Cell therapy manufacturing

Efficient CAR T cell manufacturing using a closed and integrated instrument workflow

Introduction

Chimeric antigen receptor (CAR) T cell therapeutics continue to achieve extraordinary results in treating once-fatal cancers like large B cell lymphoma [1] and acute lymphoblastic leukemia [2]. These therapeutics also show promising results for long-term antineoplastic effects, helping keep patients in remission longer [3].

Considering this, there is an increased focus on developing tools and technologies to help support standardization of cell therapy manufacturing using closed, automated workflows that reduce manufacturing failure rates, reinforce high-quality production standards, and ultimately help lead to positive clinical outcomes.

Manual processing of biotherapeutics is prone to contamination, inconsistency, and significant lot-to-lot variability. Using a closed, automated CAR T cell therapy manufacturing workflow could help overcome these challenges while reducing their cost and increasing their safety profile. Eliminating factors such as lot-to-lot variability and contamination also helps ensure that the therapies reach the patients who need them the most, quickly. To provide closed and automated workflows that support CAR T cell manufacturing, we continue to study and optimize varying unit operations for cell therapy manufacturing.

The Gibco™ CTS™ DynaCellect™ Magnetic Separation System allows for the isolation of specific cell populations based on the use of Gibco™ CTS™ Dynabeads™ CD3/CD28 magnetic beads that attach to surface-bound T cell markers.

The multipurpose Gibco™ CTS™ Rotea™ Counterflow Centrifugation System is a versatile cell processing tool that helps enable GMP manufacturing. The CTS Rotea system applies proven counterflow centrifugation to a broad range of cell processing applications. It is gentle on cells and enables over 95% cell recovery while maintaining cell viability and high throughput.

The Gibco™ CTS™ Xenon™ Electroporation System reliably delivers DNA, RNA, and protein payloads into cells, with high cell viability and recovery. This enables efficient transfection even in hard-to-transfect cell types.

The above systems can be digitally and physically integrated to create a closed workflow that can be controlled by process automation software to help minimize drawbacks associated with manual processes. Here we demonstrate the closed and integrated use of the CTS Rotea system with the CTS Xenon system to edit T cells isolated with the CTS DynaCellect system.

Methods

T cell isolation

Frozen leukopak bags containing white blood cells (WBC) from healthy donors were thawed. T cells were isolated using the CTS DynaCellect system with the isolation protocol shown in Table 1 and CTS Dynabeads CD3/CD28 magnetic beads. Isolated T cells were cultured in G-Rex™100 bioreactor vessels (Wilson Wolf Manufacturing) containing Gibco™ CTS™ OpTmizer™ Pro Serum-Free Medium (SFM) supplemented with 100 U/mL of IL-2.

Table 1. T cell isolation protocol using the CTS DynaCellect system.

Port	
A	Isolation bag
B	1,000 mL bag for 550 mL DPBS with 1% HSA
C	1,000 mL bag for output (positive fraction)
D	Empty
E	1,000 mL bag for 550 mL DPBS with 1% HSA (same bag as B)
F	600 mL bag for CTS OpTmizer Pro SFM
G	Conical input bag with thawed leukopak
H	Conical bag with CTS Dynabeads CD3/CD28 magnetic beads (3:1 bead-to-cell ratio)
I	1,000 mL bag for supernatant (negative fraction)

After 3 days of culture, cells were debeaded using the CTS DynaCellect system as outlined in Table 2.

Table 2. Removal of Dynabeads magnetic beads on day 3, following isolation and activation using the CTS DynaCellect system.

Port	
A	Bead removal bag in port
B	Bead removal bag out port
C	Empty
D	Empty
E	1 x 1,000 mL bag for export/harvest
F	Empty
G	250 mL DPBS with 0.1% HSA in 300 mL bag
H	1,000 mL input bag with cell/bead input
I	Empty

Closed integration of the CTS Rotea system with the CTS Xenon system

Once the CTS Dynabeads CD3/CD28 magnetic beads were removed, cells were washed, and electroporation buffer exchange was carried out using the CTS Rotea system. (Download the protocol here.) Both the payload (Invitrogen™ TrueCut™ Cas9 Protein v2, Invitrogen™ TrueGuide™ Synthetic gRNA, and target DNA) and the concentrated T cells were transferred to the CTS Xenon system via welded PVC tubing (connected to line H on the Gibco™ CTS™ Rotea™ Single-Use Kit), to be electroporated using the setup outlined in Table 3. T cells were genetically modified using the payload strategy outlined in Table 4 and the Gibco™ CTS™ Xenon™ Multishot Electroporation Cartridge with the parameters noted in Table 5, and with the setup as shown in Figure 1. The total electroporation volume was adjusted based on cell count and desired cell density as listed in Table 6.

Table 3. Day 3—setup for integration of the CTS Rotea system to the CTS Xenon system.

Port	
A	1,000 mL bag for waste
B	300 mL bag for 200mL DPBS with 1% HSA
C	Gibco™ CTS™ Xenon™ Genome Editing Buffer, bag
D	Cell input loop
E	2 mL payload in 10 mL squeeze pouch sample port
F	Empty
G	Cell input loop
H	Direct weld PVC line to CTS Xenon system

Table 4. Payload component concentrations.

Payload	Concentration
TrueCut Cas9 Protein v2	120 µg/mL
TrueGuide synthetic gRNA	30 µg/mL
CD19 CAR (2.4 kb)	240 µg/mL

Table 5. Electroporation conditions on the CTS Xenon system.

Voltage	Pulse width	Number of pulses	Pulse interval
2,300 V	3 ms	4	500 ms

Table 6. Electroporation volumes.

Run	Electroporation volume per 5 x 10^7 cells
Run 1	13 mL
Run 2	17 mL
Run 3	16 mL

A



B

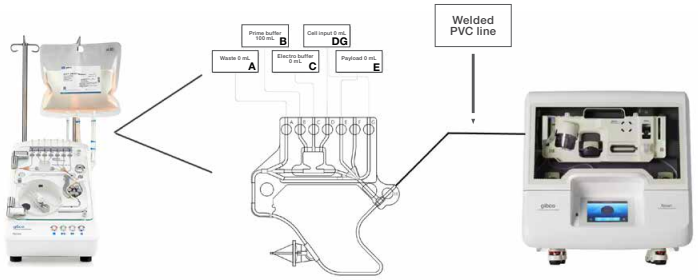


Figure 1. T cell isolation process summary. T cells were isolated using the CTS DynaCellect system and then cultured in CTS OpTmizer Pro SFM with 100 U/mL IL-2 for 3 days. Following bead removal on day 3, T cells were washed and then transferred from the CTS Rotea system to the CTS Xenon system via a closed integration. **(A)** The CTS DynaCellect system, the CTS Rotea system, and the CTS Xenon system (from left to right) are shown. **(B)** Schematic representation of the physical integration via a welded PVC line between the CTS Rotea system and CTS Xenon system used for T cell electroporation on day 3.

Post-electroporation, the edited T cells were cultured and expanded in CTS OpTmizer Pro SFM for 10 days. The cell characterization parameters measured included cell viability, immune cell phenotypes, and T cell activation marker expression on day 0 and day 3 post-activation. The viability of T cells post-electroporation and the knockout/knock-in (KO/KI) efficiency of edited T cells were analyzed as well. The data shown here represent three independent experiments. Flow cytometric analysis was done using the Invitrogen™ Attune™ CytPix™ Flow Cytometer.

Results

Three days after T cell isolation and following removal of the Dynabeads magnetic beads, we observed high (>90%) T cell viability (Figure 2A). Additionally, on day 3, debeaded cell cultures exhibited an average of 95% T cell content, with the major contaminating cell type being NK/NKT cells (1.71%, Figure 2B). As shown in Figure 2B, the percentage of all non–T cell types (NK/NKT cells, B cells, and monocytes) consistently decreased between day 0 (pre-isolation) and day 3.

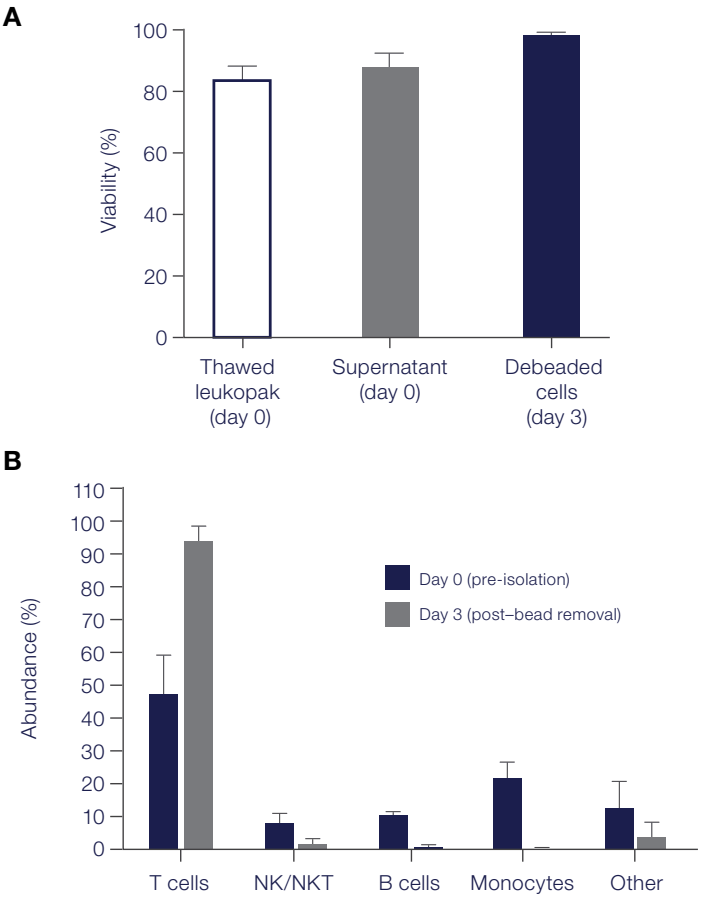


Figure 2. Viability and cell type distribution. (A) Cell viability from day 0 to day 3. **(B)** Cell type distribution for thawed leukopak cells on day 0 and on day 3 post–bead removal.

Flow cytometric analysis using the Attune CytPix Flow Cytometer, on day 0 and on day 3 following T cell activation and culture in CTS OpTmizer Pro SFM supplemented with IL-2, showed effective induction of T cell activation markers CD69, CD25, and HLA-DR by day 3 (Figure 3).

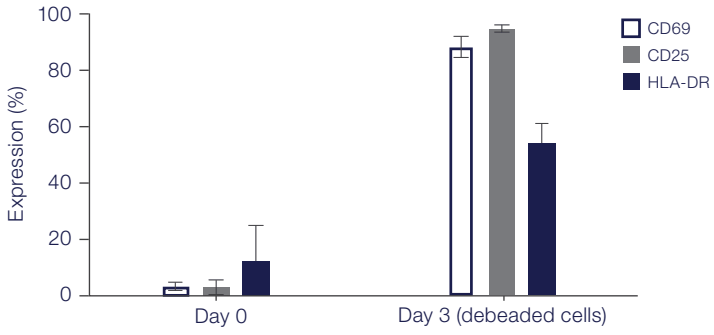


Figure 3. T cell activation marker expression on day 0 and post-activation on day 3.

Taken together, Figures 2 and 3 demonstrate that viable, activated T cells were used in subsequent processing and electroporation using the CTS Rotea and CTS Xenon systems.

Following the transfer of T cells from the CTS Rotea system to the CTS Xenon system using a closed process, cells were electroporated to deliver the gene editing payload mix of TrueCut Cas9 Protein,* TrueGuide Synthetic gRNA, and dsDNA. Then the edited T cells were grown in stirred-tank bioreactor vessels. By day 10 of culture post-electroporation, T cell viability averaged approximately 85% across the three independent runs. This demonstrates that a high percentage of T cells survived the electroporation process (Figure 4).

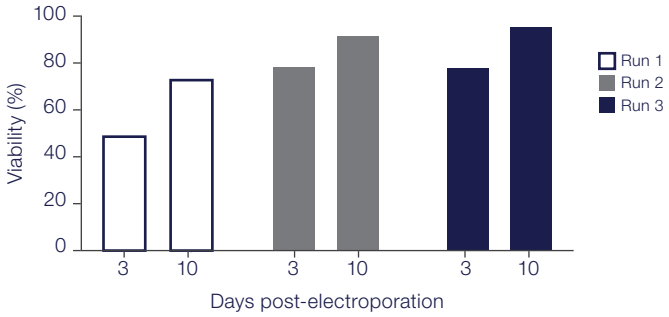


Figure 4. Viability of edited T cells on days 3 and 10 following electroporation using the CTS Xenon system.

* Gibco™ CTS™ TrueCut™ Cas9 Protein is also available. Learn more.

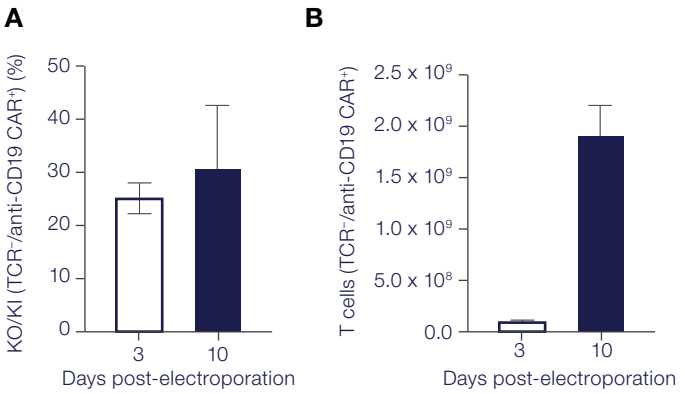


Figure 5. Post-electroporation analysis of (A) gene editing efficiency (KO/KI) of T cells and (B) total number of edited T cells.

Gene editing efficiency in the T cells was analyzed on days 3 and 10 post-electroporation. On average, 25% and 30% of T cells expressed anti-CD19 CAR with no T cell receptor expression (Figure 5A) on days 3 and 10, respectively. These percentages represented an average of 10^8 cells at day 3 with an expansion to 2 x 10^9 edited T cells by day 10 following electroporation (Figure 5B).

Conclusions

In this application note, we sought to highlight a functional, closed physical integration between the CTS Rotea and CTS Xenon systems in manufacturing CAR T cells. We successfully isolated T cells using the CTS DynaCellect system. The cells were activated and cultured for 3 days in medium supplemented with IL-2. On day 3, T cells were washed using the CTS Rotea system. Both the payload and T cells were then delivered via a welded PVC line to the CTS Xenon system, where T cells were electroporated using the CTS Xenon Multishot cartridge. T cells electroporated using this closed and integrated method resulted in an average of 2 x 10^9 anti-CD19 CAR T cells, with high viability.

Here we have demonstrated the use of our portfolio of modular CTS instruments, and effective physical integration of the CTS Rotea and CTS Xenon systems in a closed manner. The results noted here also provide evidence that the closed integration between the CTS Rotea and CTS Xenon systems can be used to help maintain consistency throughout the cell therapy manufacturing process.

The modular nature of our instruments, coupled with their digital and physical integration compatibility, enables countless configurations for a wide array of applications. An example of a future study may include a complete physical connection between the CTS DynaCellect, CTS Rotea, and CTS Xenon systems on day 3 using welded tubing, which would ideally be controlled by automation software. This closed system integrating multiple instruments could be used in the processing of various kinds of cell therapies while reducing contamination and helping to ensure consistency during the cell therapy manufacturing process.

References

1. Roschewski M, Longo DL, and Wilson WH (2022) CAR T-cell therapy for large B-cell lymphoma—who, when, and how? *N Engl J Med* 386(7):692–696. **doi.org/10.1056/NEJMe2118899**
2. Pehlivan KC, Duncan BB, and Lee DW (2018) CAR-T cell therapy for acute lymphoblastic leukemia: transforming the treatment of relapsed and refractory disease. *Curr Hematol Malig Rep* 13(5):396–406. **doi.org/10.1007/s11899-018-0470-x**
3. Lin H, Cheng J, Mu W et al. (2021). Advances in universal CAR-T cell therapy. *Front Immunol* 12:744823. **doi.org/10.3389/fimmu.2021.744823**
4. **CTS DynaCellect Magnetic Separation System** (2022).
5. **Flexibility and modularity in cell therapy manufacturing-incorporating the CTS Rotea and Xenon systems** (2022).

Ordering information

Product	Cat. No.
CTS Rotea Counterflow Centrifugation System	A44769
CTS Rotea Single-Use Kit	A49313
CTS DynaCellect Magnetic Separation System	A55867
CTS DynaCellect Isolation Kit	A52300
CTS DynaCellect Bead Removal Kit	A52301
CTS Xenon Electroporation System	A50301
CTS DPBS (without calcium and magnesium)	A1285602
CTS OpTmizer Pro SFM	A4966101
CTS GlutaMAX-I Supplement	A1286001
L-Glutamine (200 mM)	25030081
IL-2	PHC0021
TrueCut Cas9 Protein v2*	A36498 (100 mg)
TrueGuide synthetic gRNA	Multiple catalog numbers (custom)
0.5 M EDTA (pH 8.0)	15575020
CTS Dynabeads CD3/CD28	40203D
5 mL luer-lock syringe	15869152
60 mL luer-lock syringe	14955455
CTS Xenon Genome Editing Buffer	A4998001
CTS Xenon Multishot Electroporation Cartridge	A50306
CD19 CAR DNA (2.4 kb)	LineaRx (Custom)**
BioBLU™ c Single-Use Bioreactor Stirred Tank	Eppendorf (1386100600)
Recombinant Human Serum Albumin (HSA 25%)	Octapharma (ALB064302)
Vial spike with needle-free valve	OriGen Biomedical (VSV)
Spike to male luer	OriGen Biomedical (NC1313150)
Spike adapter with needle-free valve	OriGen Biomedical (NC1768513)
Spike to female luer with 50cm SCD tubing and pinch clamp	OriGen Biomedical (S-F50)
Spike to female luer with 10cm tubing and pinch clamp	OriGen Biomedical (S-F10)
T-SEAL™ III Sterile tubing sealer	Terumo (T3828)
TSCD™ II Sterile tubing welder	Terumo (3ME-SC203A)
Teruflex™ 150 mL blood bag	Terumo (1BBT015CB70)
Teruflex™ 600 mL–1 L blood bags	Terumo (1BBT060CB71)
Clamps	Terumo (T100BM)
G-Rex™100 Gas Permeable Cell Culture System	Wilson Wolf (P/N 80500)
Leukopak	Multiple donors (AllCells or HemaCare)
C-Flex™ tubing to PVC adapter	CharterMedical (CT-014-CVT)
Sterile sampling pouch	Biosafe SA (AK-101)
T-SEAL™ Mobile	Terumo (T5460)

* "CD19 CAR DNA" was a custom-designed sequence.

** CTS TrueCut Cas9 Protein is available. Learn more.

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