Cryo-processed Leukapheresis Using Automated Closed System: A High-Quality Starting Material for Autologous and Allogeneic CAR-T Cell Therapy Manufacturing

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The IntegriCell™ platform provides Automated closed cryo-process for fresh leukapheresis

Introduction

Adoptive cell therapy with chimeric antigen receptor (CAR) T cells has proven to be a highly effective treatment. Due to logistical challenges, selecting the appropriate starting material for apheresis—whether fresh or frozen—is crucial in cell therapy. Understanding these differences is vital for optimizing the manufacturing process and ensuring the highest quality and efficacy of CAR-T cell products. In this study, we demonstrate that using optimized cryopreserved starting material yields highly proliferative and potent autologous and allogeneic αCD19 CAR-T .

Figure 1. IntegriCell™ platform IntegriCell™ aims to deliver consistent, compliant, high-quality leukapheresis starting material for use in the manufacture of cell-based therapies leading to risk and cost reduction through optimized manufacturing capacity planning. Cryoport Systems, through the IntegriCell™ solution, is building a network of cryopreservation centers across the US and EU to ensure proximity to patients, and to optimize and standardize cryopreservation of leukapheresis within 24h post-collection together within an integrated storage and distribution network.

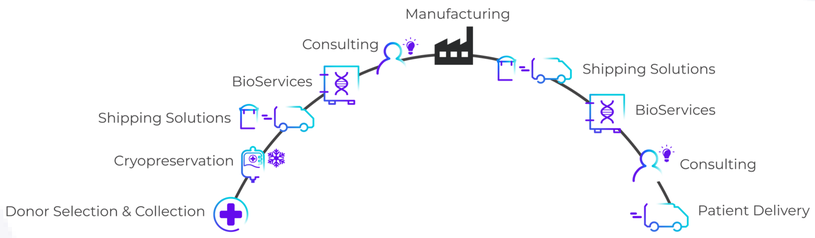
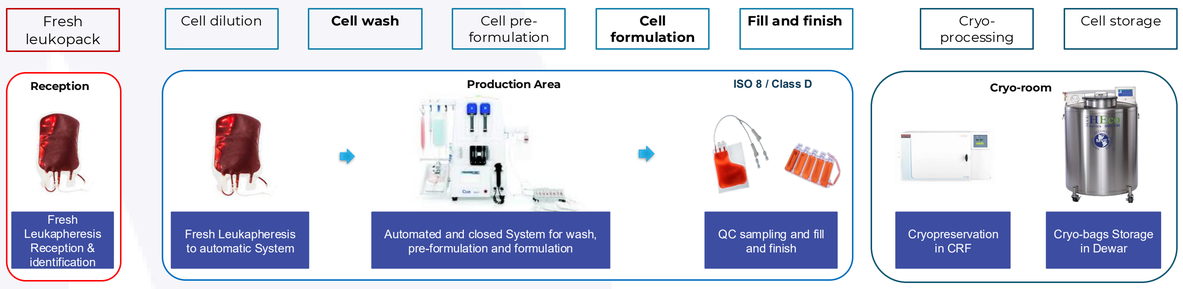


Figure 2. Automated closed Cryopreservation Process (ACP). The Automated closed Cryopreservation Process (ACP) is designed as an automated, closed system. Importantly, this standardized, automated approach ensures consistency, efficiency, and compliance with GMP requirements, enhancing the reliability of leukapheresis-based starting materials for cell and gene therapy applications. The ACP follows a structured workflow consisting of five main steps: leukapheresis preparation, cell washing, pre-formulation, formulation, and cryo-processing. This ensures that the cells maintain their viability and functional integrity when stored and later thawed for therapeutic manufacturing.



Automated closed cryo-process for fresh leukapheresis

Figure 3. Study design. The influence of Cryoport's IntegriCell® ACP cryo-processing technology on CAR-T cell starting material and subsequent manufacturing was evaluated by comparing fresh and cryopreserved leukapheresis samples. Leukapheresis products were collected from four distinct donors and transported to Cryoport cryo-processing facilities, where each sample was divided into two aliquots. One aliquot was stored at 4°C for up to 48 hours post-collection prior to initiation of CAR-T cell manufacturing (designated as Fresh Leukopack Storage, 4°C). The second aliquot underwent cryopreservation via the IntegriCell® ACP platform within 24 hours of collection (designated as Leukopack ACP Cryo-Processing). Both fresh and cryopreserved leukopacks were subsequently transported to the CAR-T cell manufacturing facility for T cell activation.

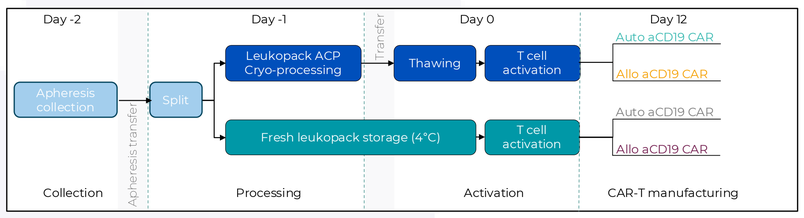


Figure 4. IntegriCell™ Standardized, Automated Closed cryo-Process (ACP) for fresh leukapheresis maintains high cell recovery, viability and lymphocytes population ratio upon processing. ACP maintains high cell viability (a.), viable nucleated cell recovery (b.), T, B, NK, NKT cells reparation (c.) and CD4+ and CD8+ T cell percentage (d.) from fresh leukopack to post-wash, post-formulation and post-thaw steps (n=4). Mean +/- SD is represented.

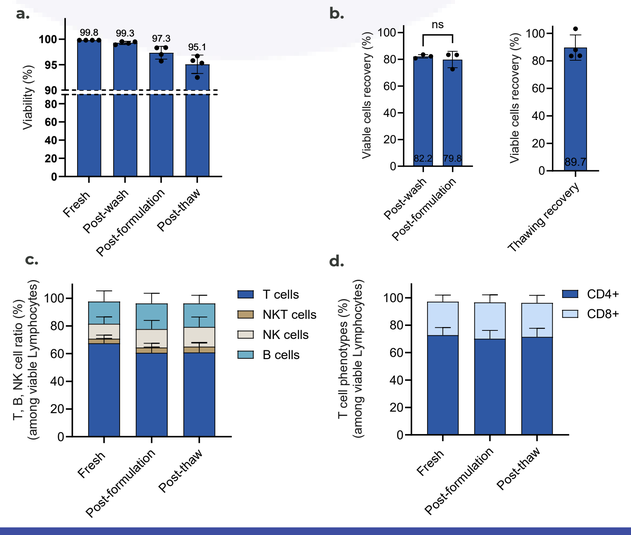
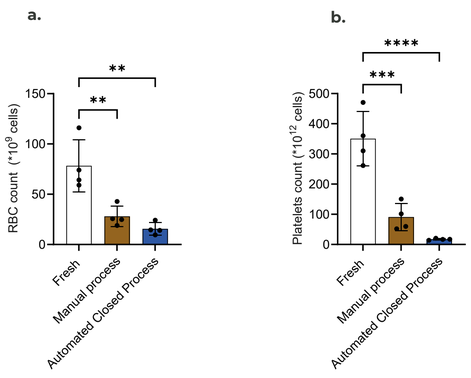


Figure 5. IntegriCell™ Standardized, Automated Closed cryo-Process (ACP) for fresh leukapheresis ensure platelet and RBC wash. Leukopack Red Blood cell (RBC) count (a.), and platelet count (b.) from 4 different healthy donors represented for fresh material (white), post centrifugation upon manual process (brown) and post spinning membrane filtration upon ACP (Dark blue). Interestingly, ACP's spinning membrane filtration improves contaminant depletion over manual centrifugation, achieving superior Red Blood Cell (RBC) and platelet clearance. Mean +/- SD is represented. ** p < 0.01; *** p < 0.001; **** p < 0.0001 upon 2way ANOVA against control (Fresh).



CAR T-cell manufacturing using ACP cryo-preserved leukapheresis as starting material

Figure 6. Overview of the CAR-T cell manufacturing process.
CAR-T cell were produced from matched ACP cryo-processed and fresh starting material from four healthy donors.
A standard 12-day process (Rossi et al., Mol Ther Nucleic Acids 2023) was used.
A direct comparison between ACP material- and fresh material-derived CAR-T cells was performed for both autologous and allogeneic CAR-T cells.

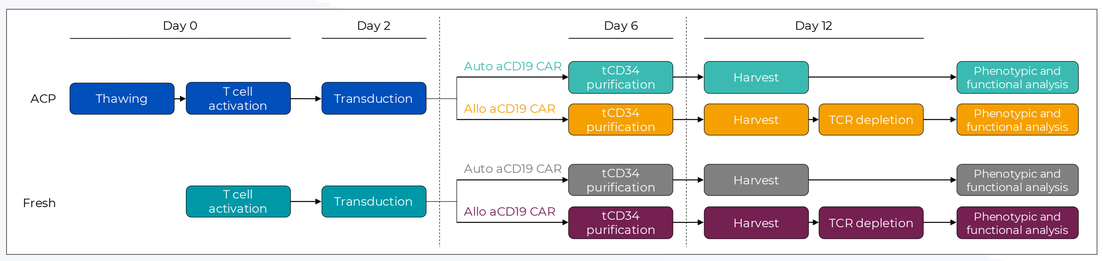
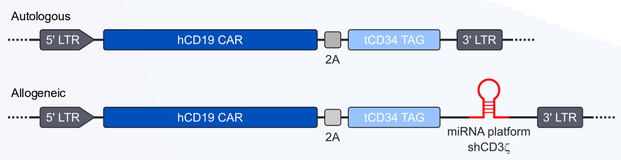


Figure 7. Scheme of the viral vectors used to produce autologous and allogeneic CAR-T cells.
Allogeneic CAR-T cells were generated by knocking down the expression of the T cell receptor, through a miRNA-based cassette carrying an shRNA targeting CD3ζ (Rossi et al., Mol Ther Nucleic Acids 2023) using Celyad Oncology's multimiR platform.



Phenotypic and functional characterization of CAR-T cell manufactured using ACP cryo-preserved leukapheresis

Figure 8. Cryopreservation of the starting material does not alter the culture parameters and the CAR-T cell phenotype.
Cell culture parameters were monitored throughout the process, and the final products were assayed for phenotype and functionality. No statistically significant differences (P>0.05) in viability (**a**), cell expansion (**b**), CD4+/CD8+ ratio (**c**), transduction efficiency (**d**) and TCR knock-down efficiency (**e**) (in the case of allogeneic CAR-T cells) were observed between CAR-T cells generated from ACP cryo-preserved and fresh starting material.
N=4 donors. Means ± SD are depicted, 2-way ANOVA with Šidák correction for multiple comparisons.

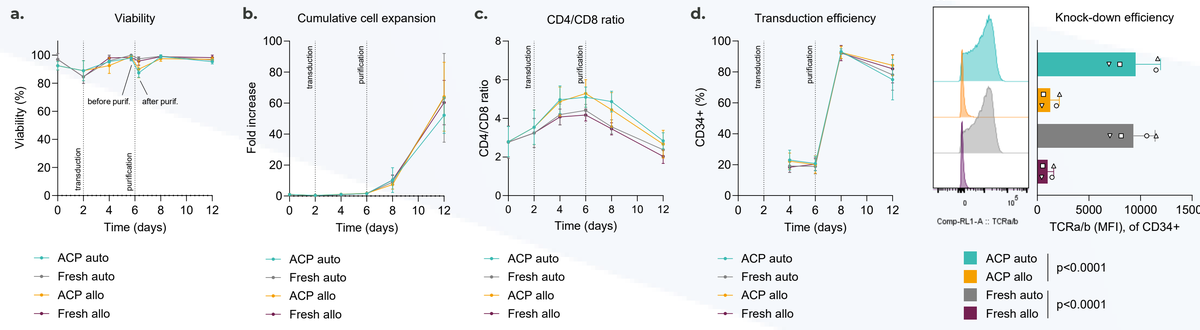


Figure 9. Cryopreservation of the starting material does not alter the expression of activation and exhaustion markers throughout the CAR-T production process.
Activation and exhaustion markers were monitored throughout the process.
Bona fide activation markers CD25 (**a**) and CD69 (**b**) and exhaustion markers PD-1 (**c**), LAG-3 (**d**) TIM-3 (**e**) and TIGIT (**f**) displayed the same expression pattern throughout the process for all conditions.
No statistically significant differences (P>0.05) were observed between CAR-T cells generated from ACP cryo-preserved and fresh leukapheresis. The biggest determinant of variability in the markers' expression was indeed the donor, rather than the processing, of the starting material.
N=4 donors. Means ± SD are depicted, 2-way ANOVA with Šidák correction for multiple comparisons.

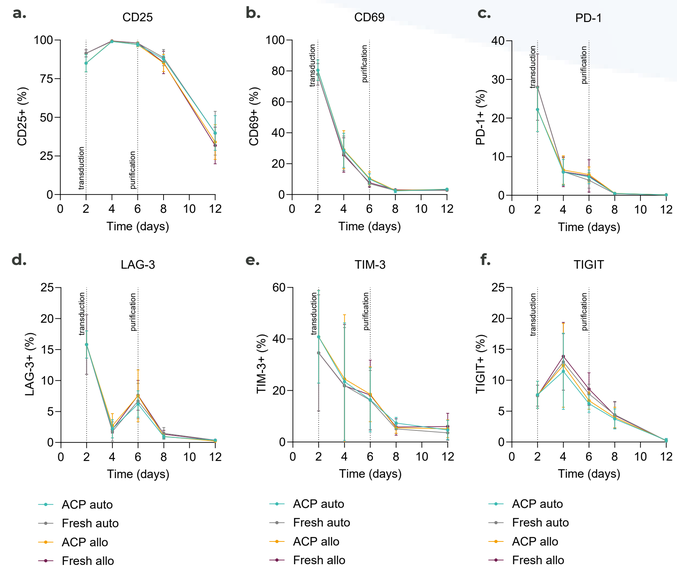


Figure 10. Cryopreservation of the starting material has a marginal impact on the memory phenotype.
The evolution of the CAR-T cell phenotype was monitored throughout the process. The percentage of naive/stem cell-like memory (N/SCM, **a**), central memory (CM, **b**), effector memory (EM, **c**) and effector (E, **d**) cells were determined by FACS analysis based on *bona fide* surface markers CD45RA and CD27.
No statistically significant differences (P>0.05) in the distribution through the various populations were observed between CAR-T cells generated from ACP cryo-preserved and fresh starting material, with the only exception of a small increase in the relative number of effector (E) cells in the CAR-T cells from ACP cryo-preserved material (**d**).
N=4 donors. Means ± SD are depicted, 2-way ANOVA with Šidák correction for multiple comparisons.

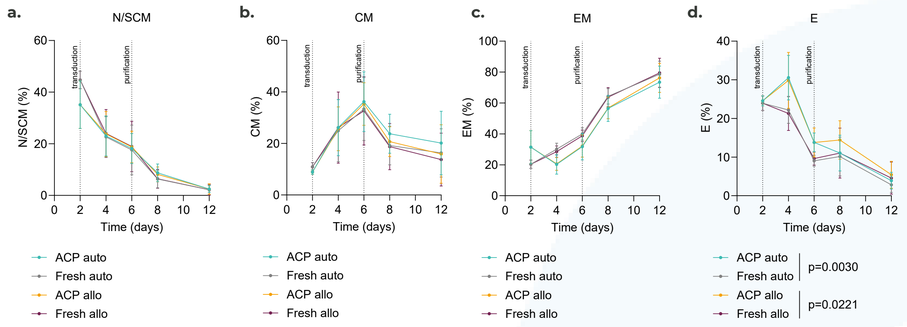
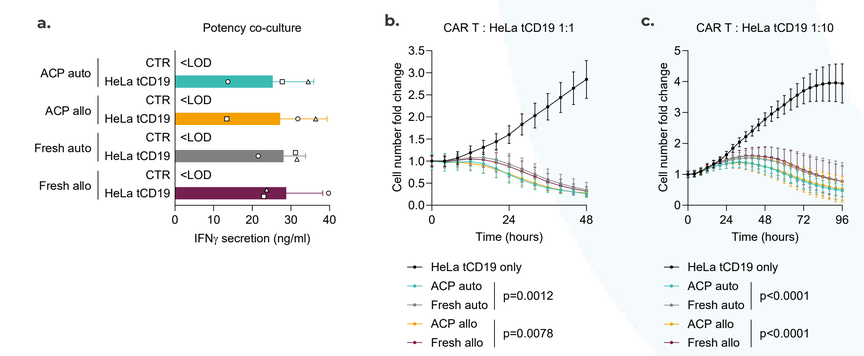


Figure 11. CAR-T cells derived from ACP cryo-preserved material are comparable to CAR-T cells derived from fresh material for cytokine secretion upon stimulation, and outperform them for killing activity.
Co-culture with HeLa cancer cells overexpressing the CAR-T cell target CD19 stimulated the secretion of interferon gamma (IFNγ) by the CAR-T cells (**a**). IFNγ secretion was monitored by ELISA. No significant differences (P>0.05) in IFNγ secretion were observed within the same treatment groups between CAR-T cells derived from ACP cryo-preserved and fresh material.
The killing activity against HeLa overexpressing CD19 was significantly higher for CAR-T cells derived from ACP cryo-preserved material than for CAR-T cells derived from fresh material, at both 1:1 (**b**) and 1:10 (**c**) effector-to-target ratio.
N=3 donors. Means ± SD are depicted, 2-way ANOVA with Šidák correction for multiple comparisons.



Conclusion and discussion

This study demonstrates that cryo-processing of fresh leukapheresis using an automated closed process (ACP) yields high-quality starting material suitable for both autologous and allogeneic CAR-T cell therapy manufacturing. The automated approach preserves cell viability and phenotype post-thawing, ensuring minimal impact on T cell subsets, activation potential, exhaustion and memory phenotypes upon CAR-T manufacturing. In addition, CAR-T cells manufactured from cryo-preserved apheresis material showed comparable transduction efficiency, expansion, and cytotoxic function to those from fresh material. Automated cryo-processing reduced operator-dependent variability and improved process reproducibility while enabling greater manufacturing flexibility and scheduling, critical for global CAR-T therapy deployment. The results presented in this study validate cryo-processed leukapheresis as a robust and reliable option for advanced cell therapies. The combination of automation and cryopreservation provides a consistent, GMP-compliant workflow. Overall, this platform enhances the feasibility and efficiency of both autologous and allogeneic CAR-T manufacturing pipelines.

Contact

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