Cold Storage of Fresh Leukopak Maintains Cell Viability and Functionality

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Abstract

Fresh leukapheresis product (LP) is a critical raw material in providing primary cells for applications in research and manufacturing. While challenging, the preservation of the integrity of the fresh material is key to successful downstream workflows but puts constraints on short-term storage and shipping durations to avoid loss of cell viability and functionality.

Multiple factors can influence the stability of the fresh product, including temperature. Whole LP products are stored and transported at controlled room temperature (CRT), however, studies have shown that when transported under these conditions these products degrade rapidly within 72 hours.

In this study, the stability of fresh LPs from unique donors was compared at 2 to 8°C and CRT setpoints over 5 days to represent worst case international shipping durations. Samples were assessed daily for cell viability, functionality and activation potency.

Results showed that LP Storage at 2 to 8°C maintains LP quality across the assessment panel after Day 2 relative to CRT storage, making shipping at 2 to 8°C a preferred condition for extending LP quality post collection. Refrigerated shipment and storage of these materials provides the added benefit of wider accessibility of fresh whole LP products for research use and clinical processing.

Methods

- Fresh LP was collected from 6 healthy donors (Fig 1, Step 1)
- Three LPs were stored at CRT or at 2 to 8°C and sampled each day for a period of 5 days (Fig 1, Steps 2 and 3)
- LP samples were characterized daily as per experimental outline in Fig 1, Step 4 and listed below:
  - Cell count and viability (AO/PI)
  - Cell metabolic status by CellTiter-Glo® Luminescent Cell Viability Assay
  - Cell distribution by immunophenotyping per gating strategy in Fig 2
- At each sampling day, CD3+ T cells were isolated by immunomagnetic isolation and enumerated. Isolated cells were stimulated for 7 days with beads coated with CD3/CD28 (Dynabeads™ Human T-Activator) and compared to cells not undergoing stimulation as per Fig 1, Step 5
- Isolated T cells were characterized daily as per Fig 2, Step 6 and listed below:
  - T cell count and viability (AO/PI)
  - T cell distribution (T cell subpopulations of Helper and Cytotoxic T cells) by immunophenotyping
  - T cell metabolic status by CellTiter-Glo® Luminescent Cell Viability Assay
  - T cell cytokine release (IL2 and IFNγ) by ELISA
  - T cell activation marker expression (CD25 and CD154) was quantified by flow cytometry

Results

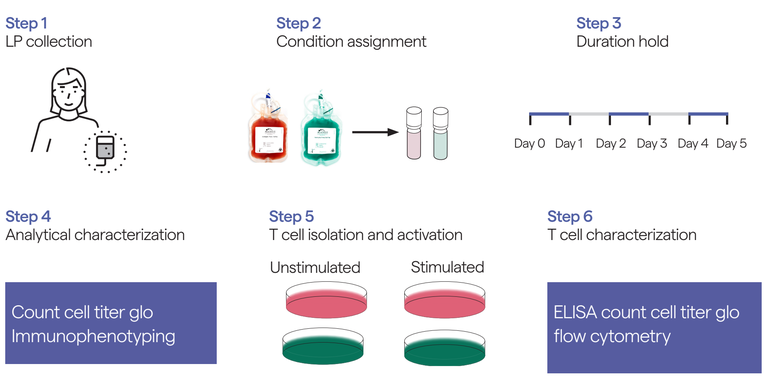

Figure 1.
Experimental outline for each leukopak product LP that was collected

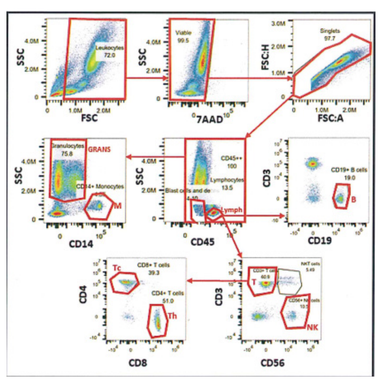

Figure 2. Gating strategy for the study.
Starting at the top left panel, acquired events were first gated for total leukocytes based on FSC and SSC that excluded debris and other small particles. Leukocytes were then sequentially gated for live cells (Viable), singlet cells (Singlets), and CD45+ expression twice (CD45++). For figure clarity, the first Cd45+ gate has been omitted. The resulting population was then gated for specific lymphocyte and granulocyte markers as shown. Lymph, Lymphocytes. Grans, granulocytes. M, CD14+ monocytes. B, CD19+ B cells. T, CD3+ pan T cells. NK, CD56+ Natural Killer cells. Tc, CD8+ cytotoxic T cells. Th, CD4+ helper T cells.

Results

Effect of hold time and temperature on cell viability and cell metabolism

LPs held at 2 to 8°C demonstrated a maintained cell viability (Fig 3A) and metabolic activity (Fig 3B) throughout the assessment period, while the CRT counterparts displayed a sharp decline in both viability and metabolic activity at Day 2. Total cell counts (not shown) remained the same for both CRT and 2 to 8°C throughout the assessment period.

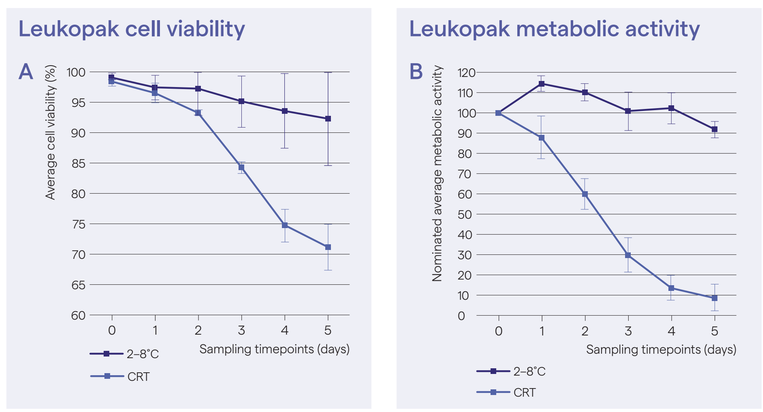

Figure 3.
Effect of hold time and temperature (CRT vs 2 to 8°C) on cell viability in fresh leukapheresis material assessed with A) AO/PI and B) CellTiter Glo.

Effect of hold time and temperature T cell viability

We observed that CD3+ Pan T cell counts in the starting material were not affected throughout the assessment period in both hold temperatures, CRT and 2 to 8°C as shown in Fig 4A. CD4+ T Helper cells and CD8+ Cytotoxic T cells viable cell counts were also not impacted throughout the assessment period and different hold temperatures (not shown). Additionally, the cell count of B cells and NK cells were not significantly affected (not shown). Interestingly, monocyte cells demonstrated a sharp decrease in cell count in CRT LPs by
Day 2 (Fig 4B).

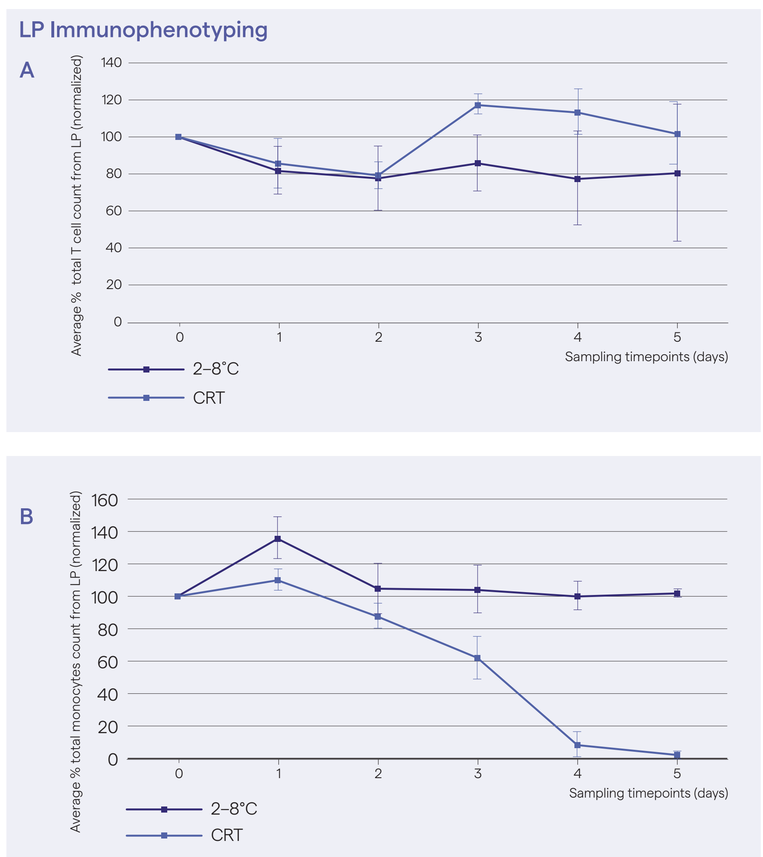

Figure 4.
Effect of hold time and temperature (CRT vs 2 to 8°C) on total viable cell count of (A) Pan T cells (B) Monocytes. Data is represented as the average percent cells in the LP and has been normalized to Day 0.

Effect of hold time and temperature on T cell function as assessed by T cell activation

Isolated T cells stimulated with CD3/CD28 Dynabeads™ demonstrated significant proliferation compared to their unstimulated counterparts. T cells isolated from LPs held at 2 to 8°C showed a proliferative response throughout the 5 day hold period, whereas T cells isolated from CRT LPs exhibited a decrease in proliferative response by Day 2 (Fig 5A). These results were identical to the observations for cell metabolism (Fig 5B), expression of activation markers, CD25 (IL2R) (Fig 5C) and CD154 (CD40L) (not shown) as well as IL2 and IFNγ cytokine release (not shown here).

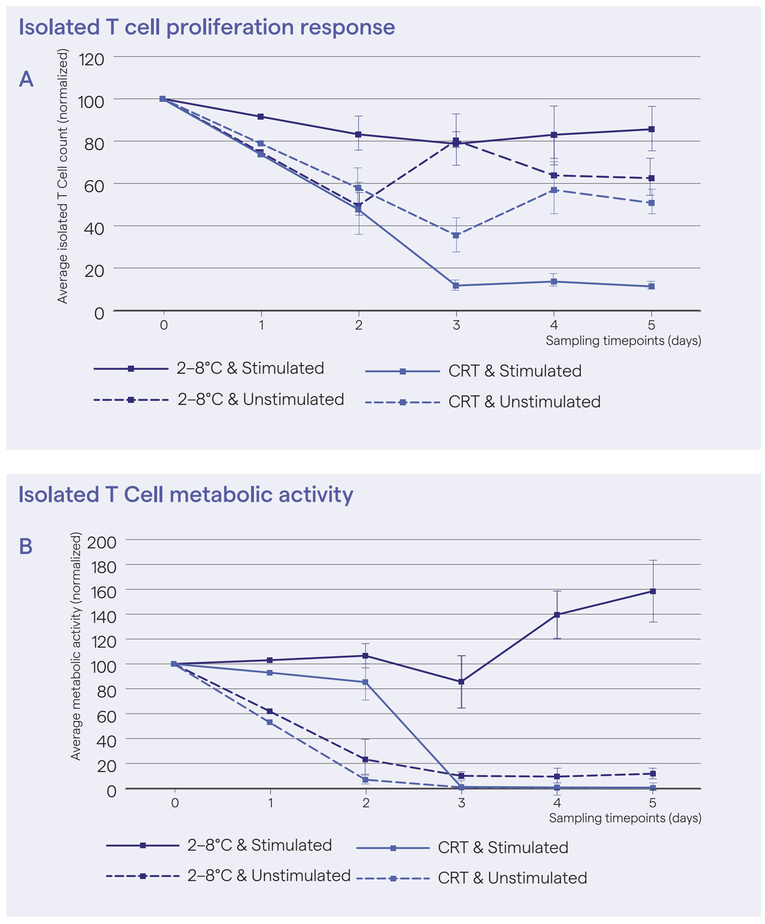

Figure 5
Effect of hold time and temperature (CRT vs 2 to 8°C) on functional T cell activation (A) proliferative response measured by T cell counts (B) cell metabolism (C) IL2 cytokine release

Conclusion

Shipping of fresh Leukopak Product (LP) at 2 to 8°C maintained LP stability throughout the 5 day assessment period and was superior to LPs maintained at controlled room temperature (CRT), of which the stability quickly degraded at Day 2.

- Overall cell viability and metabolism is maintained in LP for up to 5 days when held at 2 to 8°C compared to CRT.
- Isolated T cell functionality as measured by T cell activation, proliferation and cytokine release is maintained during the assessment period in LPs held at 2 to 8°C but drops dramatically by day 2 when held at CRT.
- NK and B cells viability was unaffected when LPs were held at 2 to 8°C, while monocytes demonstrated a sharp decline.

This study supports previous findings [1] and indicate that the stability extension from 2 to 8°C shipment extends geographic feasibility for fresh LPs for cell isolation and other primary cell applications.

References

Autologous cryopreserved leukapheresis cellular material for chimeric antigen receptor–T cell manufacture. Tyagarajan, Seshu *et al.* Cytotherapy, Volume 21, Issue 12, 1198 – 1205

Managing starting material stability to maximize manufacturing flexibility and downstream efficiency. Dominic Clarke & David Smith, Cell & Gene Therapy Insights 2019; 5(2), 303–313

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