RAPID, PRECISE, AND CONTROLLED: AUTOMATED FORMULATION AND FILLING TO SUPPORT SCALING TUMOR-INFILTRATING LYMPHOCYTE THERAPY MANUFACTURING

Donastas Sakellariou-Thompson, Lothar Lauterboeck, Tamara Griffiths, Aemilia Katzinski, Anindita Ravindran, Jane Zulovich, Katrina Adlerz, Priya Balasubramanian, Chantale Bernatchez, Marie-Andrée Forget

All authors are affiliated with CTMC, Houston, TX, USA

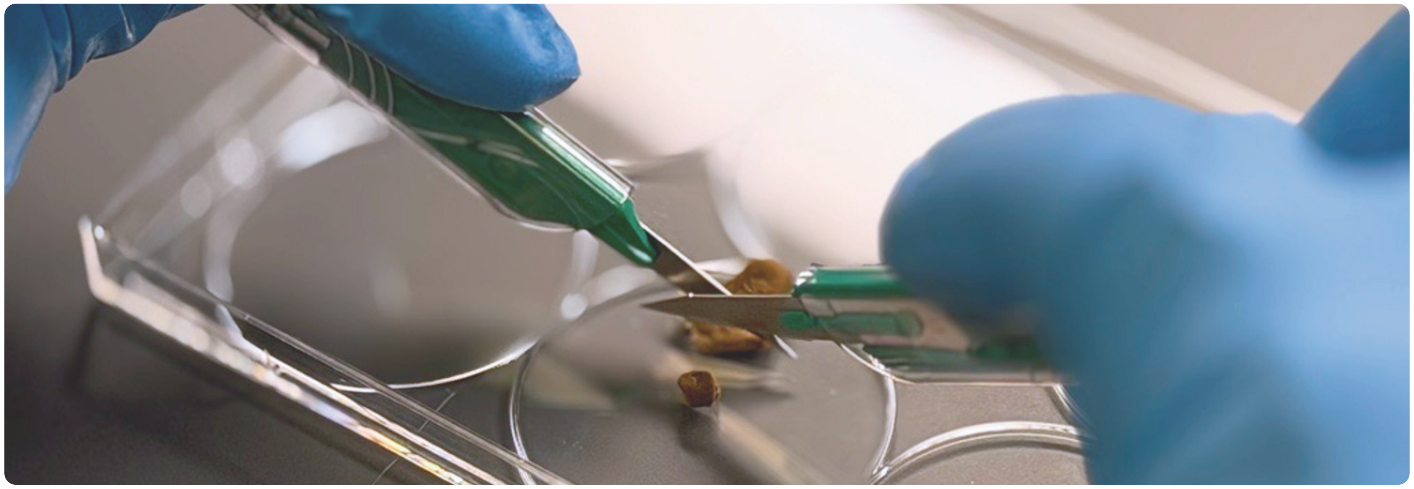
INTRODUCTION

Cell therapy products are typically formulated in a cryoprotective agent (CPA) and dispensed into cryogenic containers prior to cryopreservation. This formulation and fill-finish process encompasses the final manufacturing operations before freezing, including formulation of the drug product, aseptic dispensing into cryogenic containers, air removal from the container, container closure, and preparation for controlled-rate freezing. As a critical step in the manufacturing process, formulation and fill-finish directly influence product quality, dose accuracy, cell viability, and overall process consistency.



Ideally, formulation and fill-finish operations should be rapid, precise, and highly controlled to preserve product integrity, ensure dose consistency, and minimize cell exposure to dimethyl sulfoxide (DMSO), a widely used cryoprotectant that can adversely affect cell viability with prolonged exposure. These challenges are particularly significant for high cell-yield therapies such as tumor-infiltrating lymphocyte (TIL) products, where numerous cryogenic containers may need to be filled within a narrow processing window.

Conventional manual formulation and fill-finish processes are labor-intensive and dependent on operator skill, making it difficult to consistently maintain dispensing accuracy across multiple containers while introducing opportunities for process variability and limiting scalability.



THE CTMC TIL PROCESS

CTMC's TIL platform is designed to jumpstart the manufacturing process.

The platform has five key processes:

- **Tumor Procurement:** A tumor sample is obtained from surgical resection or core needle biopsy and transported to the GMP facility.
- **Tumor Dissection, TIL Propagation in Pre-Rapid Expansion Process (Pre-REP):** Fragmented tumor or unprocessed tumor core needle biopsies are combined with CTMC TIL reagents, such as TR-41, to produce rapid and robust growth.
- **Rapid Expansion Process (REP):** TIL are engineered and expanded using a proprietary feeder system (iTR-56).
- **Harvest and Formulation:** Cells are washed, concentrated, formulated in cryoprotectant, filled into cryobags or cryovials, and cryopreserved. This is where the ARES™ X20+ enters the process.
- **TIL Analytics and Quality:** Final product testing is performed to assess release criteria and product characterization, including identity, purity, potency, viability, and safety.

BENEFITS OF THE CTMC TIL PLATFORM INCLUDE:

- Simplified and robust TIL manufacturing platform informed by decades of experience in TIL therapy development and manufacturing across academic and industry settings
- 95% manufacturing success rate
- Minimal tumor starting material is required
- Reduced time in culture, operator interventions, and number of processing days
- Increased number of TIL, particularly $CD8^+$ TIL



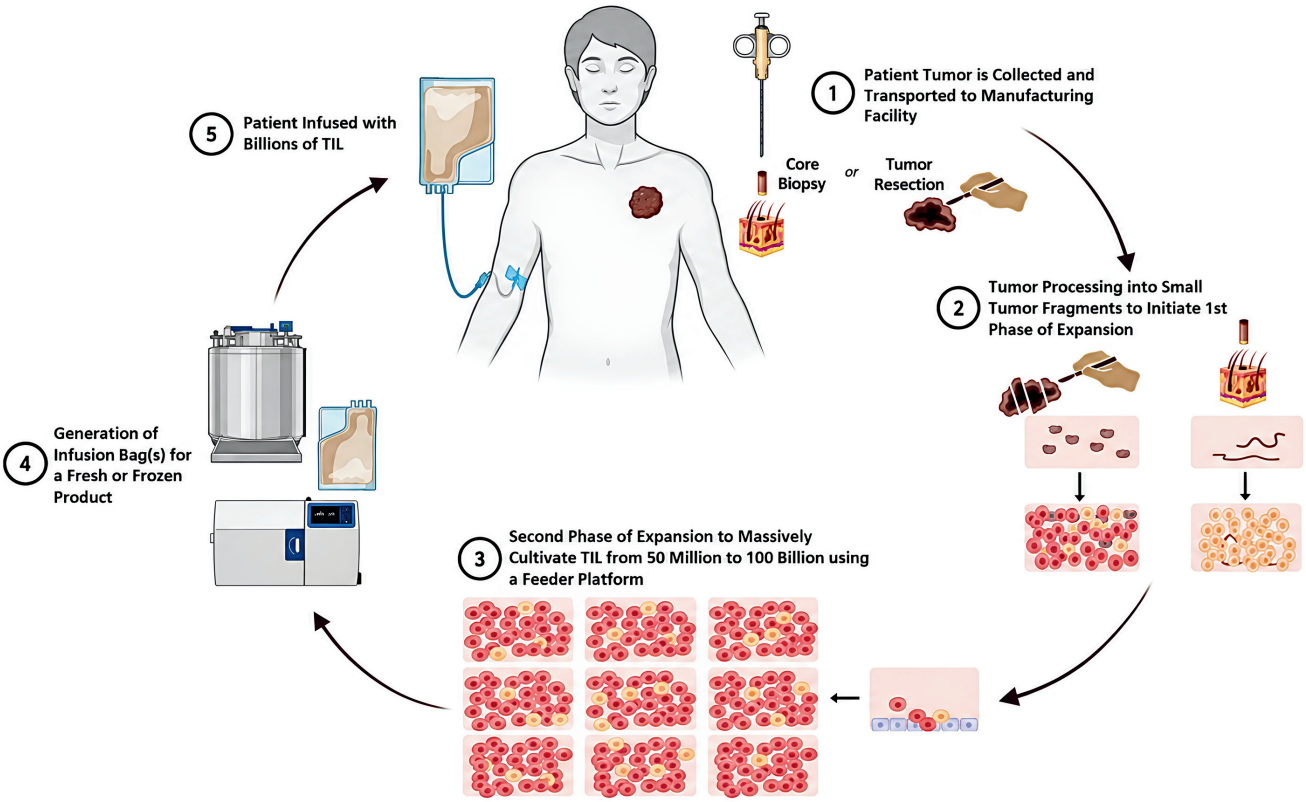


Figure 1.

THE TIL MANUFACTURING PROCESS INCLUDES:

1) Patient's tumor is collected through surgical resection or core needle biopsy, and then transported to CTMC's manufacturing facility. 2) Tumor fragments are used to initiate first phase of TIL expansion (pre-REP) which can generate up to, but not limited to, 100s of millions of cells. Prior to Step 3, TIL can be engineered via viral or non-viral methods depending on the TIL program. 3) Cells undergo a second phase of expansion (REP) using our proprietary feeder platform which can generate up to, but not limited to, 100 billion cells. 4) TIL are harvested, formulated with a CPA, and filled in cryo-containers. TIL final drug product (FDP) then goes through cryopreservation process. 5) Shipped FDP is thawed and infused at the patient's bedside.

The CTMC Process Development team continuously evaluates emerging technologies that have the potential to improve process development and GMP manufacturing for its partners. The objective of this study was to compare the standard manual formulation and fill-finish process with the ARES X20+ automated system to determine whether the automated process could achieve performance comparable to the manual method. To do so, we assessed accuracy, processing time, full-time employee (FTE) requirements, and key product quality attributes, including cell viability and viable cell density.

THE ARES X20+ AUTOMATED FORMULATION & FILL-FINISH SYSTEM

The ARES X20+, a closed and automated formulation and fill-finish system developed by Xiogenix, was designed to address these fill-finish challenges by improving process control, dispensing precision, and operational efficiency while reducing reliance on manual intervention. To assess its performance in a cell therapy manufacturing environment, the ARES X20+ was evaluated at CTMC through a series of studies focused on dispensing accuracy, process consistency, and operational efficiency. These studies were performed using the ARES X20+ Generation 1 system. Xiogenix has now released an updated Generation 2 system. The second-generation ARES X20+ platform builds upon the proven core process architecture of the Generation 1 system. The fundamental process flow and primary operating mechanisms remain consistent between both generations, including the use of the same pumping technology, cooling approach, and homogenization methodology. Generation 2 introduces targeted enhancements to the formulation and final fill sequences, which improve overall consistency while maintaining continuity with Generation 1.

Additional Benefits of the ARES X20+ System
Fully customizable recipes (pump speed, agitation, cooling, and more)
Easily cleanable surfaces
Automatic pre-fill and semi-automatic post-fill air removal
High-throughput capability
Functionally closed
Built-in sampling

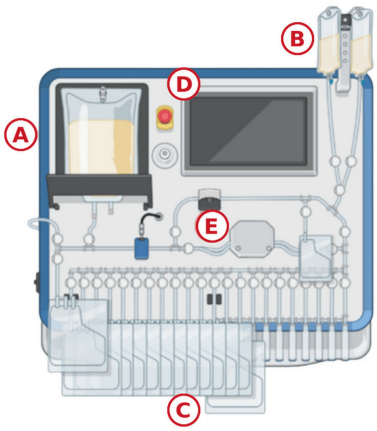
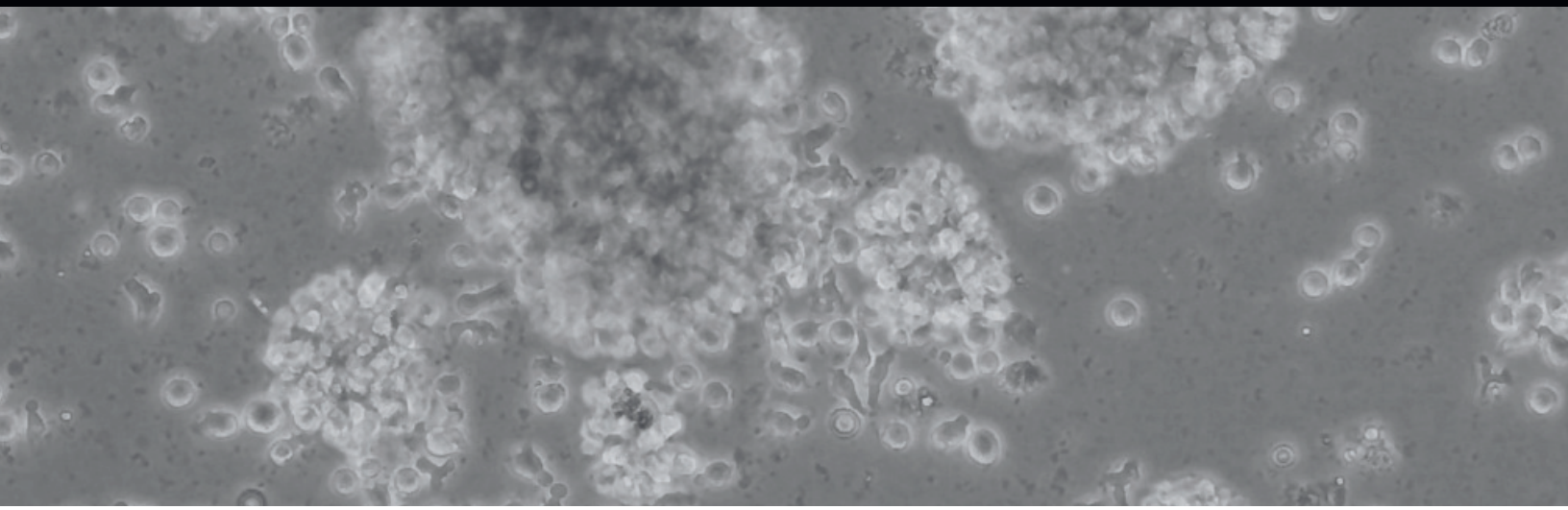


Figure 2.

A) Accommodates up to a 5 L source bag with product cooling to < 8°C and agitation for product homogenization **B)** Automatic product formulation with two solutions (e.g., buffer, cryoprotectant) **C)** Fill up to 20 FDP containers at once using single-use kits customizable for any mix of bags and vials **D)** Software / User Interface: large touchscreen, extensive recipe library, tiered user accounts, network connectivity **E)** Two peristaltic pumps to control air evacuation, formulation, and container filling.





METHODS

The ARES X20+ (Generation 1) was calibrated using a 1:1 mixture of Plasmalyte-A + Human Serum Albumin (PLA-H) buffer and CS10. Cell-free runs were conducted to evaluate the system's ability to achieve a homogeneous mixture following CPA formulation at three different volumes. Following the addition of CS10 to the PLA-H buffer, automated mixing was compared to conventional manual mixing by measuring osmolarity at multiple time points as an indicator of solution homogeneity. Testing was performed using representative single-use kits with the agitation bar operating at its maximum speed of 60 RPM and the deepest pitch setting. As a result of the mixing study, all subsequent cell runs included a short manual mixing addition to accelerate homogenization.

To assess manufacturing flexibility, the ARES X20+ was configured with custom fill recipes and single-use kits representative of CTMC's TIL manufacturing processes. Fill recipes supported formulation volumes ranging from 50 mL to 2400 mL and dispensing volumes from 1 mL to 100 mL. Single-use kits were configured for multiple final container formats, including 2 mL cryovials to CS750 cryobags. High-throughput runs using the ARES X20+ extension manifolds were performed using CTMC's proprietary TIL expansion reagent, iTR-56, with additional strips of cryo-containers welded onto the upper section of the original manifold.

Platform performance was evaluated by assessing fill accuracy and precision both immediately after filling and following cryopreservation and thaw. Additionally, the ability of the system to maintain product concentration was measured, as well as cell fitness attributes such as proliferation potential and viability. For iTR-56 automated formulation and filling, functional performance was also assessed by measuring ability to support TIL expansion in a rapid expansion process.

Comparability studies were conducted to benchmark the performance of the ARES X20+ against CTMC's standard manual fill-finish process. Endpoints included post-fill and post-thaw fill accuracy and precision, consistency of product concentration, cell proliferation potential, cell viability, and overall process reproducibility.

TIL used in this series of studies were generated, in general, following the process outlined in the above "CTMC TIL Process" section.



RESULTS & DISCUSSION

AUTOMATED MIXING POST FORMULATION PRODUCES HOMOGENEOUS MIXTURE, WHICH IS ACCELERATED WITH MANUAL INTERVENTION.

Maintaining formulation homogeneity throughout automated fill-finish is critical to ensuring consistent cell suspension and cryoprotectant composition in every cryobag. Continuous mixing prevents cell settling and cryoprotectant gradients, reducing variability in cell concentration and DMSO exposure. This preserves cell viability, post-thaw recovery, and functional performance while improving process reproducibility and dose consistency across the final drug product.

A cell-free study was conducted using the formulation function to mix a 1:1 ratio of PLA-H and CS10 to evaluate the ability of ARES X20+ to create a homogeneous solution after formulation. For these experiments, the instrument was programmed for the addition of a specified volume of CPA (e.g., CS10) to the source bag containing buffer (e.g., Plasmalyte-A + Human Serum Albumin) as shown in Figure 3.

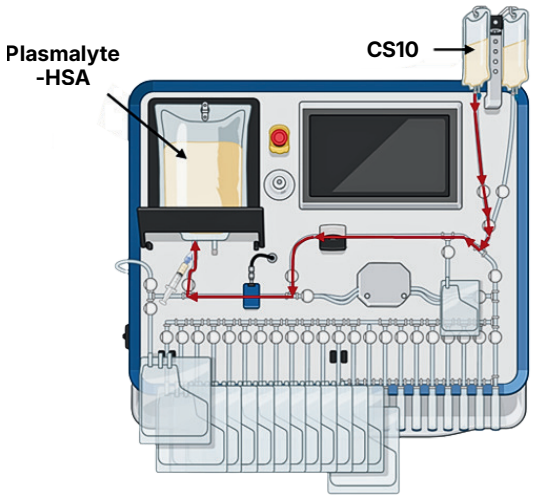


Figure 3.
For the mixing study, a source bag with Plasmalyte + HSA and a bag of CS10 were welded onto a single-use kit. The red arrows trace the path of the CS10 as it is added to the source bag during formulation. The agitation bar continuously mixes the solution in the source bag during this formulation process. The machine was programmed to continue agitating the source bag for a set period prior to filling, ensuring an even mix.

Cryoprotectant solutions are typically a mix of a basal buffer with a concentrated cryoprotective agent. Each component has a different osmolarity, which is the measure of the total concentration of osmotically active solutes in the solution. The osmolarity of each component plus a manually, well-mixed sample were measured to establish the baseline osmolarity for PLA-H (~0.3 Osm) and CS10 (~2.8 Osm) plus the target osmolarity of the mixture (~1.3 Osm) (Figure 4). The change in osmolarity as the solution mixed was used as a surrogate indicator of cryoprotectant homogeneity following formulation.

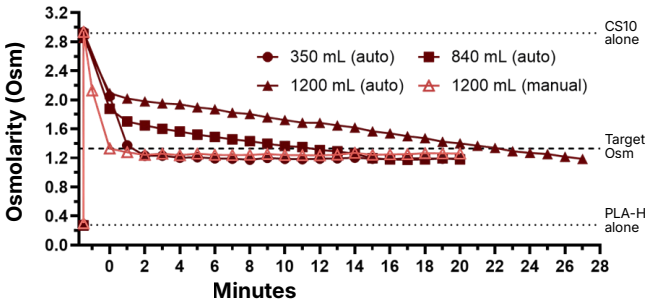


Figure 4.
Cell-free runs at three final formulation volumes were performed to characterize the mixing function of the ARES X20+. CS10 was added to PLA-H using the formulation function of the machine and homogenized solely with the agitation bar (auto, dark red) or mainly with manual agitation (manual, light red) prior to maintaining homogeneity with the agitation bar. The agitation bar was set to 60 RPM (max) and the deepest pitch.

Three final formulation volumes relevant to manufacturing-scale operations at CTMC were selected: 350 mL, 840 mL, and 1200 mL (Figure 4). The buffer and CS10 were mixed at a 1:1 ratio (e.g., the 1200 mL run started with 600 mL of PLA-H buffer and 600 mL CS10). The entire volume of CS10 was added to the PLA-H buffer using the machine's formulation function and allowed to mix without intervention (auto, dark red lines) or with a short manual mixing addition (manual, light red line) (Figure 4). Manual agitation was performed by massaging the bag 50 times over 30 seconds. Since the agitation bar runs continuously during operation, the manual mixing condition also had some automatic mixing during CS10 addition and then over the sampling time-course following CS10 addition. However, homogeneity was achieved mainly via manual agitation.

Volume / Process	Time to Reach Homogenization
350 mL (auto)	2 minutes
840 mL (auto)	14 minutes
1200 mL (auto)	22 minutes
1200 mL (manual)	2 minutes

Table 1.
Summary of volumes tested and times to reach homogenization.

For the automatic mixing conditions, the osmolarity was measured for each solution prior to mixing (t = -1), immediately after the entire volume of CS10 was added (t = 0), and every minute after until 20 min (350 and 840 mL) or 27 min (1200 mL) (Table 1). For the manual mixing condition, the osmolarity was measured for each solution prior to mixing (t = -1), immediately after the entire volume of CS10 was added (t = -0.5), immediately after manual mixing (t = 0), and every minute after until 20 min. Samples were pulled from the inlet line at the bottom of the source bag where CS10 was introduced and measured in triplicate using an osmometer.

The results show that the ARES X20+ can achieve homogenization over a range of operating volumes, with volumes around 350 mL or less mixing quickly within a couple of minutes (Figure 4 and Table 1). As the formulation volume increases, the time to reach homogeneity increases. The 840 mL volume took 14 minutes, and the 1200 mL volume took 22 minutes (Figure 4 and Table 1). So, while larger volumes can reach a well-mixed state without operator intervention, the time to do so may be longer than is desired for a given process. Therefore, addition of a well-defined, manual mixing step can greatly accelerate homogenization for large volumes. For the 1200 mL formulation, addition of a short manual mixing step reduced the time required to achieve a homogeneous solution from approximately 22 minutes to 2 minutes, representing a ten-fold improvement over automatic mixing alone (Figure 4 and Table 1).

HIGH-THROUGHPUT FORMULATION AND FILLING ON THE ARES X20+

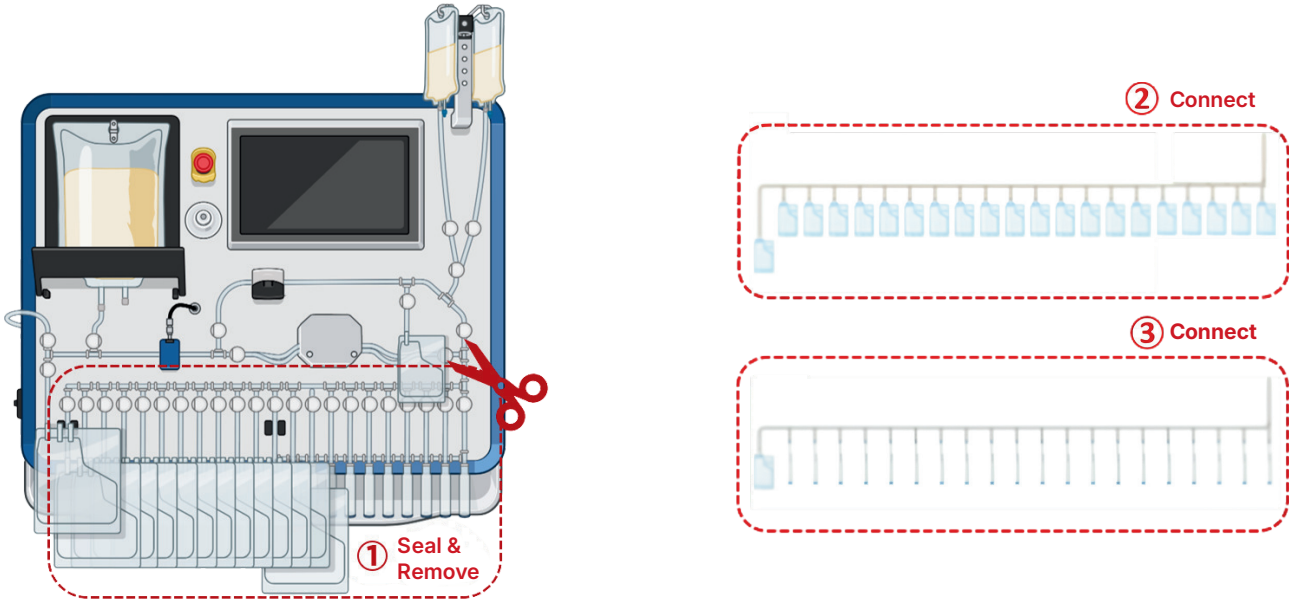


Figure 5.
A fully setup ARES X20+ is shown loaded with an "extension" manifold. After the **1)** first set of FDP containers are filled, the entire section of bags can be sealed and removed to make room for **2)** a second set of 20 FDP bags and **3)** a set of 20 cryovials to be welded on in succession.

CTMC's proprietary reagent, iTR-56, is used to greatly expand TIL from millions to hundreds of billions of cells. This reagent has ligands that engage receptors on T cells to enhance activation, improve proliferation, support survival, and reduce activation-induced cell death. The production of iTR-56 is a large-scale process that generates an end-product comparable to the scale of an allogenic product which can lead to the formulation and filling of two-digit to three-digit final containers such as cryobags or cryovials.

To assess the ability of the ARES X20+ to manage a high-throughput process like iTR-56, the next phase of our study compared high-throughput filling of iTR-56 in CS250 bags and 2 mL cryovials using extension kits compared to manual filling. The general setup is shown in Figure 5.

For this high-throughput run, iTR-56 feeder cells were washed and collected in a 5 L source bag which was welded to an extension manifold with 20 CS250 cryobags. A separate high-volume aliquot of CS10 was similarly welded to the manifold, and the complete assembly was installed on the ARES X20+. Following automated 1:1 formulation with CS10, the source bag was manually mixed as previously described to ensure a homogeneous solution before filling each cryobag at a target volume of 65 mL determined for this study. After the first 20 cryobags were filled, the manifold was sealed between the source bag and the filled bags, and then removed (Figure 5, #1). The second manifold of 20 additional CS250 cryobags was welded onto the same tubing kit, filled, and then replaced with a strip containing 20x 2 mL cryovials following the same steps (Figure 5, #2 and #3). All containers were cryopreserved using a controlled-rate freezer (CRF). Only results from the cryobags are presented in Figure 6.

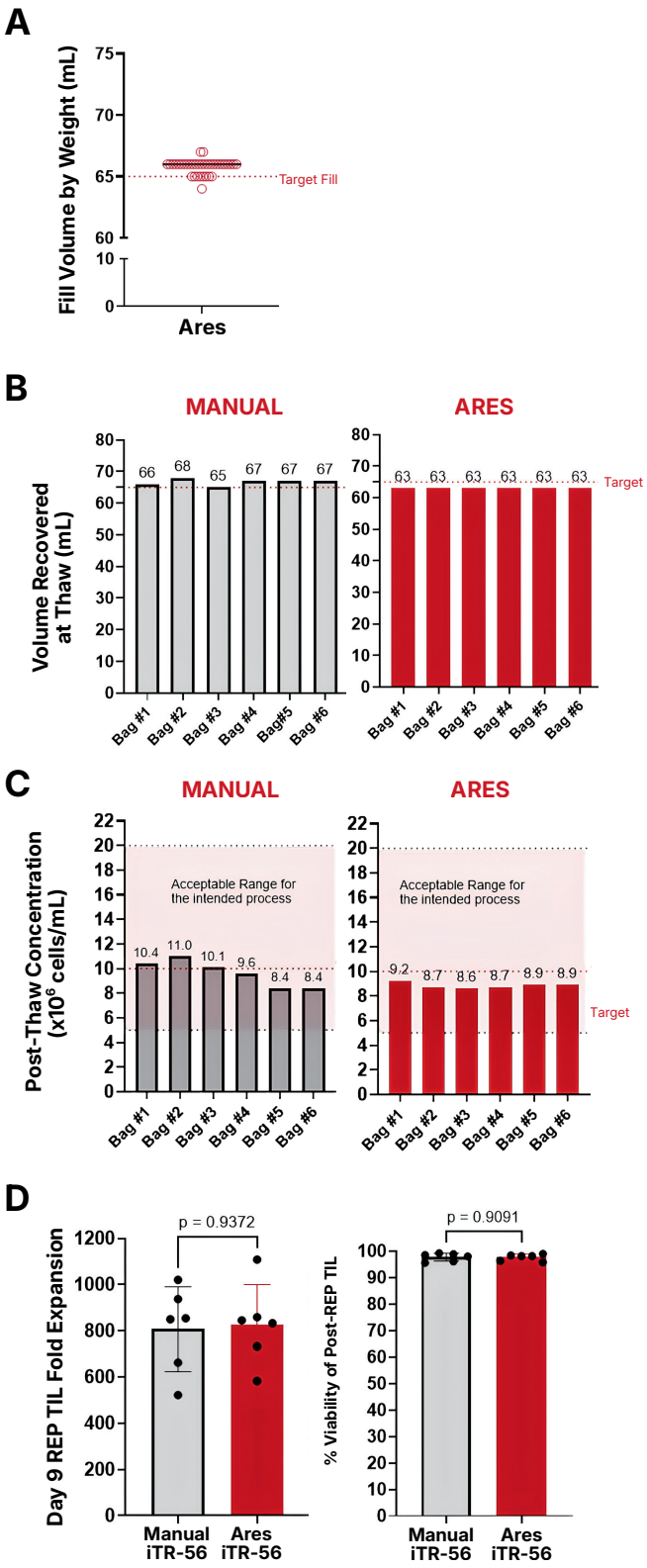


Figure 6.
A) 36 CS250 cryobags were filled with a 65 mL target volume, with 20 bags filled using the first strip of bags and 16 bags with the second strip. The weight of each filled bag is presented where 1 g = 1 mL. **B)** Comparison of the volume recovered after thaw from a set of manually-filled bags (gray, n=6) and ARES-filled bags (red, n=6). **C)** Comparison of the iTR-56 concentration recovered after thaw from a set of manually-filled bags (gray, n=6) and ARES-filled bags (red, n=6). The light red box represents the acceptable range of recovery for the intended process. **D)** Functional characterization of the ability of iTR-56 to support robust TIL growth in a REP (fold expansion and viability) that were filled manually (gray) or with the ARES X20+ (red) (n=6). Bags filled with the ARES X20+ for (B-D) were pulled at random with half from the first strip and half from the second strip. All cell counts and viability were assessed with the NC-200.

In total, 36 CS250 cryobags were filled using the ARES X20+. Before cryopreservation, each bag was weighed to determine fill volume, demonstrating accurate and precise dispensing at the target volume of 65 ± 2 mL (Figure 6A). The slight overfill ensures sufficient product retrieval after thaw. The bags filled with the ARES X20+ were subsequently compared with manually filled cryobags produced during a previous iTR-56 manufacturing run using syringe-based filling (Figure 6B). Six cryobags from each filling method were randomly selected for evaluation. For the ARES X20+ process, three bags were selected from the first manifold and three from the second. Following thaw, the contents of each cryobag were extracted by syringe and measured volumetrically. Manually filled cryobags contained 65–68 mL retrievable volume, whereas all cryobags filled with the ARES X20+ contained 63 mL, demonstrating the high filling precision and consistency of the ARES X20+.

For this study, the iTR-56 reagent was filled at a target concentration of $10x10^6$ cells/mL, and samples from each thawed bag were also taken to assay the concentration post-fill and post-thaw (Figure 6C). The manually filled bags ranged from $8.4x10^6$ to $11.0x10^6$ cells/mL, and the bags filled with the ARES X20+ ranged from $8.6x10^6$ to $9.2x10^6$ cells/mL. **While both sets of bags are within the acceptable range for this process, the bags filled with the automated system demonstrated greater precision. Furthermore, because the bags filled with the ARES X20+ were pulled from both the first and second manifold, these results demonstrate that the system's automated agitation function can maintain homogeneity of a formulated product over the course of filling even at large volumes.**

Finally, to verify that iTR-56 processed using the ARES X20+ retained its biological activity, TIL were expanded during a Short-REP process using iTR-56 from either manually filled bags or bags filled with the ARES X20+ (Figure 6D). TIL fold expansion and viability at the end of the Short-REP were equivalent, demonstrating that the fill-finish process had no impact on iTR-56 performance. **Together, these results demonstrate that the system is a suitable alternative to manual fill-finish for large-volume, high-throughput manufacturing.**





FILL & RECOVERY OF CRYOPRESERVED TIL PRODUCT IN CS250 CRYOBAGS

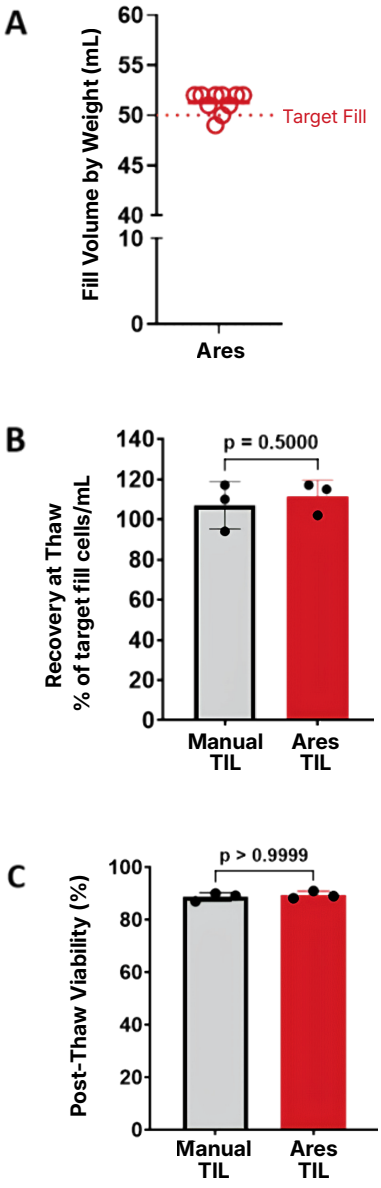
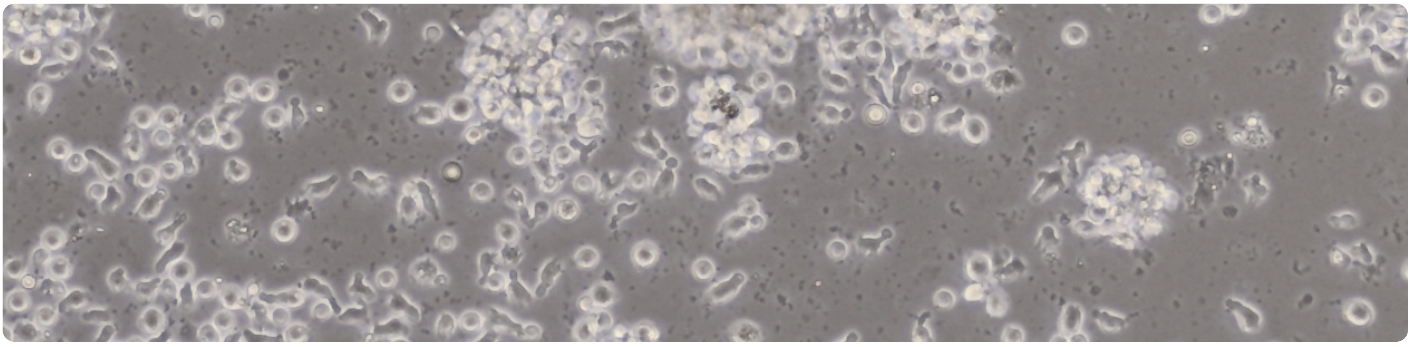


Figure 7.
A) 10 CS250 cryobags comprising 3 different runs were filled with a target volume of 50 mL. The weight of each bag's content is presented in the graph where 1 g = 1 mL. **B)** Immediate post-thaw recovery based on target formulation concentration prior to formulation and fill either manual (gray) or with the ARES X20+ (red) (n=3). **C)** Immediate post-thaw viability based on target formulation concentration prior to formulation and filled either manually (gray) or with the ARES X20+ (red) (n=3). All cell counts and viability were assessed with the NC-200.

To evaluate automated fill-finish using a clinically relevant TIL drug product, CTMC compared the ARES X20+ with its standard manual process to fill CS250 cryobags (Figure 7 and 8). This study examined whether automated formulation and dispensing could achieve equivalent product filling, recovery, viability, and post-thaw performance following cryopreservation.

TIL were generated in three separate runs, each with a different donor, where they were harvested, washed into PLA-H buffer, and collected in a CS2500 source bag. For each run, the source bag and an appropriate aliquot of CS10 were connected to an ARES X20+ single-use kit with five CS250 bags. Across the three runs, the final formulation volume ranged from 100 – 500 mL. The entire set-up was installed on the ARES X20+ as previously shown (Figure 3). Following 1:1 formulation with the entire aliquot of CS10, the source bag was mixed manually as previously described to achieve a homogeneous solution before filling each cryobag at a target of 50 mL/bag. In total, 10 cryobags were filled across the three runs using three different donors (Figure 7A). Prior to cryopreservation, each bag was weighed to determine the fill volume, showing precise filling at the target volume of 50 ± 2 mL (Figure 7A). A slight overfill of the bags ensures a sufficient volume can be extracted from the bags after thaw. **Results fall within the target fill precision for the manual process using syringes.**

The quality of cells in bags filled with the ARES X20+ was compared with bags from the same manufacturing process that were manually filled via syringe (Figure 7B and 7C). Three bags from separate runs were selected, thawed, and evaluated for cell recovery (Figure 7B) and viability (Figure 7C). **These results demonstrate that the ARES X20+ filling process produces comparable recovery and viability to the manual filling process.**



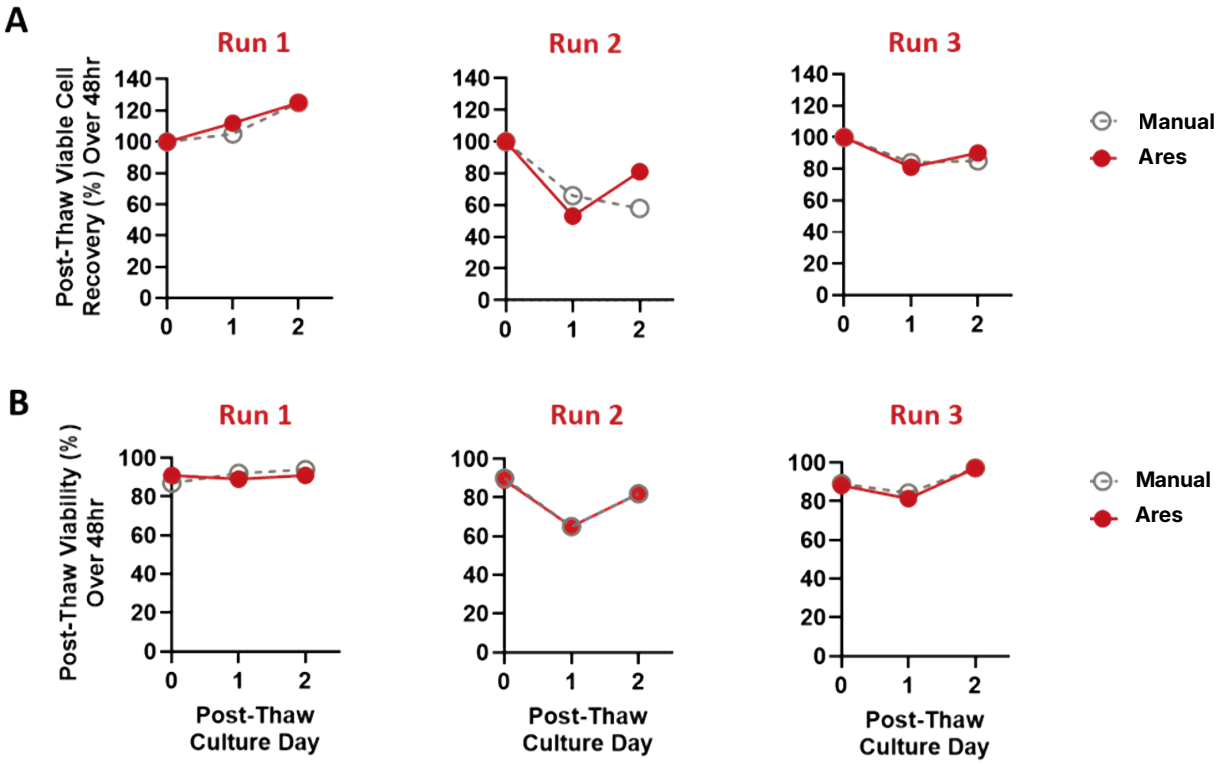


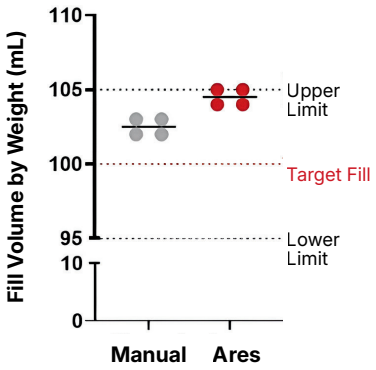
Figure 8.
Graphs showing **A)** cell recovery and **B)** viability over time of three individual runs formulated and filled either manually (gray) or with the ARES X20+ (red). The cells were thawed, washed, and seeded at a fixed concentration in culture media in replicates in a culture plate. One replicate well was sampled each day. All counts and viability assessments were done using the NC-200.

For each run, post-thaw cells were also put in culture to measure post-thaw recovery and viability over 48 hours to better assess the health of the cells (Figure 8). After two days of culture, the cells were collected to assess recovery and viability relative to the starting value on Day 0. **The recovery (Figure 8A) and viability (Figure 8B) of the TIL processed with the ARES X20+ were comparable to the manual process.**

FILL & RECOVERY OF CRYOPRESERVED TIL PRODUCT IN CS750 CRYOBAGS

To further evaluate the ARES X20+ for TIL manufacturing, a clinically relevant TIL drug product, this time filled with the system into CS750 cryobags was compared with the manual process. TIL were generated in a single run, harvested, washed into 250 mL of PLA-H buffer, and collected in a CS2500 source bag. The source bag and a 250 mL aliquot of CS10 were connected to an ARES X20+ single-use kit with five CS750 cryobags, yielding a final formulation volume of 500 mL. After the product was installed on the ARES X20+ (Figure 3), the cell suspension was formulated 1:1 with CS10, manually mixed to ensure homogeneity, and dispensed into the cryobags at a target fill volume of 100 mL per bag.

A



B

Process	FTE	Total Time (min)	CS10 Time (min)	Post-Formulation VCD & Viability
Manual (syringe fill)	3	58	52	$39.4x10^6$ cells/mL; 92.9%
Manual (weight fill)	2	61	52	N/A (cell-free)
ARES X20	2	35	26	$40.2x10^6$ cells/mL; 93.1%

Figure 9.
A) Four CS750 cryobags were filled with a 100 mL target volume via manual process with syringes (gray) or the ARES X20+ (red). The weight of each filled bag is presented where 1 g = 1 mL. **B)** Key metrics for the manual vs ARES X20+ runs are summarized, including a comparison to manual gravity filling. FTE = Full-time employee; VCD = viable cell density. All cell counts and viability were assessed with the NC-200.

Four cryobags were filled using the ARES X20+ and four cryobags were filled manually by syringe (Figure 9). Prior to cryopreservation, each bag was weighed to determine the fill volume, showing precise filling (Figure 9A). A slight overfill of the bags ensures a sufficient volume can be extracted from the bags after thaw. Results fall within the target fill accuracy for the manual process. The key process metrics comparing filling CS750 bags via the manual or ARES X20+ processes are summarized in Figure 9B. Due to the large fill volume, some processes may opt for a gravity drain fill method, which is included as a comparison. The automated system requires no more than two operators (Figure 9B Table, FTE), reducing labor requirements while streamlining the fill-finish process. Compared with CTMC's standard manual methods, the automated system reduced total processing time by approximately 40% and CS10 exposure time by 50%, while maintaining comparable viable cell density (VCD) and post-fill viability.



The time savings enabled with the ARES X20+ are well illustrated when these results are extrapolated to a theoretical manufacturing process that produces 2-5 CS750 FDP bags with 100 mL fill/bag. In this scenario, filling five bags can be completed in less than 50 minutes of CS10 exposure time, whereas manual filling results in progressively longer CS10 exposure times as the number of bags increases (Figure 10).

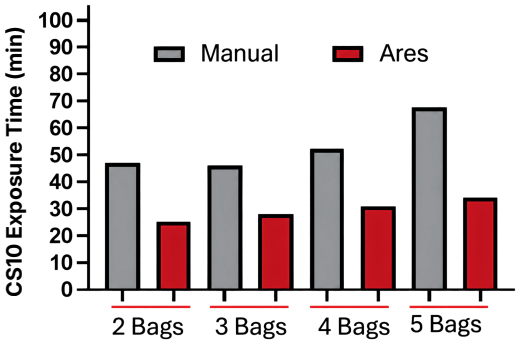


Figure 10.
Theoretical processing times for filling 2-5 CS750 bags at 100 mL/bag are compared between the manual process and the ARES X20+ process (extrapolated from the PD data in Figure 9). The data also includes the average time it takes to transport the drug product from the clean room to the CRF and start the CRF.

Faster processing times result in less CS10 exposure time, which can be beneficial since DMSO may be detrimental to cells' health. To assess the impact of reduced CS10 exposure time on product quality, TIL from two donors were formulated in cryoprotectant solution (1:1 PLA-H + CS10) and held for three hours at room temperature while being sampled periodically (0, 30, 45, 60, 90, 120, and 180 min) over the hold period and cryopreserved. Each timepoint was later thawed and assessed for post-thaw viable cell recovery (Figure 11).

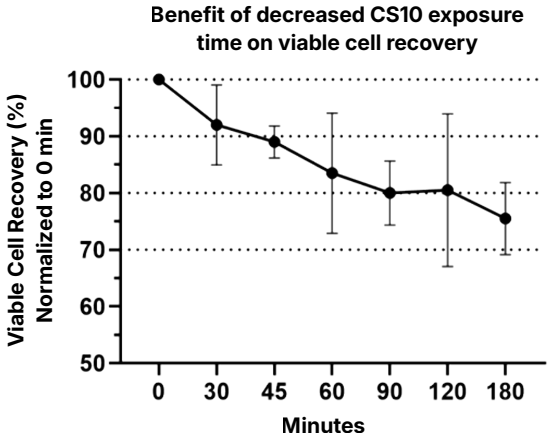


Figure 11.
The benefit of decreased CS10 exposure time to viable cell recovery after cryopreservation is presented. TIL from two donors were formulated, held for 180 min, and sampled periodically to be cryopreserved. The post-thaw viable cell recovery of each timepoint is shown normalized against the 0 min timepoint. All cell counts and viability were assessed with the NC-200.

The 0 min timepoint (i.e., TIL are cryopreserved immediately after formulation) is considered the best-case scenario and all values are normalized to the recovery of this timepoint. The viable cell recovery gradually decreases as CS10 exposure time increases, reaching about 75% recovery by 180 min. However, the data shows that keeping the CS10 hold-time around 45 min or less, which can be achieved with the ARES X20+ as shown in Figure 10, results in recovering 90% or more of cells that were recovered after no CS10 exposure (Figure 11). This supports a tangible product quality benefit of the use of an automated formulation and filling equipment to accelerate and standardize final drug product filling.



The quality of the fill for the bags filled with the ARES X20+ compared to manual syringe-filled bags from the same process (Figure 9) was characterized by assessing the recovery at thaw of the target fill concentration (Figure 12A). The bags filled with the ARES X20+ had 10% better recovery of the target concentration, indicating the ARES X20+ performed well the mixing and distribution of the formulated cell product. The TIL recovered after thaw of the CS750 bags were also put in culture to measure post-thaw recovery and viability over 48 hours to assess the health of the cells (Figure 12B and C). After two days of culture, the cells were collected to assess growth and viability relative to the starting value on Day 0. The recovery (Figure 12B) and viability (Figure 12C) of the TIL processed with the ARES X20+ were identical to the manual process.

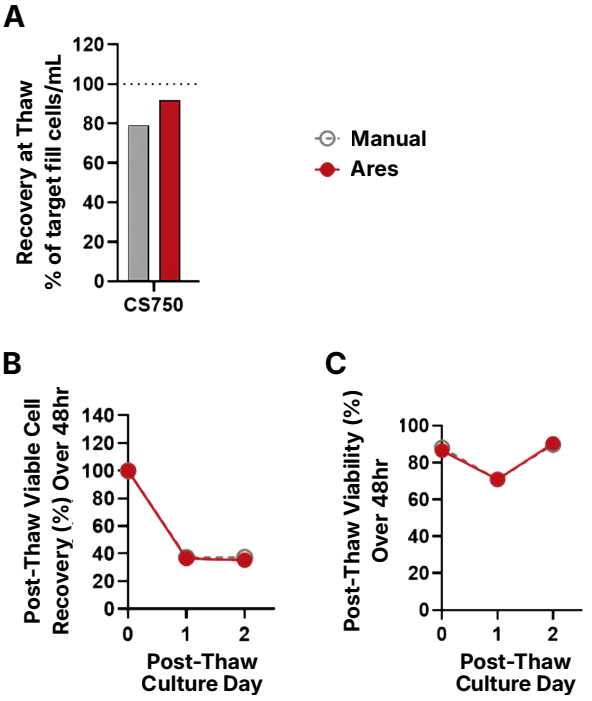


Figure 12.
A) Immediate post-thaw recovery based on post-formulation concentration for either manual (gray) or the ARES X20+ (red) (n=1). **B)** Cell recovery and **C)** viability respectively over 48 hours is shown. The cells were thawed, washed, and seeded at a fixed viable cell concentration in culture media in replicates in a culture plate. One replicate well was sampled for cell count and viability testing each day. All cell counts and viability were assessed with the NC-200.

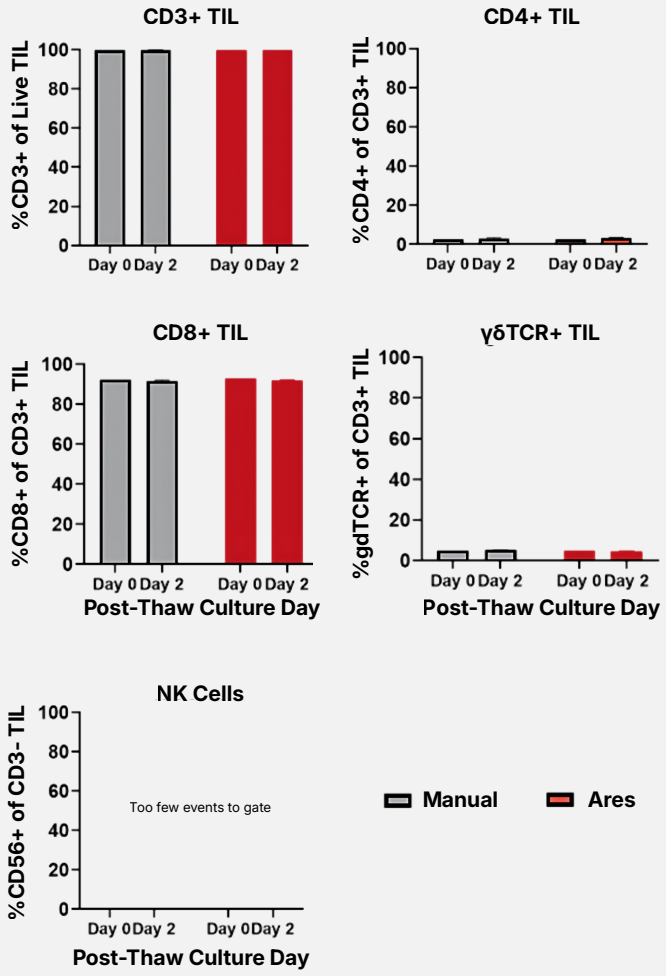


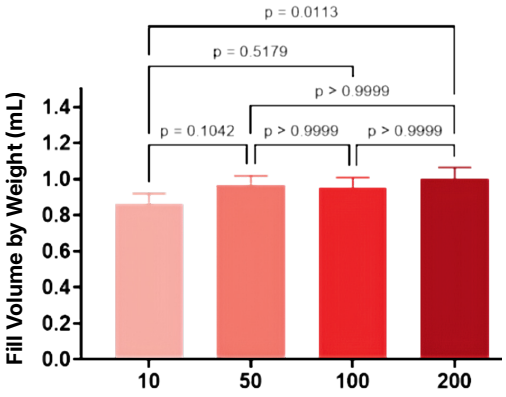
Figure 13.
Cells taken for the recovery assay (Figure 12) were also assessed via flow cytometry for %CD3+ TIL, %CD4+ TIL, %CD8+ TIL, %γδ TCR+ TIL, and %NK cells.

Cells taken for the recovery assay (Figure 12) were also assessed for general T-cell phenotype via flow cytometry for the manual and ARES X20+ conditions, compared between Day 0 and Day 2 (Figure 13). The results show that phenotypes are identical between the manual and automated process. **Collectively, these findings demonstrate that the ARES X20+ can improve manufacturing efficiency and process consistency without compromising critical product quality attributes.**

CONSISTENT PERFORMANCE ACROSS WIDE CELL CONCENTRATION RANGE

The ARES X20+ performed consistently across multiple runs with varying cell concentration using the same TIL donor. This study compared FDP from a single donor that was formulated on the ARES X20+ at four different concentrations in a final volume of 50 mL and automatically filled into six 2 mL cryovials (1 mL fill). The goal of this study was to show that one fill recipe is applicable regardless of FDP concentration. Each concentration represented a separate run with all runs performed in succession. After each set of vials were filled, they were weighed to assess the fill volume against a target fill of 1 mL. Regardless of the concentration, the ARES X20+ could fill the vials within 10% of the target (Figure 14A).

A



B

Condition (e6/mL)	Post-Formulation VCD (x10^6/mL)	Post-Formulation Viability
10	11.3	91.6%
50	46.7	91.3%
100	82.8	93.6%
200	195	87.6%

Figure 14.
The same TIL donor was formulated at four different concentrations and filled into six 2 mL cryovials using the ARES X20+, each concentration representing a separate run and all the runs were performed in succession. **A)** Cryovials were filled with a target volume of 1 mL via the ARES X20+ at 10x10^6 cells/mL, 50x10^6 cells/mL, 100x10^6 cells/mL, 200x10^6 cells/mL. The weight of each of the filled vials is presented in the graph where 1 g = 1 mL. **B)** The post-formulation concentration and viability are summarized.

To further assess the fill quality, the content of one vial from each run was extracted to characterize the cell concentration and viability in the vial (Figure 14B). The target concentration and the measured fill concentration were within 10% of each other apart from the 100x10^6 cell/mL concentration condition, which was within 20% of the target. The fact that the 10x10^6 cell/mL and 200x10^6 cell/mL conditions (with their vastly different cell concentrations) are so close to the target concentration indicates that the discrepancy of 100x10^6 cell/mL condition is likely due to operator-introduced variability as opposed to variability of the machine's performance. The post-formulation viability of each condition was also very similar to each other, again showing comparability in performance.

The post-thaw recovery after cryopreservation was assessed also for the cryovials filled at different concentrations (Figure 14). Over 48 hours, the post-thaw viable cell recovery (left) and the post-thaw cell viability (right) are identical between the conditions (Figure 15).

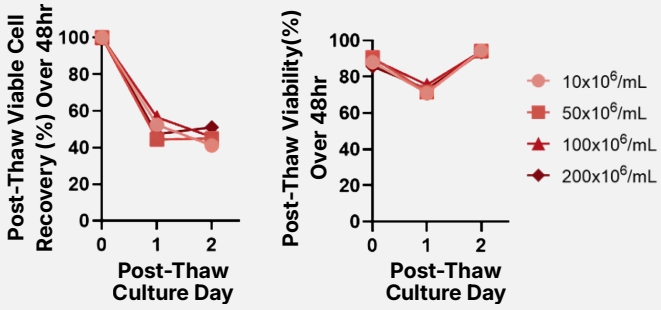


Figure 15.
Cell recovery and viability respectively over 48 hours is shown. The cells were thawed, washed, and seeded at a common concentration in culture media in replicates in a culture plate. One replicate well was sampled each day. Counts and viability assessed with the NC-200.

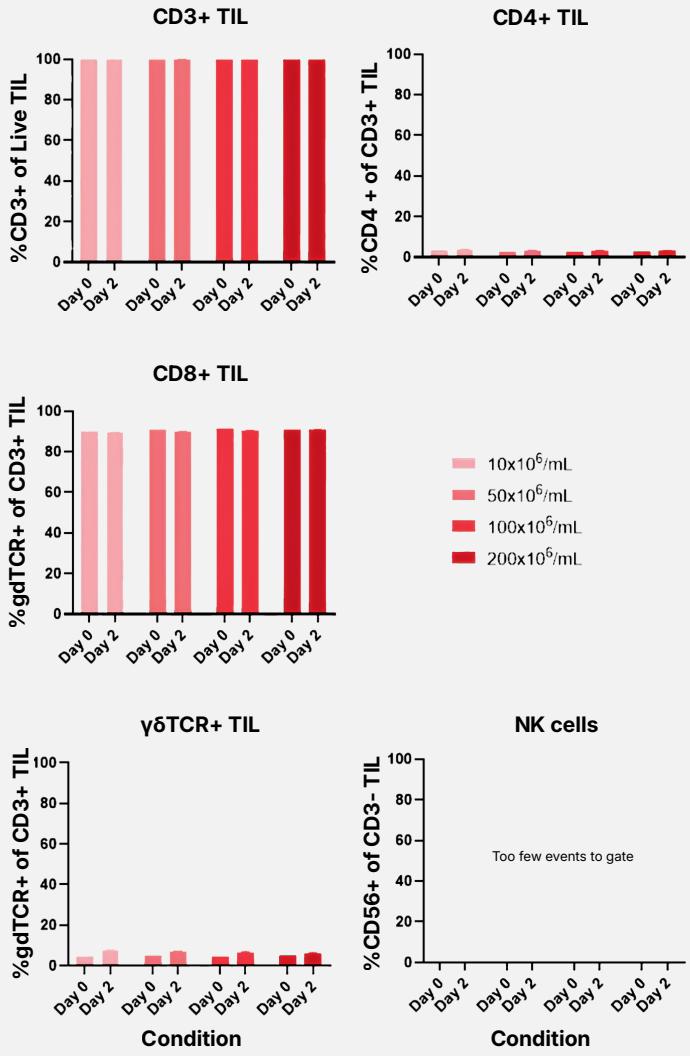
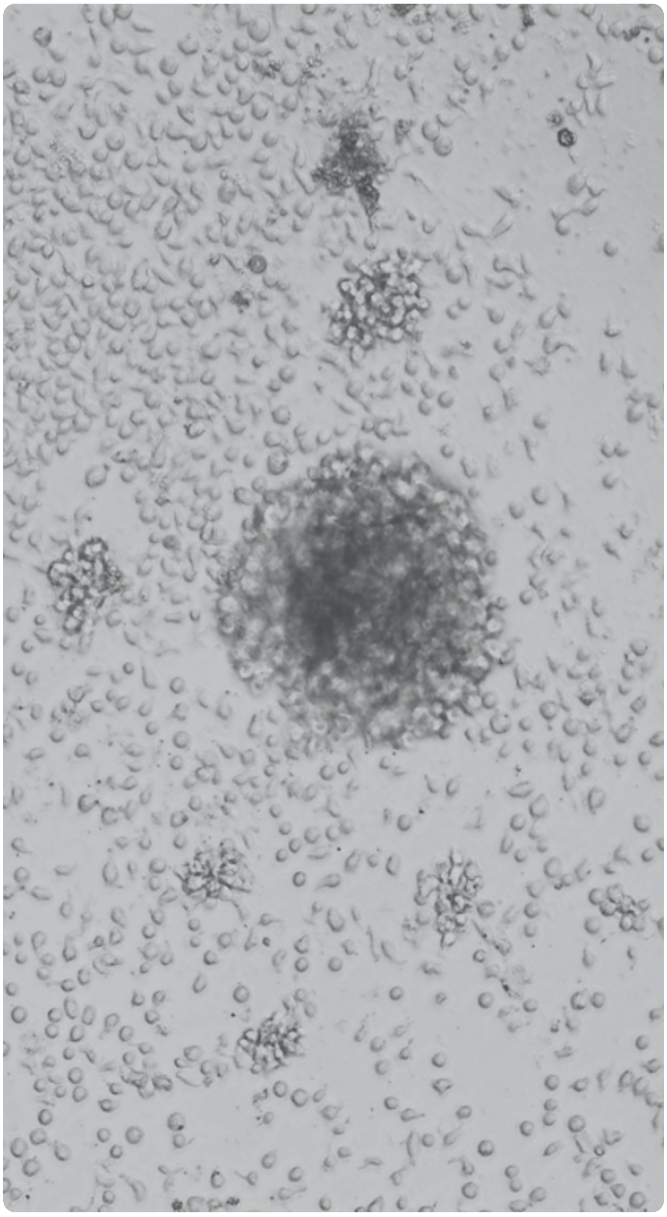


Figure 16.
Cells taken for the recovery assay (Figure 15) were also assessed via flow cytometry for %CD3+ TIL, %CD4+ TIL, %CD8+ TIL, %γδ TCR+ TIL, and %NK cells.

Cells taken for the recovery assay (Figure 15) were also assessed for general T-cell phenotype via flow cytometry for the different concentration conditions, compared between Day 0 and Day 2 (Figure 16). The results show that phenotype is identical across the conditions. **Collectively, these findings demonstrate that the ARES X20+ performs consistently across a wide range of cell concentrations without compromising critical product quality attributes.**





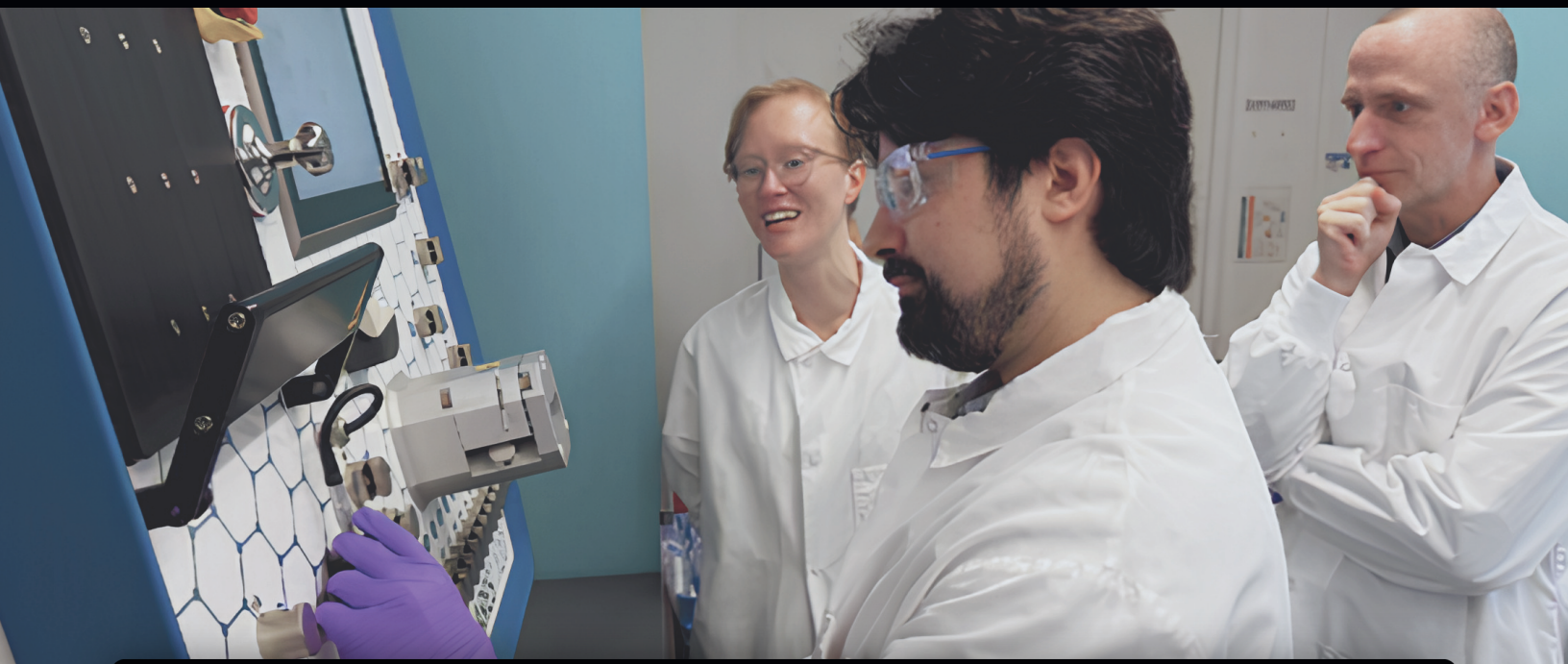
CONCLUSIONS

As TIL therapies, and cell therapies in general, continue to advance toward broader clinical adoption, manufacturing technologies must evolve to support increasing production demands while maintaining product quality and process consistency. In this evaluation, the ARES X20+ automated fill-finish system demonstrated performance comparable to CTMC's standard manual process across multiple critical quality attributes, including fill accuracy and precision, post-thaw recovery, viability, cell concentration, proliferative capacity, and T-cell phenotype. Functional assessment of CTMC's proprietary TIL expansion reagent, iTR-56, further confirmed that automated formulation and dispensing preserved biological activity.

Beyond maintaining product quality, the ARES X20+ delivered meaningful operational improvements. Automated dispensing reduced total processing time, shortened DMSO exposure during fill-finish, decreased labor requirements, and supported reproducible processing across a broad range of formulation volumes and final container formats, from cryovials to large-volume CS750 cryobags. These capabilities address key limitations of conventional manual formulation and fill-finish operations by improving process control while increasing manufacturing throughput and scalability.

The studies also identified an opportunity to further optimize the process. While the automated agitation system achieved homogeneous cryoprotectant formulation across all evaluated volumes, incorporating a brief, standardized manual mixing step following formulation significantly accelerated homogenization at larger volumes, allowing the platform to fully realize its operational advantages without compromising process consistency.

Collectively, these findings demonstrate that the ARES X20+ provides a robust and flexible automated formulation and fill-finish solution for cell therapy manufacturing. By combining precise formulation, reproducible dispensing, scalable throughput, and preservation of product quality, the platform has the potential to reduce manufacturing variability while supporting the efficient production of next-generation TIL and other advanced cell therapies as they move toward broader clinical and commercial implementation.



PARTNER WITH CTMC +

Access fully integrated 360° Solutions that connect cell therapy development, manufacturing, quality, regulatory strategy, and clinical readiness under one roof.

ABOUT CTMC

CTMC is a co-development accelerator formed through a joint venture between Resilience + UT MD Anderson. Our mission is to fast-track the development and manufacturing of innovative cell therapies for cancer patients. Located in the heart of the Texas Medical Center, we combine deep expertise in TIL and CAR-T platforms with 360° Solutions spanning process development, GMP manufacturing, and regulatory support. As a true partner, we work side-by-side with cell therapy innovators to advance programs into the clinic faster and chart a clear course to commercial readiness.

CONNECT WITH US

Interested in learning more about CTMC's capabilities or exploring a potential collaboration?

We'd welcome the opportunity to discuss your program and how we may be able to support your development goals.

Contact us at info@ctmc.com or visit CTMC.com to start the conversation.

Follow us on LinkedIn @ctmcplus for more.

CTMC+ a joint venture between Resilience + UT MD Anderson