CELL & GENE THERAPY INSIGHTS

DOWNSTREAM PROCESSING

INNOVATOR INSIGHT

Proof of concept of a fully enclosed CAR-T process without use of a biosafety cabinet

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This proof-of-concept study evaluated the feasibility of using CellSeal Connect vials as closed-system alternatives to conventional cryovials, with the goal of eliminating reliance on biosafety cabinets (BSCs) during CAR-T manufacturing. Enriched T cells from healthy donors and lentiviral vectors were filled and cryopreserved in both CellSeal Connect vials and standard cryovials, then used in a CAR-T process. Additionally, BioLife Solutions' CellSeal® CryoCases, which are a rigid and transparent primary storage container, were evaluated as a closed system option for filling final drug product. Results demonstrated comparable cell expansion, viability, and transduction efficiency between cells cryopreserved in the CellSeal Connect vials and standard cryovials. Additionally, post-thaw final drug product was similar between cryovials and CellSeal CryoCases. These findings support the feasibility of eliminating BSC use in standard CAR-T manufacturing, which can potentially reduce contamination risk, facility complexity, and cost in CAR-T production.

Cell & Gene Therapy Insights 2025; 11(7), 897–907 · DOI: 10.18609/cgti.2025.100

INTRODUCTION

CAR-T cell therapy has emerged as a transformative approach in cancer treatment, offering hope to patients with refractory disease. However, CAR-T manufacturing is often complex and labor-intensive, posing significant logistical and operational challenges [1]. While multiple automated systems are available for closing CAR-T processing, a major limitation of these systems is that they can only enable a truly end-to-end enclosed process if all materials are packaged appropriately [2,3]. Almost universally, cryopreserved starting cells and viral vectors are packaged in containers that require the use of a biosafety cabinet (BSC) to access and transfer their contents into weldable containers to avoid sterility breach. Although BSCs are

effective in maintaining aseptic conditions during open processing steps, this approach introduces other challenges related to spatial constraints and the need for increased environmental monitoring during manufacturing [4].

Typically, BSCs are used for several key processing steps, including compounding media and accessing thawed cells and thawed viral vectors. Solutions exist for compounding media without the use of a BSC, including sterile filtration (e.g., in-line weldable filters) and cytokines packaged in weldable containers. If thawed cells and viral vectors can also be accessed in a sterile manner, an entire CAR-T manufacturing process can be performed without requiring a BSC. This study aimed to demonstrate the feasibility of a fully enclosed CAR-T process by using BioLife Solutions' CellSeal® Connect vials, which allow for a fully closed, sterile pathway for not only filling of critical materials, including viral vectors and starting cells, but also for retrieval at time of use.

STUDY DESIGN

This study was designed to compare a traditional CAR-T manufacturing process using cryovials and BSCs with a fully closed system utilizing CellSeal Connect vials for containment of starting cells and viral vector and CellSeal CryoCases for containment of final drug product (Figure 1). The objective was to determine whether the closed-system process could produce similar results in terms of expansion and transduction compared to a standard process while eliminating the need for a BSC.

MATERIALS & METHODS

Lentiviral vector

Anti-CD19 CAR/GFP lentiviral vector was generated following standard CTMC protocols and filled into either 1.8 mL cryovials or 2 mL CellSeal Connect vials using a BioLife Solutions' Signata CT-5™ fluid handling platform. Following fill, vials were stored at ≤-80 °C until time of use.

Starting cells

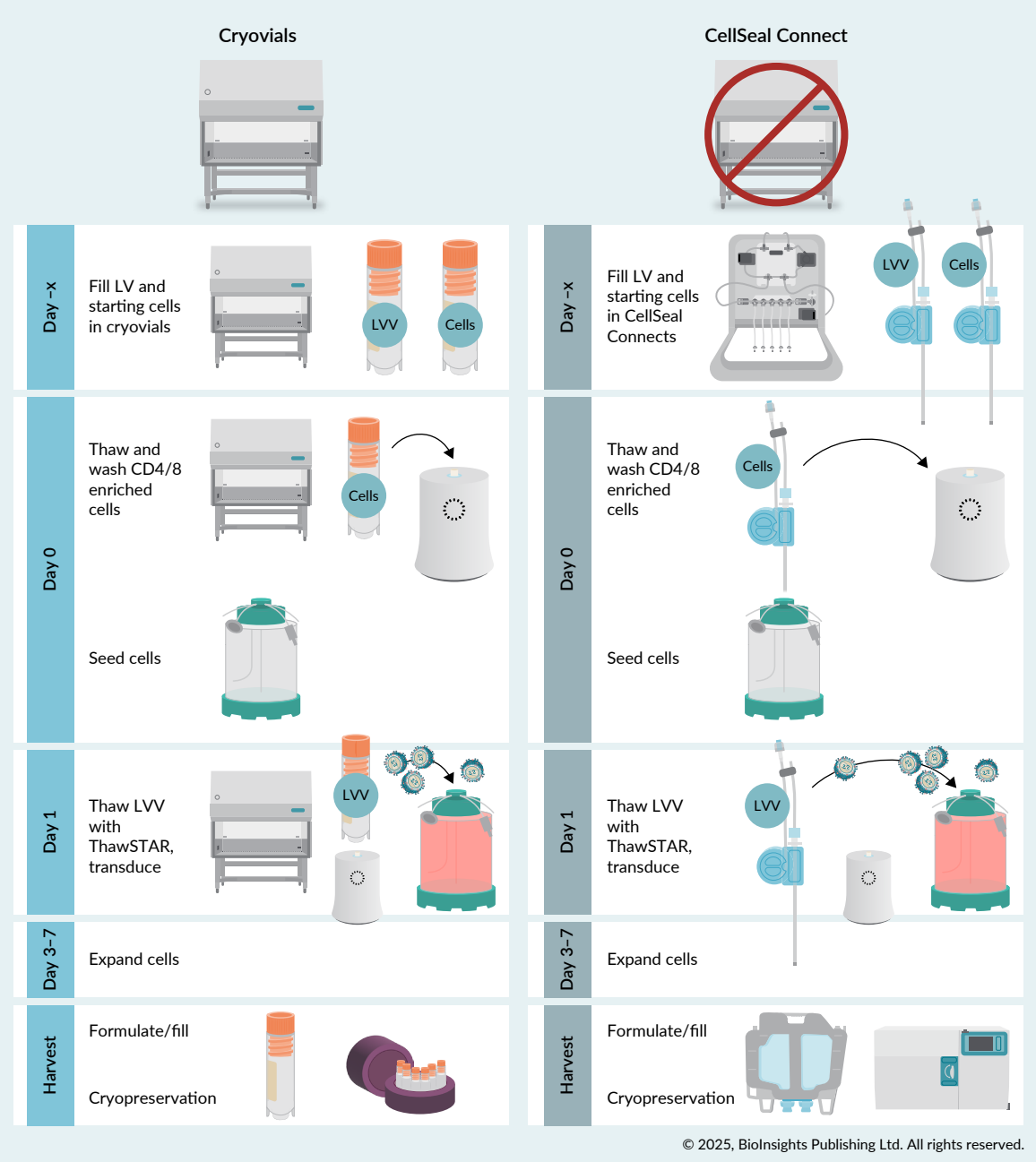
Three healthy donor leukapheresis collections were procured from Gulf Coast Regional Blood Center and processed fresh. T cells were positively selected with $CD4^+/CD8^+$ paramagnetic beads using an enclosed and automated system. Following enrichment, isolated cells were formulated into cryopreservation solution and then filled into either 1.8 mL cryovials, or 2 mL CellSeal Connect vials. Cells were then cryopreserved using a controlled rate freezer and stored in cryogenic freezers.

Thawing, activation, transduction, and expansion of cells

Containers were thawed using an automated, water-free tool, BioLife Solutions' ThawSTAR® thawing system. A ThawSTAR® CFT was used for cryovials, and a ThawSTAR® CSV was used for CellSeal Connect vials. Vials were inserted into each ThawSTAR unit and automatically released upon completion of thawing (~2 minutes, 30 seconds). Cells were then washed, resuspended in culture media, and activated using STEMCELL Technologies' ImmunoCult™ Human CD3/CD28/CD2 T Cell activator in G-Rex flasks. The day after activation, each culture was transduced using the aCD19 CAR/GFP Lentiviral Vector (LVV). For cultures initiated with T cells cryopreserved in CellSeal Connect vials, LVV filled in CellSeal Connect vials was used. For cultures initiated using T cells in cryovials, LVV from 1.8 mL cryovials were used. LVV was thawed using the ThawSTAR system and added at an MOI of 1.9, followed by a 48 hour incubation. Cultures were then fed with complete media and incubated an additional 96 hours.

FIGURE 1

Each workflow was executed in parallel using cells from three healthy donors.



Aside from the enclosures for starting cells and viral vector, all conditions were kept the same for each process. Following expansion, cells were cryopreserved in either cryovials or CellSeal CryoCases.

Harvest, formulation, and cryopreservation

At the end of expansion, each culture was harvested and washed into cryopreservation solution. Formulated cells were filled into either cryovials or CellSeal CryoCases. Cryovials were cryopreserved using Corning® CoolCell® containers in a -80°C freezer, while CellSeal CryoCases

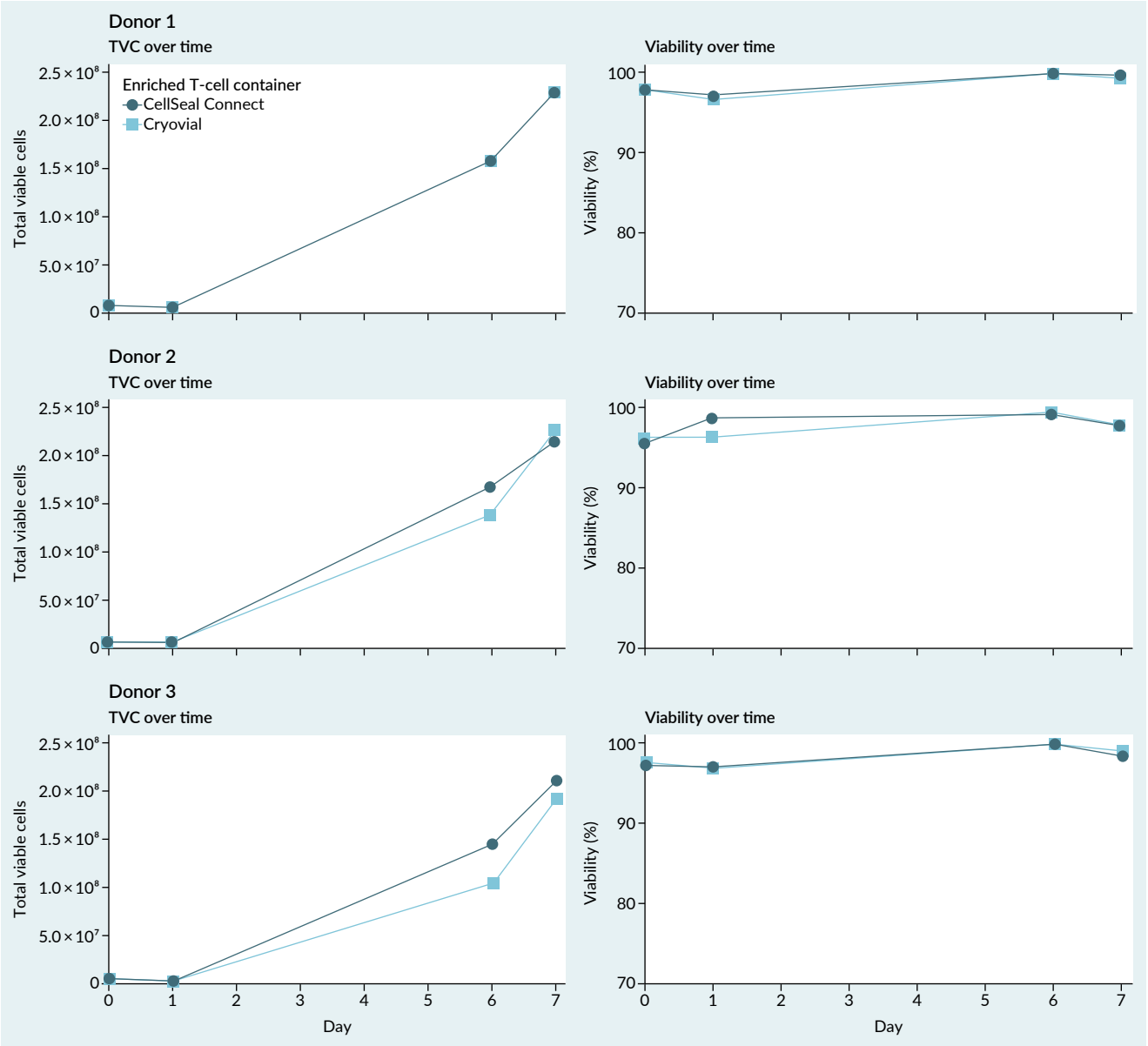
were cryopreserved using a controlled rate freezer. All samples were stored in cryogenic freezers post-cryopreservation.

Post thaw testing

CellSeal CryoCases and cryovials were thawed using a water bath. Cell count and viability were measured post thaw. 1.5×10^6 cells from each donor and container were washed into complete medium and seeded into 6 well plates at a concentration of 5×10^5 viable cells/mL for further culture. Cells were counted using a ChemoMetec NucleoCounter® NC-200™ at 24 and 48 hours post thaw.

FIGURE 2

In process cell counts and viability.



Across all donors, cultures initiated with enriched T cells cryopreserved in either CellSeal Connect vials or standard cryovials showed similar performance. Cell counts and viability were assessed using NucleoCounter NC-200.

Transduction testing

Transduction was measured by flow cytometry using a Cytek Aurora following standard CTMC protocols.

RESULTS

Expansion, viability, and transduction rate is similar for cells cryopreserved in CellSeal Connect vials or standard cryovials

Expansion kinetics were similar for cultures initiated with T cells cryopreserved in both CellSeal Connect vials and standard cryovials across all donors (Figure 2). Viability was high for all time points measured across all donors and conditions.

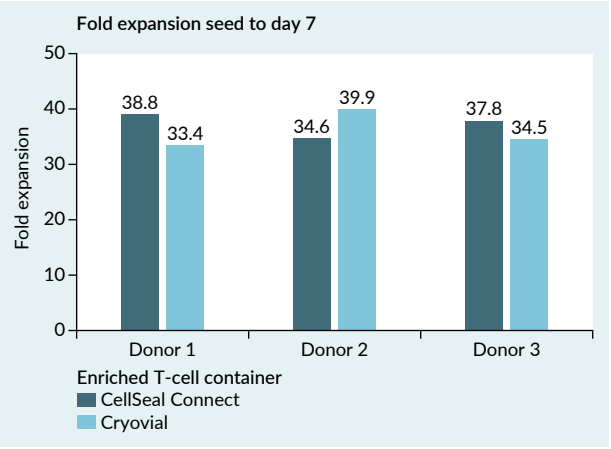
Fold expansion from seed to day 7 was consistently high across all donors and container types (Figure 3). Cultures initiated with cells cryopreserved in CellSeal Connect vials had expansions of 38.8×, 34.6×, and 37.8× for Donors 1, 2, and 3, respectively, compared to 33.4×, 39.9×, and 34.5× for cultures initiated with cells cryopreserved in cryovials. These results suggest no negative impact with the use of CellSeal Connect vials.

Transduction efficiency (Figure 4), measured as the percentage of CAR^+ (GFP^+) viable cells, was comparable between conditions. For cultures initiated with cells cryopreserved in CellSeal Connect vials, transduction rates were 43%, 54%, and 43% for Donors 1, 2, and 3, respectively. Cultures initiated with cells cryopreserved in cryovials yielded 49%, 59%, and 46% in the same donors. While some inter-donor variability was observed, the relative differences between container types were minor.

The number of CAR^+ cells generated through day 7 had some variability across donors but was similar regardless of the container used for cryopreservation of starting cells. Cultures initiated with cells cryopreserved in CellSeal Connect vials yielded 9.8×10^7, 1.2×10^8, and 9.1×10^7 CAR^+ cells for Donors 1–3, respectively. Cultures initiated with cells cryopreserved in cryovials yielded 1.1×10^8, 1.3×10^8, and 8.9×10^7 CAR^+ cells (Figure 5).

FIGURE 3

Fold expansion from seeding to day 7 was similar between conditions and across donors.



Total fold expansion was calculated by dividing the number of cells harvested on day 7 into the number of cells seeded on day 0.

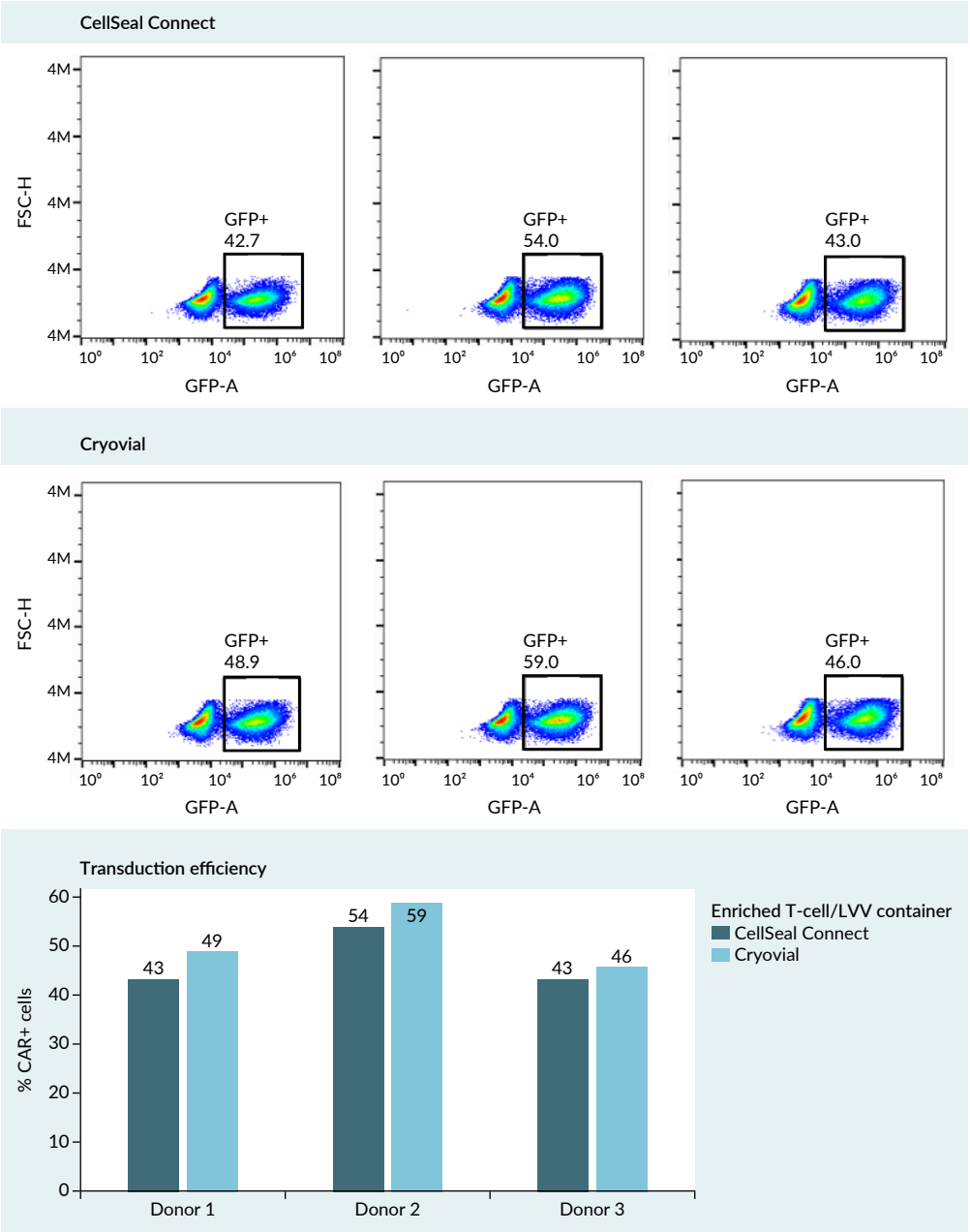
Post-thaw viability and recovery over 2 days is similar for final products expanded from cells cryopreserved in CellSeal Connect vials or standard cryovials

Immediate post thaw viability was high for final drug product cells cryopreserved in both CellSeal CryoCases and cryovials (Figure 6) across all donors. CellSeal CryoCase results ranged from 94.8% to 96.9%, while cryovials ranged from 97.1% to 97.5%.

Post rest viability was high at each timepoint measured across donors and conditions. Post rest cell recovery was measured by dividing the number of viable cells counted at 24 and 48 hours post rest into the total number of viable cells seeded into culture post thaw. Post rest recovery was high across donors and conditions with 2 of 3 donors achieving higher post rest

FIGURE 4

Transduction efficiency was variable across donors (D1, D2, D3) but similar across conditions.



Transduction efficiency was measured using a Cytek Aurora with CAR+ cells determined by the percentage of live single cells being GFP+. LVV: lentiviral vector.

expansion with cells cryopreserved using CellSeal CryoCases.

CONCLUSIONS

This study demonstrates that CAR-T manufacturing can be fully enclosed when appropriate containers are used for both starting cells and viral vectors. CellSeal Connect vials provide a functionally equivalent alternative to conventional screw cap cryovials, with comparable cell viability, expansion, and transduction efficiency (due to the small sample size, statistical analysis

was not performed). Additionally, CellSeal CryoCases can be used as a closed-system option for filling of final drug product. By enabling sterile welding for the extraction of thawed starting cells and viral vectors, the need for biosafety cabinets during CAR-T manufacturing can be eliminated. Taken together, these innovations reduce contamination risks, streamline operations, and simplify facility design.

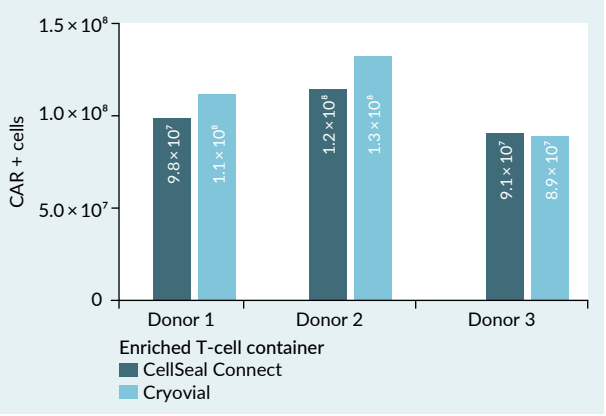
DISCUSSION

Although many manufacturing platforms are marketed as closed-system solutions for CAR-T manufacturing, all of these systems require an ancillary BSC if materials, primarily cryopreserved starting cells and viral vectors, are packaged in containers that do not support product extraction via sterile docking. The use of a BSC requires manual cleaning and adherence to aseptic techniques to ensure microbial-free culture. The front opening of the BSC used for transferring equipment and materials presents similar cleaning limitations and can disrupt air flow, increasing contamination risk. Additionally, BSCs have a large footprint, increase operating costs, and limit the number of subsequent operations due to the decontamination procedures required. In some instances, the use of a BSC may require special accommodation to the facility and is vulnerable to failures in the facility's infrastructure. Elimination of a BSC from CAR-T manufacturing can help simplify operations, reduce costs, and ultimately improve patient access to these therapies. CellSeal Connect Vials are a feasible option that can allow for both filling and retrieval of viral vector or starting cells for a CAR-T process without entering a BSC.

All BioLife primary containers used in this study, including CellSeal Connect Vials and CellSeal CryoCases, have undergone extractables and leachables testing and container closure integrity validation to meet CGT manufacturing requirements. These tests confirmed no reaction from leachable constituents, which is considered baseline qualification for all primary containers in clinical and commercial cell therapy processes. The data is available from the manufacturer to support evaluation and regulatory submissions.

FIGURE 5

The total number of CAR⁺ cells produced at harvest on day 7 was variable across donors but similar whether CellSeal Connect vials or cryovials were used.



The total number of CAR⁺ cells was calculated by multiplying the percentage of transduced (GFP⁺) cells by the total number of viable cells at harvest.

FIGURE 6

Post thaw viability was high across conditions and donors from both CellSeal CryoCases and cryovials.

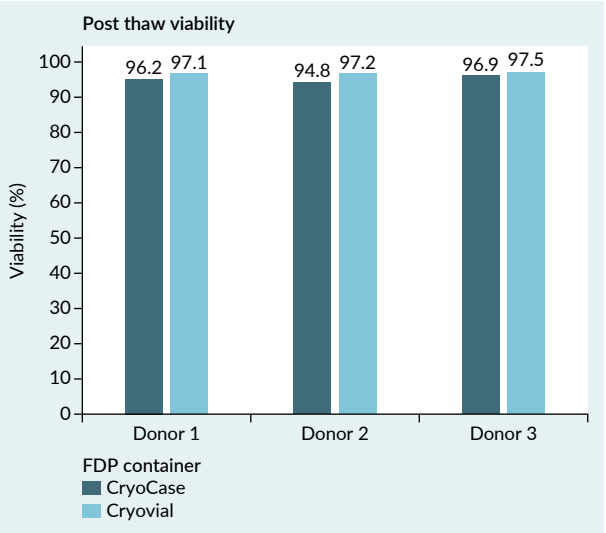
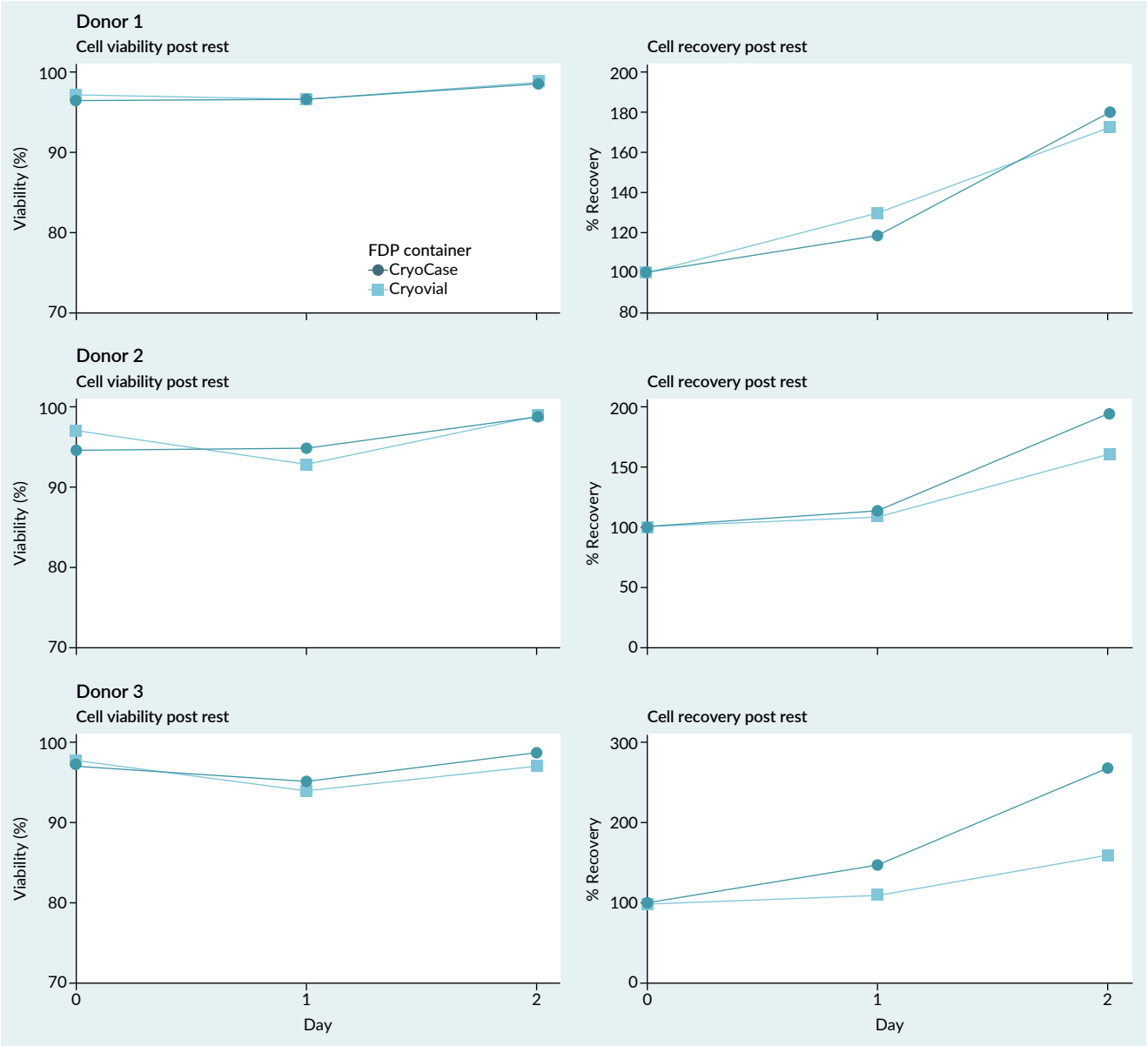


FIGURE 7

Post rest cell viability was high across all donors, conditions and timepoints.



Post-rest cell recovery was as good or better with cells cryopreserved in CellSeal CryoCases compared to cryovials. Post rest cell recovery was measured by dividing the number of viable cells counted at 24 and 48 hours post rest into the total number of viable cells seeded into culture post thaw.

It is noted that the CellSeal Connect Vials have not yet been validated for storage at temperatures below -80°C as the weldable lines are composed of PVC, which can become fragile at cryogenic temperatures. During the execution of this study, no damage or deformity was noted as a result of storing vials at liquid nitrogen temperatures, however, additional testing may be needed to better assess the impact of cryogenic storage on container integrity. Proper handling and storage of CellSeal Connect vials to prevent fracture of these lines at time of use following cryopreservation would be critical.

One limitation of the CellSeal Connect vials is the volume capacity, as vials can

only hold a maximum of 5 mL of solution. In the context of standard autologous CAR-T manufacturing, these volume limitations need to be considered for both containment of cryopreserved starting cells and viral vectors. For many programs, $1–2 \times 10^8$ cells are needed for initiation of a manufacturing batches and if starting cells are cryopreserved at concentrations at or above 2×10^7 viable cells/mL, then only 1–2 CellSeal Connect vials would be needed per manufacturing run. For lentiviral vector containment, the volume limitations of the CellSeal Connect vials are not thought to be problematic as most programs use considerably less than 5 mL of LVV for transduction. However, this could be restrictive for programs using retrovirus depending on the volumes needed for transduction, which are routinely above 5 mL and can sometimes exceed 50 mL for transducing under 1×10^8 cells. For closed system filling and retrieval of retroviral vectors, it may be more practical to use weldable bags to reduce the number of containers needed.

In summary, while the CellSeal Connect vials can be used to further reduce the need for a BSC in CAR-T manufacturing, there are limitations that should be considered and evaluated to ensure their suitability for specific programs. While the volume capacities of the vials are well within the ranges of what are typically used for starting cells and lentiviral vectors, CellSeal Connect vials may not be well suited for retroviral vectors. Additional studies assessing the impact of cryogenic storage on container integrity would also be needed prior to integration of the vials into a program for use as a container for starting cells. Nevertheless, the use of CellSeal Connect vials represents an important step toward enabling a fully closed, BSC-independent CAR-T manufacturing process, which could ultimately improve scalability, reduce manufacturing costs, and expand patient access to these life-saving therapies.

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Chantale Bernatchez has overseen the process development of multiple CAR-T projects in collaboration with academic and industry partners. She has licenses related to IP on the process of expanding and engineering tumor-infiltrating T cells, and two patents related to methods to expand or engineer TIL as well as critical reagent needed in TIL expansion. She is also a Scientific Advisory Board member for KSQ Therapeutics and Obsidian Therapeutics.

Funding declaration: Biolife Solutions provided support for study materials and article processing.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; externally peer reviewed.

Submitted for peer review: Jun 16, 2025.

Revised manuscript received: Aug 5, 2025.

Publication date: Aug 19, 2025.

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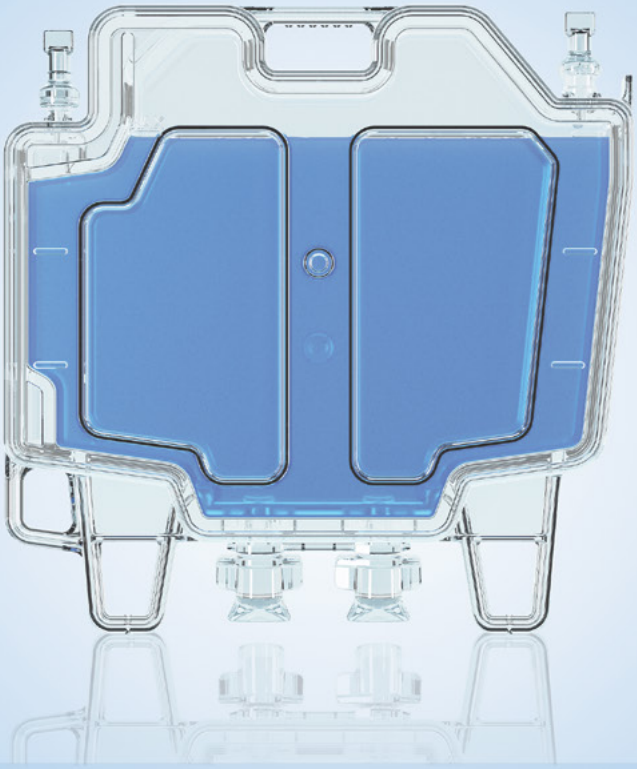

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