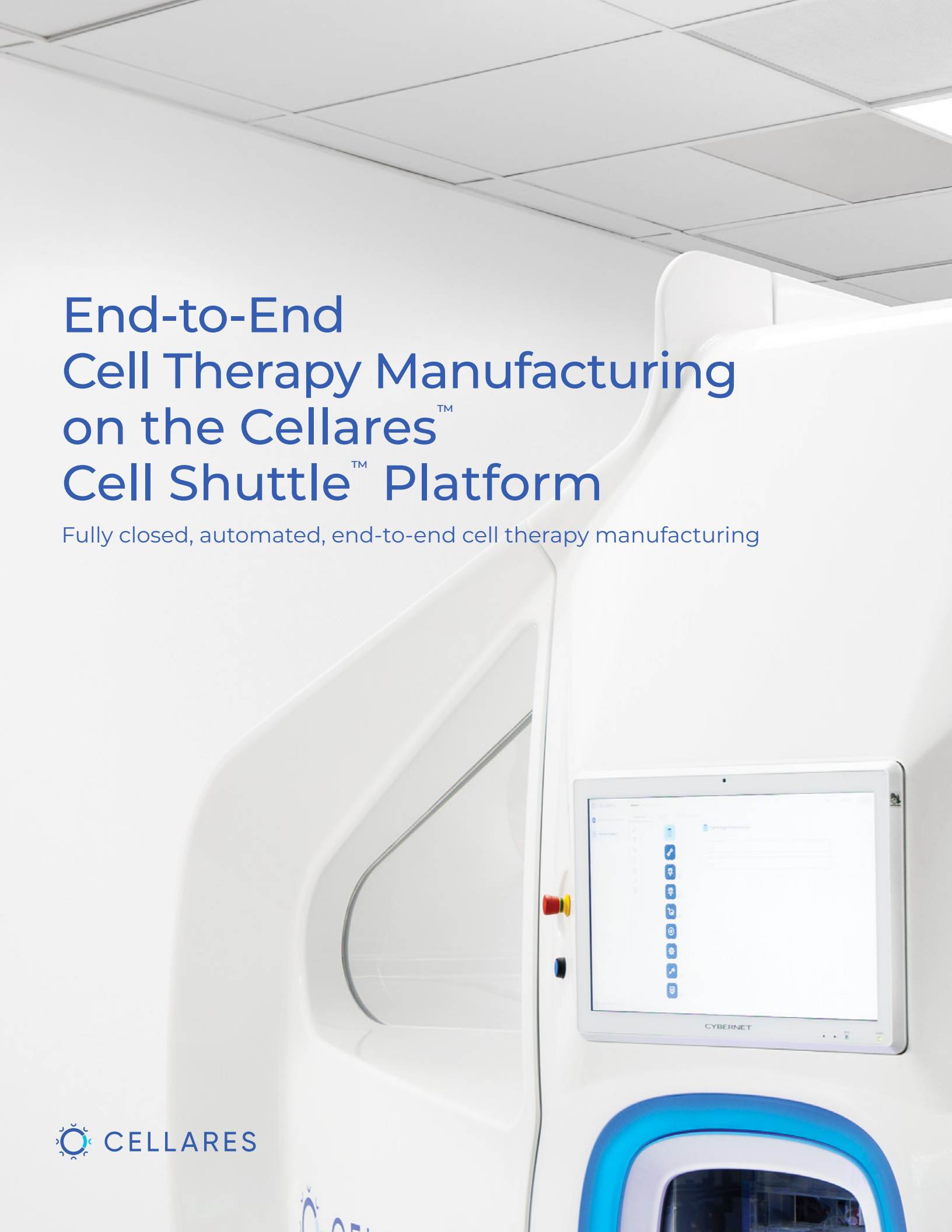
End-to-End Cell Therapy Manufacturing on the Cellares™ Cell Shuttle™ Platform

Fully closed, automated, end-to-end cell therapy manufacturing



CELLARES

Cellares Cell Shuttle

Integrated Cell Therapy Manufacturing

- Capable of manufacturing 16 cell therapy batches in parallel, which equates to 800 batches per year per Cell Shuttle (7-day process) or 2,800 batches per year per Cell Shuttle (2-day process)
- Compact automation reduces the facility size and workforce required by 90%
- Automation efficiencies decrease the total manufacturing costs by up to 65% and per-patient labor, CapEx, and OpEx costs by more than 90%*
- Closing and automating the process reduces process failure rates by 75%
- Maintains an ISO8 internal environment and can be deployed in Controlled Not Classified (CNC) space

The Cell Shuttle offers a path to automated, reproducible, and scalable cell therapy manufacturing.

*See Cost-Benefit Analysis of Using the Cellares Cell Shuttle platform for Autologous CAR-T Cell Therapies

Cell Shuttle

Online and Offline QC

Each Cell Shuttle enables automated sample collection for online and offline QC. Online QC is accomplished via a cell culture analyzer that supports real-time process monitoring of 16 assays, including metabolites, cell count, and viability.

Sterile Liquid Transfer Systems

Each Cell Shuttle contains 4 sterile liquid transfer systems that facilitate automated reagent addition, waste removal, and sampling from the fully closed consumable cartridge.

Reagent Vault System

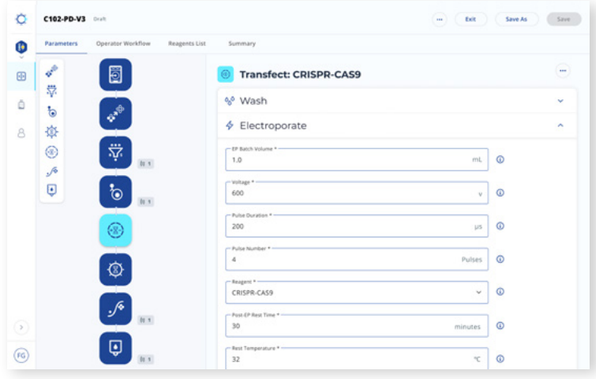
Each Cell Shuttle is equipped with a reagent vault system that enables storage and tracking of reagents using the Sterile Liquid Transfer Devices (SLTDs) in a 2-8°C environment. SLTDs are automatically delivered to the Sterile Liquid Transfer System when requested by a process. The exterior of each SLTD is automatically decontaminated prior to entry into the ISO8 environment of the Cell Shuttle.

Bioprocessing System

Each Cell Shuttle contains 16 bioprocessing systems that facilitate all cell therapy manufacturing unit operations, including enrichment, selection, activation, transduction, electroporation, expansion, and formulation.

Software

The Process Design Studio provides the flexibility to support 90% of autologous and allogeneic cell therapy manufacturing processes and offers complete control over critical process parameters. The software integrates seamlessly with existing Laboratory Information Management Systems (LIMS) for closed-loop tracking of reagents.



Consumable Cartridge

The Cellares Consumable Cartridge is fully-closed, single-use, and equipped with modules to support critical unit operations of the cell therapy manufacturing process. The modules include a counterflow centrifugal elutriator, magnetic cell sorters, electroporation chambers, bioreactor system, and smart containers for reagents and cellular material. The integrated fluidic bus facilitates the transfer of cells and fluids from any module to any other module, meaning there is no need for manual and failure-prone consumable setup.

Smart Containers

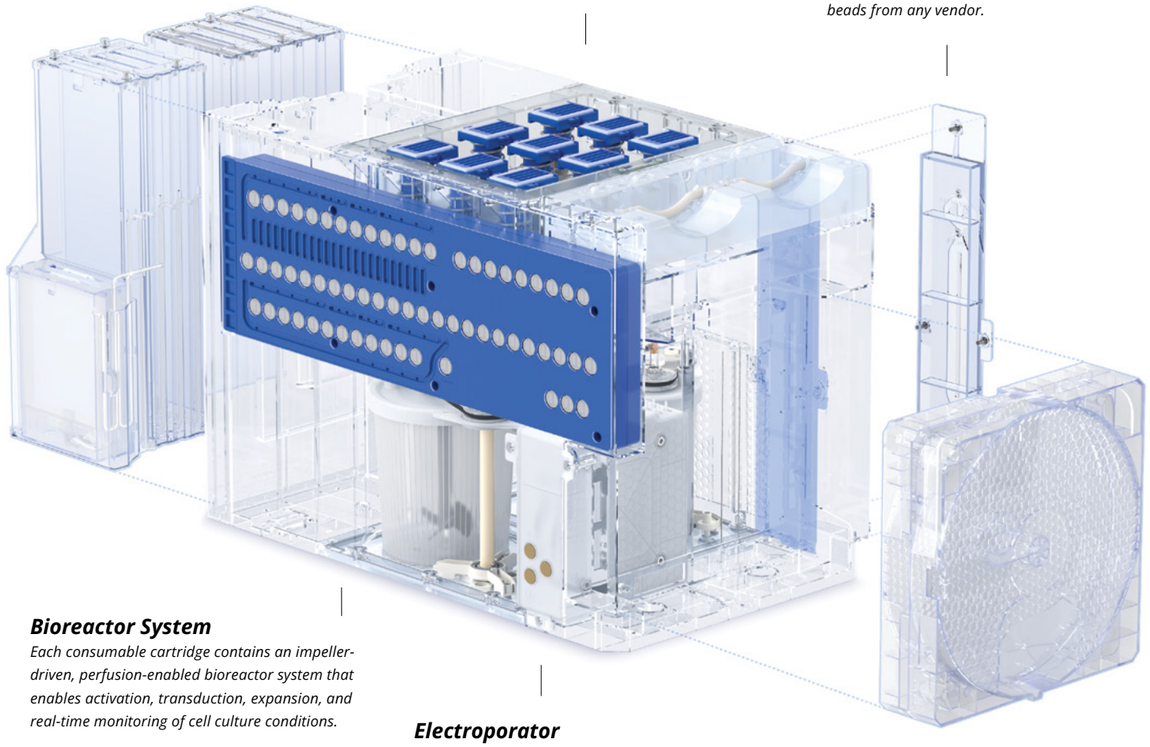
Each consumable cartridge contains multiple smart containers that enable storage and real-time volume tracking of starting material, reagents, and formulated cellular products.

Sterile Liquid Transfer Ports

Each consumable cartridge contains Sterile Liquid Transfer Ports (SLTPs) that facilitate the automated transfer of samples, reagents, and waste into and out of the cartridge by interfacing with the automation-friendly reagent bottles, called sterile liquid transfer devices (SLTDs).

Magnetic Cell Sorter

Each consumable cartridge contains two magnetic selection flowcells that operate in batch mode and facilitate positive, negative, and serial selection of target cell populations. The sorter is reagent agnostic, meaning it will accommodate magnetic beads from any vendor.



Bioreactor System

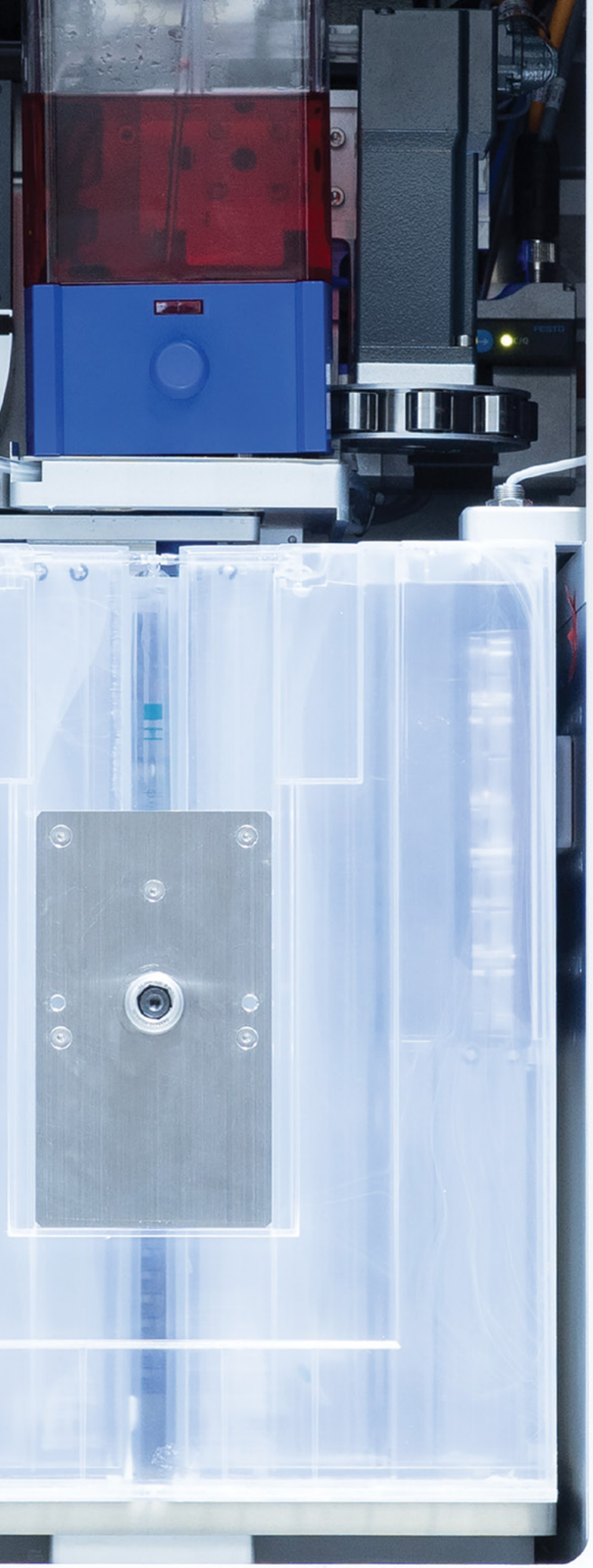
Each consumable cartridge contains an impeller-driven, perfusion-enabled bioreactor system that enables activation, transduction, expansion, and real-time monitoring of cell culture conditions.

Electroporator

Each consumable cartridge contains two electroporator chambers that operate in batch mode and enable customization of all electroporation parameters through the Process Design Studio software.

Counterflow Centrifugal Elutriator

Each consumable cartridge contains an elutriator that operates in batch mode and enables ficoll-free white blood cell enrichment, cell concentration, and buffer exchange using user-defined reagents and process parameters.



AUTOLOGOUS CAR-T CELL THERAPY PROCESS

Starting Material
Fresh or Frozen

DAY 0

WBC Enrichment
with Lysis Buffer

SAMPLE

Label and Selection
with CD4 & CD8 Magnetic Beads

SAMPLE

Activation
with CD3 & CD28 Activation Reagent

Transduction
with GFP or CAR19 Lentiviral Vector

DAY 1

Expansion
with T Cell Culture Media

DAY 1-6

SAMPLE

Formulation
with Formulation Buffer

SAMPLE

Cell Shuttle End-to-End Performance Data

The Cellares Cell Shuttle successfully executed numerous fully-automated manufacturing runs using representative commercial autologous CAR-T processes developed with industry partners.

All processes were executed in a fully automated manner *without* manual intervention

The CAR-T product maintained sterility while the Cell Shuttle was operated in a Controlled Not Classified (CNC) space

The final products exceeded release specifications for commercial CAR-T products with values observed as high as:

- Cell Viability **86%**
- Transduction Efficiency **62%**
- Viable CAR+ T Cells **17×10^7**
- T Cell Purity **99%**

Final Product Phenotype

Fresh, healthy donor starting material was processed through the counterflow centrifugal elutriator to remove undesired plasma, platelets, and red blood cells, resulting in an enriched white blood cell population. This population was then labeled with magnetic beads and subsequently selected for CD4+ and CD8+ T cells through the magnetic flowcell.

While the starting material had a mixed population of T cells, B cells, monocytes, and NK cells, the final cell therapy product achieved greater than 97% T cell purity, which exceeded the final product release specification of commercial CAR-T products (Figure 1B). T cell phenotype analysis demonstrated that the final product manufactured on the Cell Shuttle platform maintained favorable stem cell memory (Tscm) and central memory (Tcm) phenotypes (Figure 1C) and 2:1 CD4+ to CD8+ T cell ratio, consistent with the starting material (Figure 1D).

Figure 1.

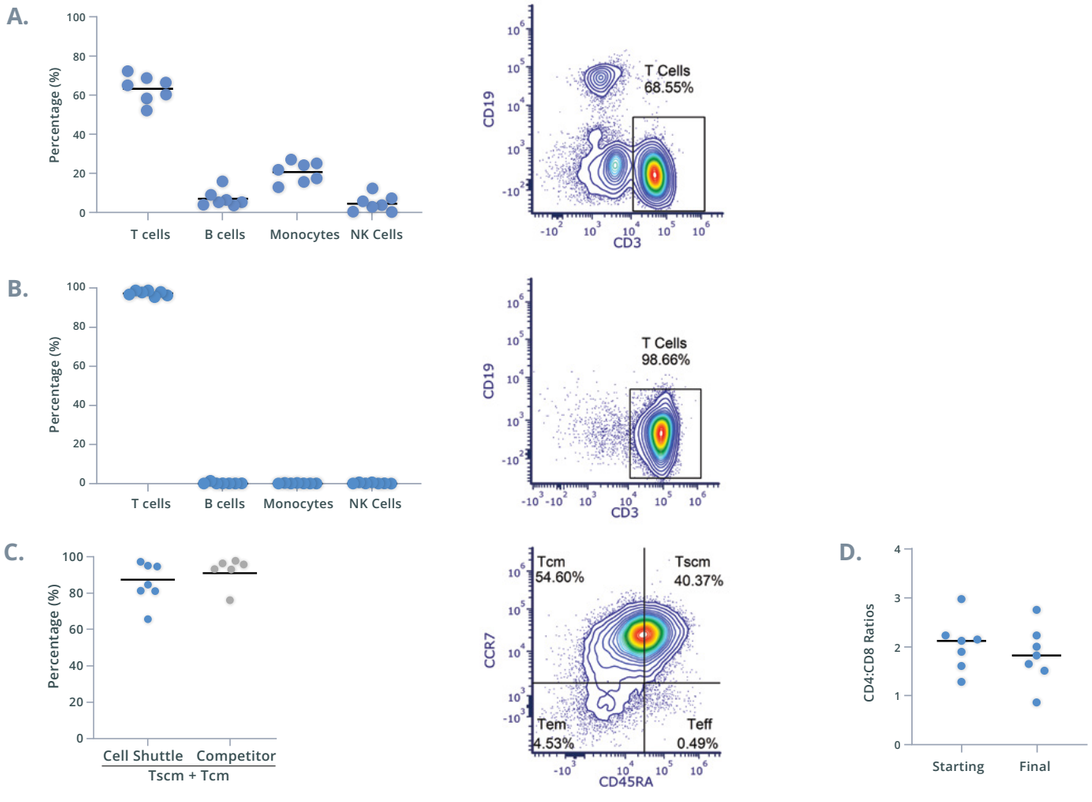


Figure 1. Cell Shuttle selected CD4+ and CD8+ T cells and achieved high purity and stemness phenotype in the final product.
(A) Starting material consisted of a heterogeneous cell population containing 63.2% ± 6.8% CD3+ T cells.
(B) Final product consisted of a homogeneous cell population of 97.6% ± 1.3% CD3+ T cells, as measured by flow cytometry.
(C) Final product demonstrated comparable stemness phenotype to the competitor system.
(D) 2:1 ratio of CD4:CD8 T cell population was maintained after the 7-day Cell Shuttle process. Bars represent the mean frequency of 7 healthy donors.

Final Product Transduction Efficiency and Expansion

Following the selection step, T cells were activated and transduced with two different types of off-the-shelf lentiviral vectors (LVV-A or LVV-B). The Cell Shuttle automatically transferred and incubated lentivirus with cells at user-defined time and temperature.

After 18 hours of incubation, the cell culture was diluted and expanded in static mode for 5 days. Identical process steps, reagents, and seeding parameters were replicated on a competitor system. Experimental data demonstrated that cell viability, transduction efficiency, and number of viable CAR+ T cells all exceeded the final product release specifications of commercial CAR-T products (Figures 2 and 3).

Figure 2.

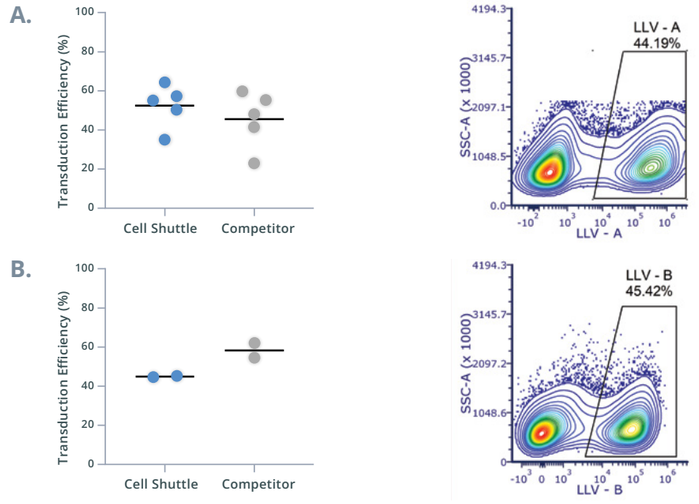


Figure 2. Cell Shuttle effectively transduced T cells using two different off-the-shelf lentiviral vector constructs. *Transduction efficiency was comparable between the Cell Shuttle and the competitor system for both (A) LVV-A and (B) LVV-B.*

Figure 3.

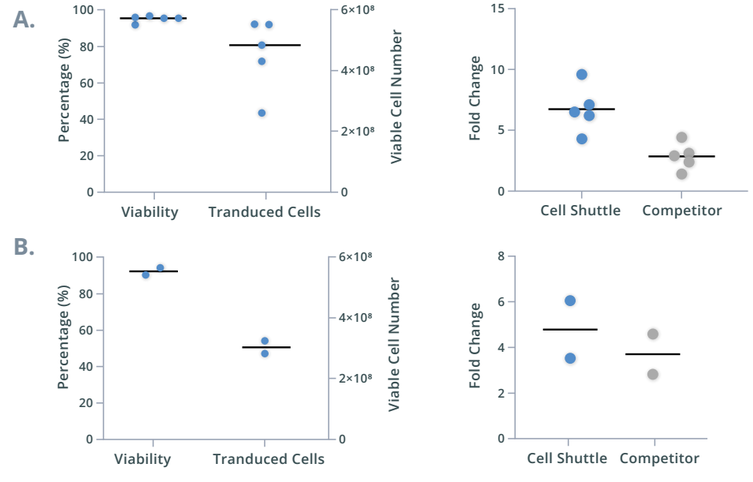


Figure 3. Cell Shuttle effectively manufactured final product with high viability and sufficient number of transduced cells to meet commercial patient dose requirements. *(A) LVV-A and (B) LVV-B transduced T cells manufactured on the Cell Shuttle demonstrated higher expansion fold-change when compared to the competitor system.*

Final Product Potency

Once the target cell number was achieved, the Cell Shuttle automatically exchanged cells into user-defined formulation buffer and cell concentration, followed by delivery of the consumable cartridge to the operator for downstream fill and cryopreservation.

The final product potency was evaluated by co-culturing LVV-B transduced T cells with the intended cancer cell line *in vitro* at specific effector to target (E:T) ratios. Secretion of interferon gamma (INF-g) and killing of the cancer cell line indicated that the final products manufactured on the Cell Shuttle were functional and cytotoxic.

Figure 4.

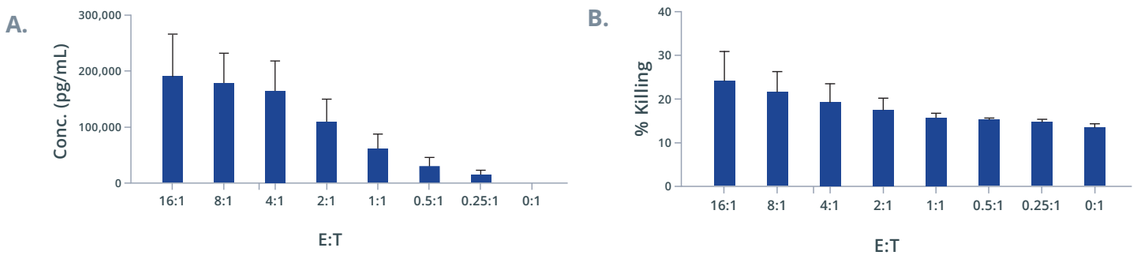


Figure 4. Cell Shuttle effectively manufactured functional CAR-T cells capable of secreting INF-g and killing cancer cells.
LVV-B transduced T cells (A) secreted pro-inflammatory cytokines in a dose-dependent manner when co-cultured with cancer cells, and (B) achieved >20% killing of cancer cells.

The performance data demonstrated that CAR-T cells manufactured on the Cellares Cell Shuttle met the commercial autologous cell therapy release specifications, including cell viability, T cell purity, transduction efficiency, and number of viable transduced T cells. In addition, the CAR-T cells demonstrated clinically-relevant characteristics with a high percentage of stem cell memory and central memory populations, as well as potency against tumor cells.

The Cell Shuttle and purpose-built consumables maintained sterility during automated manufacturing while operating in a CNC environment.

Additional automated cell therapy manufacturing runs on the Cell Shuttle are underway, including representative allogeneic processes.

For inquiries related to partnerships or IDMO services, please contact the Cellares Business Development team at **bd@cellares.com.**

CELLARES

Accelerating Access to Life-Saving Cell Therapies

345 Allerton Ave
South San Francisco, 94080