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

Fully closed, automated, end-to-end 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 delivery, 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.

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**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.

**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.

TRULY CLOSED AND AUTOMATED FROM END-TO-END

**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, 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.

**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).

**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.

**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.

**Counterflow Centrifugal Elutriator**
Each consumable cartridge contains a counterflow centrifugal 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.

**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.
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: $1.7 \times 10^7$
  - T Cell Purity: 99%
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).

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.

Cell Shuttle demonstrated higher expansion fold-change when compared to the 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).
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.

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.
