Whitepaper

APPLICATION OF LEUKOSTOR™ TO CAR-T CELL THERAPY MANUFACTURING

LEUKOSTOR™

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1. Introduction

This report provides data to support the integration of our technology into the CAR-T manufacturing process. Our technology, known as LeukoStor™, offers an alternative to freeze-thaw by storing gel-encapsulated cells at room temperature (25°C). The manufacturing process of autologous CAR-T cells begins with the collection of non-mobilised peripheral blood mononuclear cells (PBMCs) from a patient-by leukapheresis[1] (Figure 1). This leukapheresed material is cryopreserved within 24h after collection and stored below -120°C. Cryopreserved material is then shipped to the manufacturing facility and stored below –120°C. Upon availability of a manufacturing slot, material is thawed under controlled conditions and washed to remove cryoprotectant. Where clinical sites are geographically close to sites of manufacture, fresh material can be shipped directly to the manufacturing facility without cryopreservation but must be used within a restricted time schedule. At the start of manufacture T cells are enriched, selected, and activated using anti-CD3/CD28 antibody-coated paramagnetic beads, followed by transduction to incorporate CARs. Following transduction, excess vector and other residuals are washed from the culture and the cells are expanded ex vivo until there are sufficient cells to meet the final product dose requirements. For cell harvest, the transduced T cells are isolated by separating them from the beads, washed, formulated in infusible media, transferred into infusion bags, and cryopreserved. The finished product is then transported back to the treatment centre and administered to the patient when required.

This period of processing, referred to as vein-to-vein time (V2V), ranges between three and four weeks for CAR-T cell therapies, including Yescarta (17 days) and Kymriah (22 days)[2]. This period can be daunting for the patients awaiting treatment and renders these CAR-T cells unsuitable for patients with rapidly progressing disease. Moreover, a long and potentially variable manufacturing time dramatically increases Cost of Goods as well as logistical complications.

2. Methods

In order to obtain the freshest material possible for T cell stability studies, leukocyte reduction system cones (LRSCs, or leukocones) were diluted to an appropriate cell density and used as a substitute for leukapheresis (LRSC-leukapheresis). All preservation studies were performed within 4 hours of donor collection. Material from 5 healthy donors was stored with/without LeukoStor™ at room temperature (25°C) before assessing cell recovery and quality at day 5. The no-gel control (NGC) group represented non-manipulated starting material (i.e. without LeukoStor™ preservation). Freeze-thaw samples were preserved in 1 mL cryovials at a 1:1 dilution with CS10 cell freezing medium, while LeukoStor™ and NGC samples were diluted in RPMI medium with 10% FBS and stored at 10 mL volumes. Following cell release at day 5, recovery and viability were examined by trypan blue exclusion. T cell quality at day 5 was assessed by flow cytometry

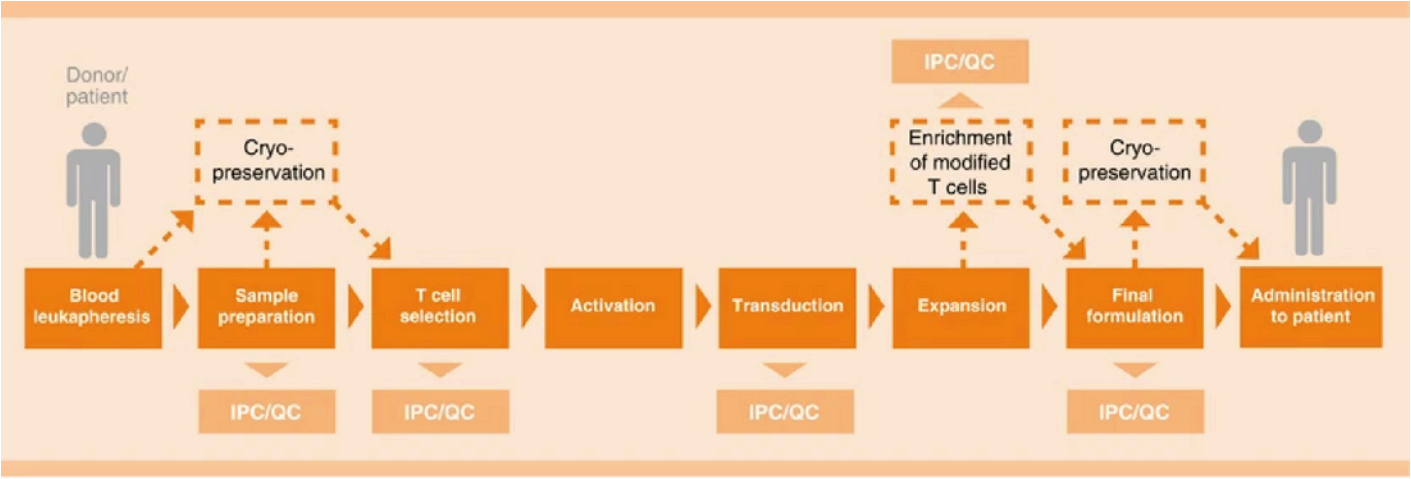

Figure 1. The V2V (Vein-To-Vein) process during CAR-T manufacturing.

staining of CD45+ CD3+ live (unstained), apoptotic (Annexin V+) and apoptotic (7AAD+) cells. Isolated PBMCs were cultured overnight to assess post-rest recovery, viability (CAM live stain, PI dead stain), and metabolic activity (Alamar blue assay). For the assessment of monocyte stability, refrigerated leukapheresis material was used from 5 healthy donors and preserved within 2-24 hours post-collection. Leukapheresis was stored for 5 days at 2-8°C either with no preservation (no-gel control (NGC)), LeukoStor™ or competitor solution, all at 70 mL (quarter-pack) volumes.

3. T cell Preservation using LeukoStor™

3.1 Preservation of LRSC-leukapheresis material

One of the critical drivers of CAR T cell manufacturing delay is the number of usable cells in the patient starting sample when entering the manufacturing process; higher counts and more importantly, higher quality of cells of interest at this stage reduces expansion time to yield the target number for cell therapy. We assessed cell recovery and viability of LRSC-leukapheresis material at day 5 post-storage, followed by post-rest assessment of recovery, viability, and metabolic activity overnight rest under standard culture conditions (day 6). LRSC material, also known as a buffy cone, is the waste product of plateletpheresis, a process by which thrombocytes are separated and collected from drawn blood for later transfusion into patients with platelet-related disorders[3]. The separated platelet fraction still contains leukocytes, which must be removed prior to platelet transfusion to prevent rejection problems. Leukocytes are therefore filtered out by leukoreduction and collected into an LRSC. Thus, the LRSC contains a high density of leukocytes with a low concentration of neutrophils. This differs from leukapheresis, which is specifically designed to remove leukocytes from the participant and return the remaining cells (i.e., granulocytes, platelets, erythrocytes) and plasma back to the participant[4]. The constituents/cell composition of LRSC and leukapheresis material are almost identical making it a suitable substitute.

Specific investigation of the T cell population was performed by flow cytometry. We show that storage using LeukoStor™ at 25°C delivers, on average, a greater viable cell yield (~40%) compared to the no-gel control (NGC) (~30%) (Figure 2A). In addition to a lower viable cell yield, the NGC showed greater variability in yield compared to LeukoStor™, suggesting that storage using our technology may improve predictability in yield. Cells stored using freeze-thaw have an average of 55% viable cell recovery, though this recovery rate is hugely variable (~60% variability), compared to that of LeukoStor™ 25°C, which has a much more consistent (~30%) variability, and therefore predictable recovery rate. The variability displayed in the freeze-thaw cell yield renders it difficult to determine the expansion time required to achieve the target number of cells for a successful therapeutic dose. Moreover, LeukoStor™ preserved cells are of a higher 'fitness and quality', showing greater viability (~70%) than control cells (~42%) (Figure 2B).

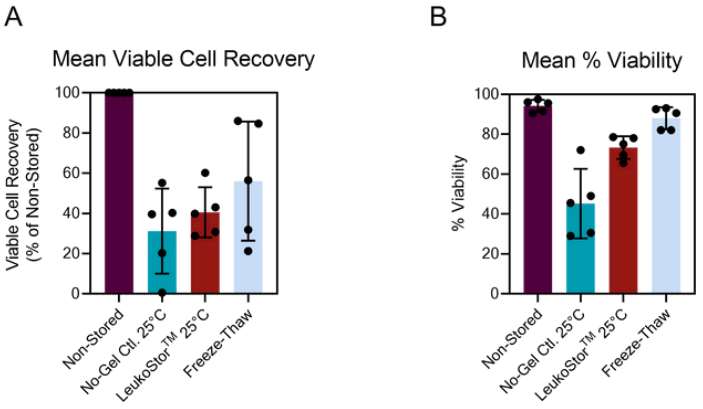

Figure 2. LeukoStor™ improves predictability of cell yield following 5 days of storage at 25°C. Cell recovery and viability of leukocytes using different preservation methods was evaluated at day 5 following storage. A. LeukoStor™ 25°C improved cell recovery compared to NGC. Cell yield was highest in the freeze-thaw condition however, LeukoStor™ 25°C showed greater consistency in recovery rate. B. LeukoStor™ 25°C improved viability over the control. Viability was assessed by trypan blue exclusion. (n=5)

3.2 Preservation of T cell starting material

Evaluation of the T cell population by Flow Cytometry showed that preservation of these cells from LRSC-leukapheresis material using LeukoStor™, 25°C improved 'starting' yield (~48%) compared to the NGC (~35%) (Figure 3A). Notably, the viable T-cell population was vastly improved in LeukoStor™, 25°C by approximately 2-fold compared to NGC control (~90%, ~40% respectively) (Figure 3B, C). LeukoStor™ also shows reduced variation in T-cell viability compared to NGC, suggesting this technology can improve predictability in T cell viability. Multiple other storage temperatures (5°C, 20°C, 30°C, data not shown) were also examined, however it was found that 25°C was optimal for T cell preservation. Establishing these optimum conditions for T cell stabilisation and preservation is critical, multiple approved T cell therapies, >100 registered T cell therapy developers, and intensive research activities all depend on a high quality of T cells from starting leukapheresis starting material. The high competency of T cells preserved using LeukoStor™, 25°C, shown in this study, encourages integration of this technology into the V2V process.

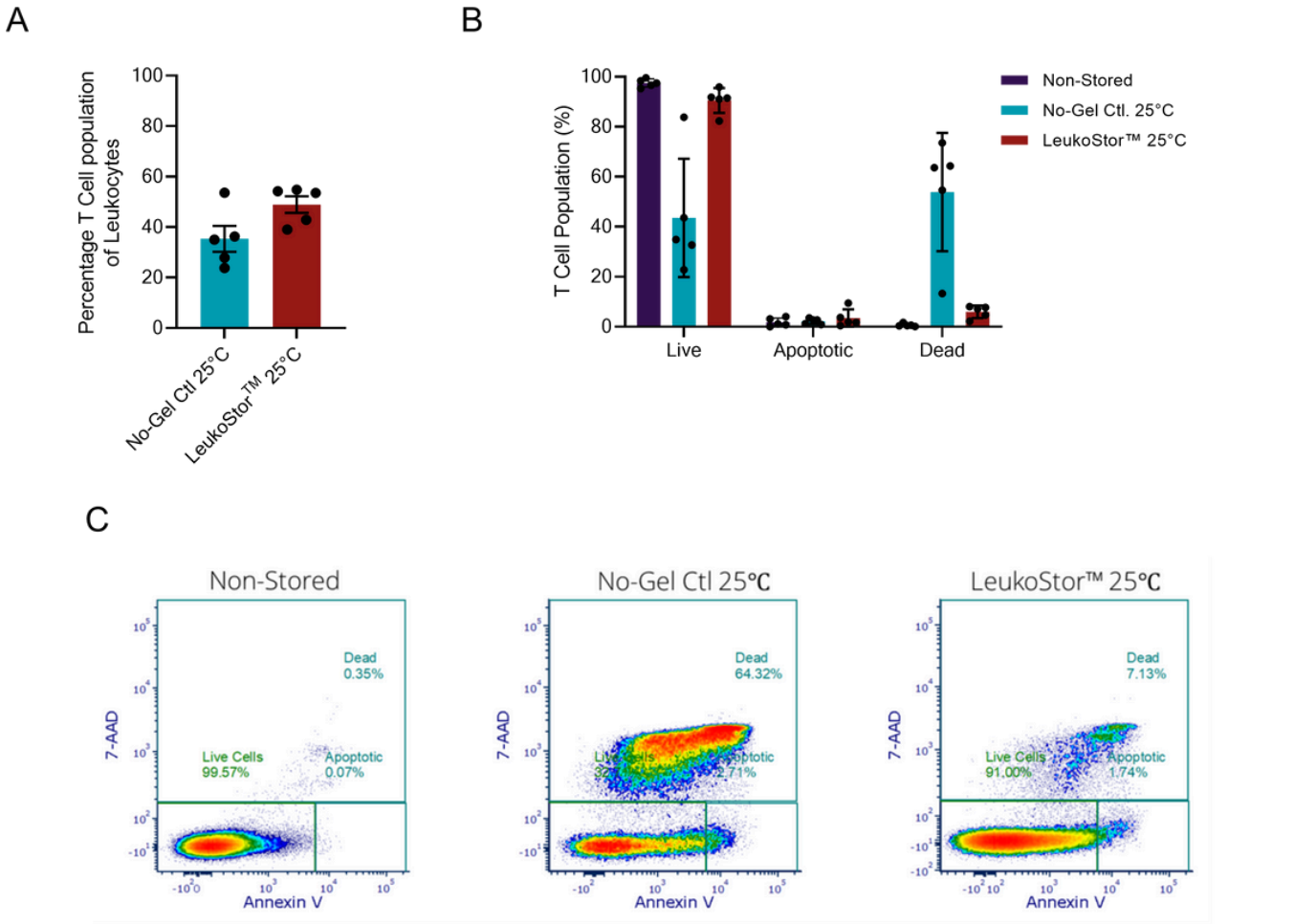

Figure 3. LeukoStor™ preserves T-cell yield and viability after 5 days of storage at 25°C. LRSC-leukapheresis material was stored with or without LeukoStor™ before assessing quality by flow cytometry. A. Total T-cell yield (CD3+ cells as a % of total single CD45+ cells) at day 5 following storage was improved by LeukoStor™ compared to the no-gel control (NGC) which represented non-manipulated LRSC-leukapheresis material. B. The quality of T cells severely deteriorated when left unprotected over 5 days at 25°C. Aside from one donor, live cells corresponded to 20-40% of the T cell population. In starting material encapsulated in LeukoStor™, live T cells corresponded to 80- 90% of the population with very few apoptotic or dead cells. C. Flow Cytometry of T cells from leukocone material. T cells were defined as single, CD45+ (CD45-BUV395), CD3+ (CD3-BV421) cells. % live (unstained), apoptotic (AnV+) and dead (7AAD+) cells in the T cell population were assessed. (n=5)

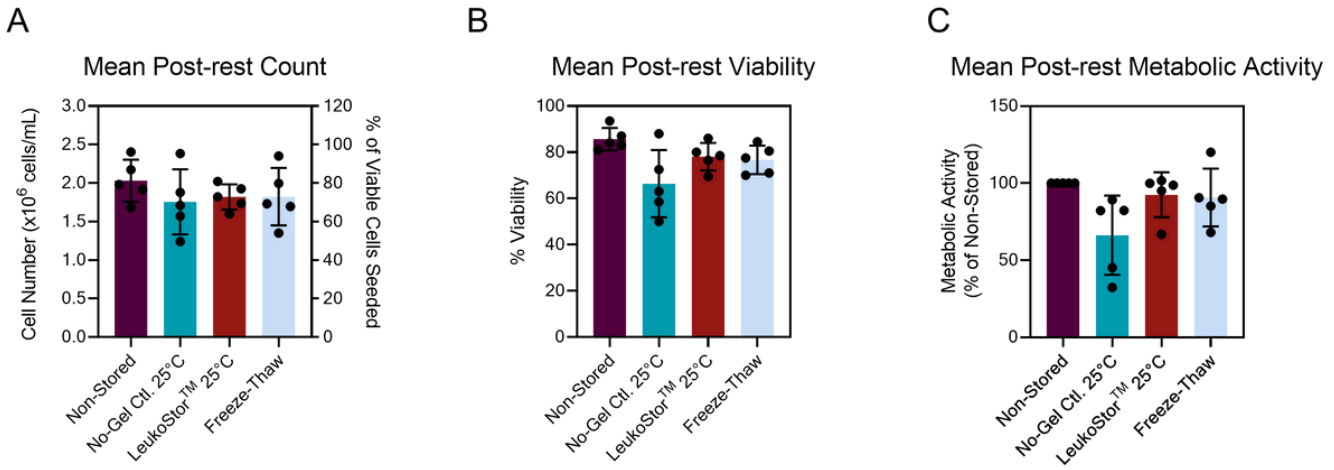

Figure 4. LeukoStor™ preserves cell viability and competency under culture conditions. A. Preservation of PBMCs in LRSC-leukapheresis using LeukoStor™ 25°C improves predictability of post-rest cell yield compared to freeze-thaw. B, C. Viability and metabolic activity are enhanced by LeukoStor™ encapsulation compared to the no-gel control (NGC) and are uncompromised compared to freeze-thaw. Viability was assessed by trypan blue exclusion while metabolic activity was assessed using Alamar Blue. (n=5)

3.3 Preservation of LRSC-leukapheresis material post- rest

When placed in culture conditions overnight, we show that leukocone-derived cells preserved using LeukoStor™ 25°C have high viability and metabolic activity compared to the NGC (Figure 4B, C). Compared to freeze-thaw, LeukoStor™ 25°C preserved cells are uncompromised and show greater predictability in yield post-rest as observed at day 5 (Figure 4A). Due to the freeze-thaw process, cells may be weaker and therefore take longer to adapt to the culturing environment and get into exponential growth. The improved ability of cells to grow in culture using our technology may offer the possibility to reduce manufacturing delay.

4. Preservation of Leukapheresis Monocytes

Evaluation of monocytes from leukapheresis material stored for 5 days' by Flow Cytometry showed that preservation using LeukoStor™ 2-8°C maintained 80-85% viability (Figure 5). Monocyte yield was dramatically improved by preservation in LeukoStor™ compared to Competitor Solution and NGC.

This data has important implications for clinical use, clinical trials, and research that relies on the use of fresh, non-cryopreserved monocytes and PBMCs. Importantly, LeukoStor™ offers a solution to avoid the biological disadvantages of cryopreservation and freeze-thaw.

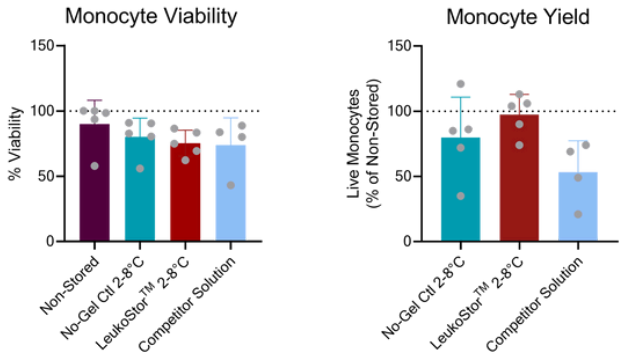

Figure 5. LeukoStor™ preserves Monocyte yield and viability after 5 days of storage at 2-8°C. Apheresis material was stored with or without Leu- koStor™ before assessing quality by flow cytometry. Total Monocyte yield (CD11b+ CD14+ cells as a % of Day 0 non-stored) at day 5 following storage was improved by LeukoStor™ compared to the no-gel control (NGC) which represented non-manipulated apheresis material, and Competitor Solution. Live monocytes corresponded 80-85% of total monocytes following 5 days' storage in LeukoStor™. Monocytes were defined as single, CD45+ CD11b+ CD14+ cells, and as live (unstained), apoptotic (AnV+) or dead (7AAD+) (n=5).

5. Conclusions – Leukapheresis preservation for CAR-T Manufacturing

One of the biggest challenges faced by CAR-T cell manufacturers is the variability among apheresis product starting material, which can negatively impact T cell manufacturing success and therefore patient treatment. A solution is required to minimise this variability such that the CAR-T manufacturing process can be standardised in accordance with Good Manufacturing Practices (GMP). LeukoStor™ 25°C enhances predictability of starting T-cell yield compared to cryopreservation and NGC, and dramatically improves viability of T cells after 5 days of storage by 2-fold compared to unprotected material (NGC). This data addresses one of the critical drivers of CAR-T manufacturing delay by demonstrating how LeukoStor™ 25°C T cell preservation can provide consistent, high-quality starting material, offering a step towards standardisation. For CAR-T developers, this reduced variability in T cell starting material will translate to a more consistent manufacturing time and in turn, reduced manufacturing cost.

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