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Sustained Cell Quality in Cryopreserved Leukopaks: Viability, Phenotype, and Expansion Potential

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BACKGROUND

- Cryopreservation of leukopaks (**Fig. 1**) is essential for flexible manufacturing.
- Its impact on cell quality remains critical for cell-based therapies[1-3].
- Current characterization of frozen leukopaks primarily relies on cell viability and recovery.

FIGURE 1. Leukopak.

AIM

- Investigate the impact of cryopreservation on PBMC composition and T cell characteristics.

METHODOLOGY

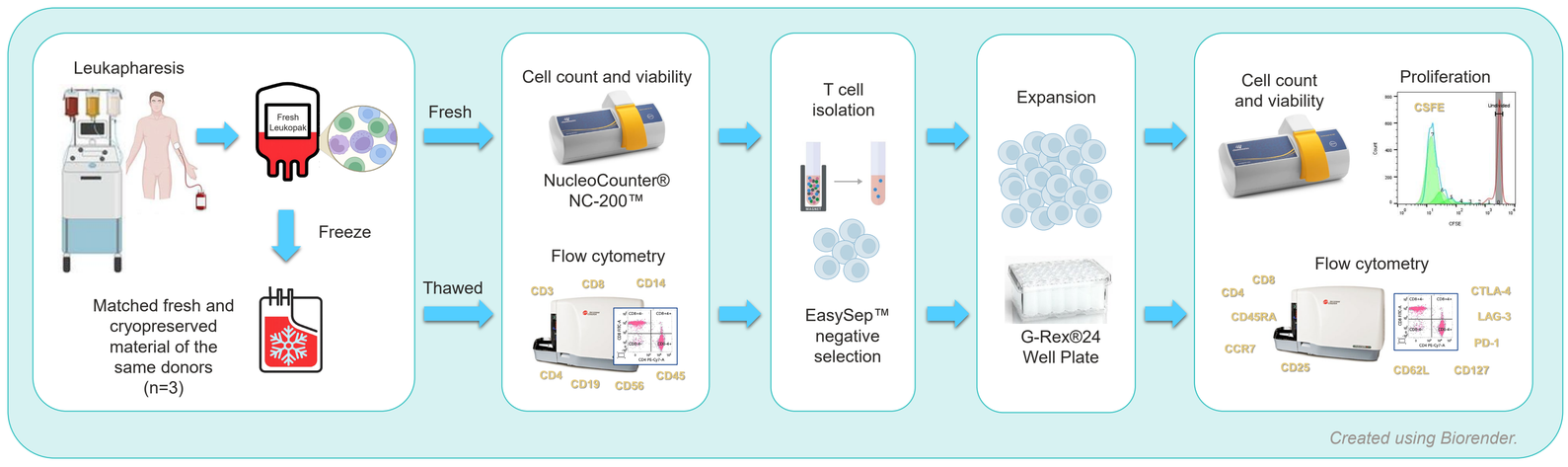

FIGURE 2. Experimental workflow.

RESULTS

The overall PBMC composition was comparable between fresh and cryopreserved material (**Fig. 3A**). While T cell isolation yielded comparable purity across conditions (**Fig. 3B**), recovery was reduced in frozen samples, likely due to diminished CD3 expression following thawing.

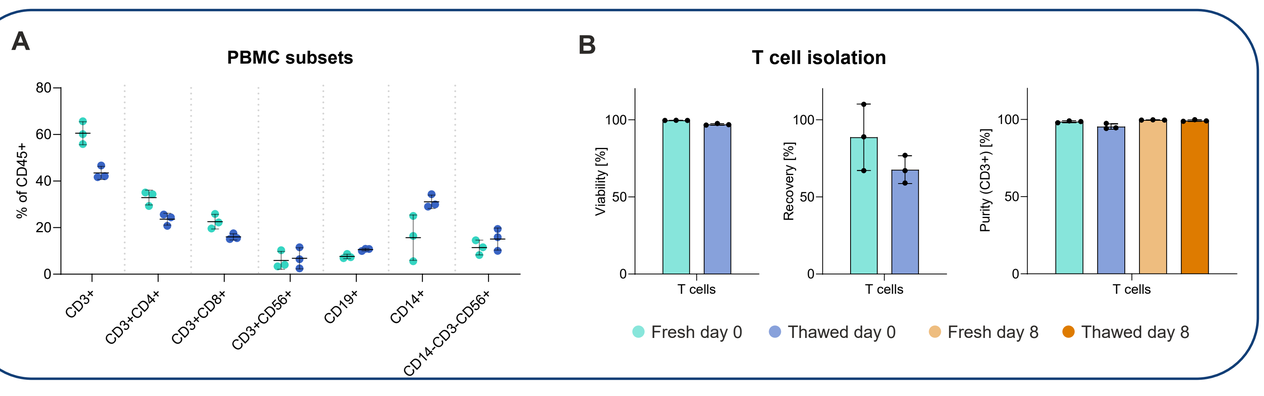

FIGURE 3. PBMC phenotype and T cell isolation in fresh vs. thawed leukopak material. (A) PBMC surface markers of leukopak material. (B) Viability, recovery and purity after T cell isolation.

Post-thaw viability of T cells remained high after thaw, cell isolation and cultivation (>94%) (**Fig. 4A**). Cryopreservation did not affect viability (**Fig. 4A**), CD4/CD8 ratios (**Fig. 4B**), expansion (**Fig. 4C**) or proliferation (**Fig. 4D**) after culture for up to 8 days.

Activation and exhaustion markers remained low and were not induced by freeze-thaw (**Fig. 5**).

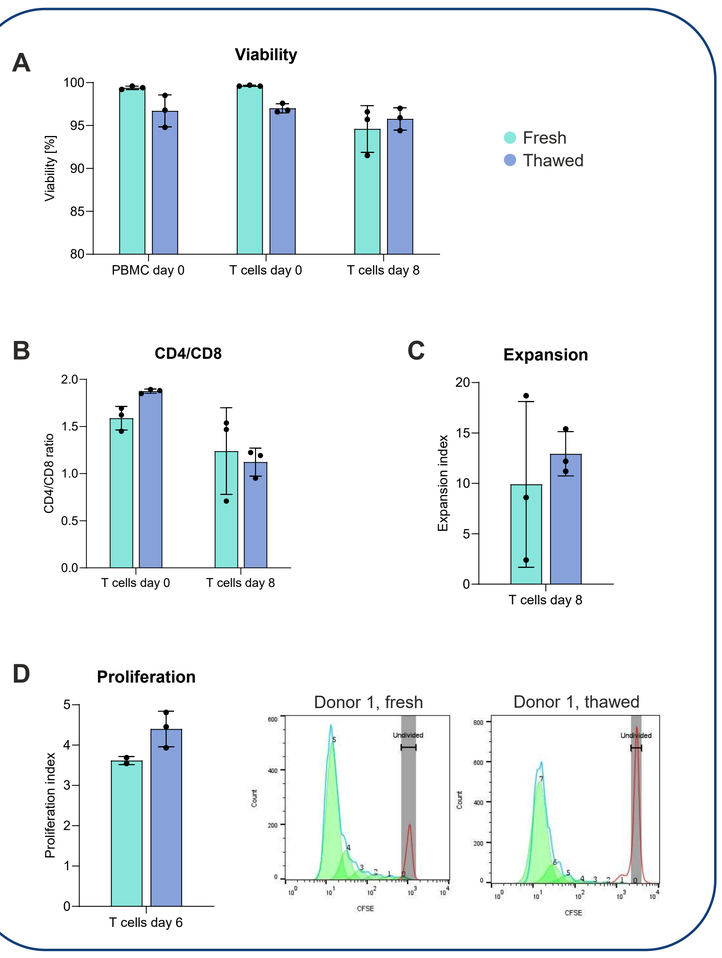

FIGURE 4. Expansion and proliferation of T cells isolated from fresh vs. thawed leukopak material. (A) Viability of PBMCs and T cells. (B) CD4/CD8 ratio. (C) Expansion index determined by cell count. (D) Proliferation index determined by CFSE staining and exemplary deconvolution plots.

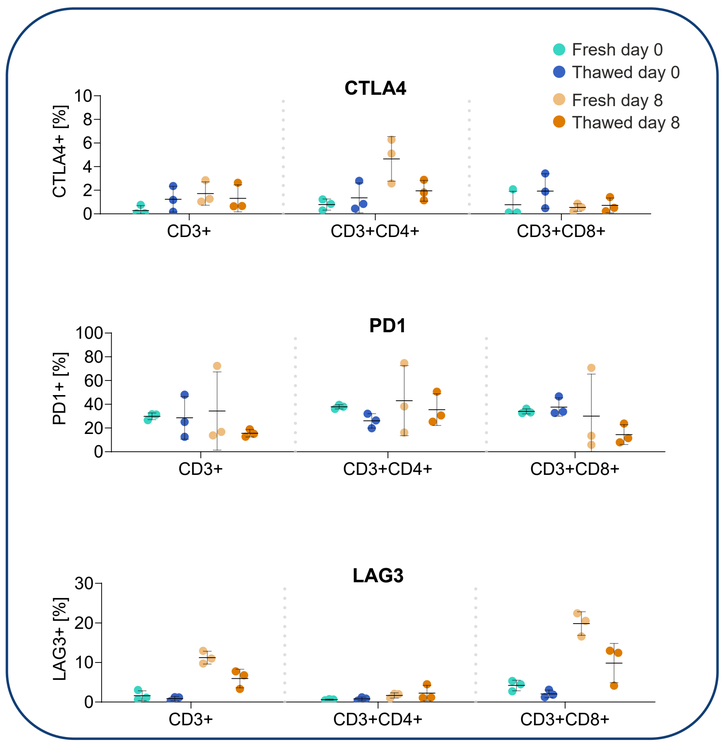

FIGURE 5. Activation status of T cells isolated from fresh vs. thawed leukopak material CD3+, CD3+CD4+ or CD3+CD8+ T cells directly after cell isolation and after 8 days of culture were further analyzed for markers of activation and exhaustion using flow cytometry.

CONCLUSION

- Cryopreservation maintained viability, CD4/CD8 ratios, proliferation, and expansion.
- Minor memory shifts post-thaw normalized after culture.
- Activation/exhaustion markers remained low throughout.
- Our cryopreservation protocol preserves key CAR T–relevant T cell attributes, throughout freeze–thaw processing.

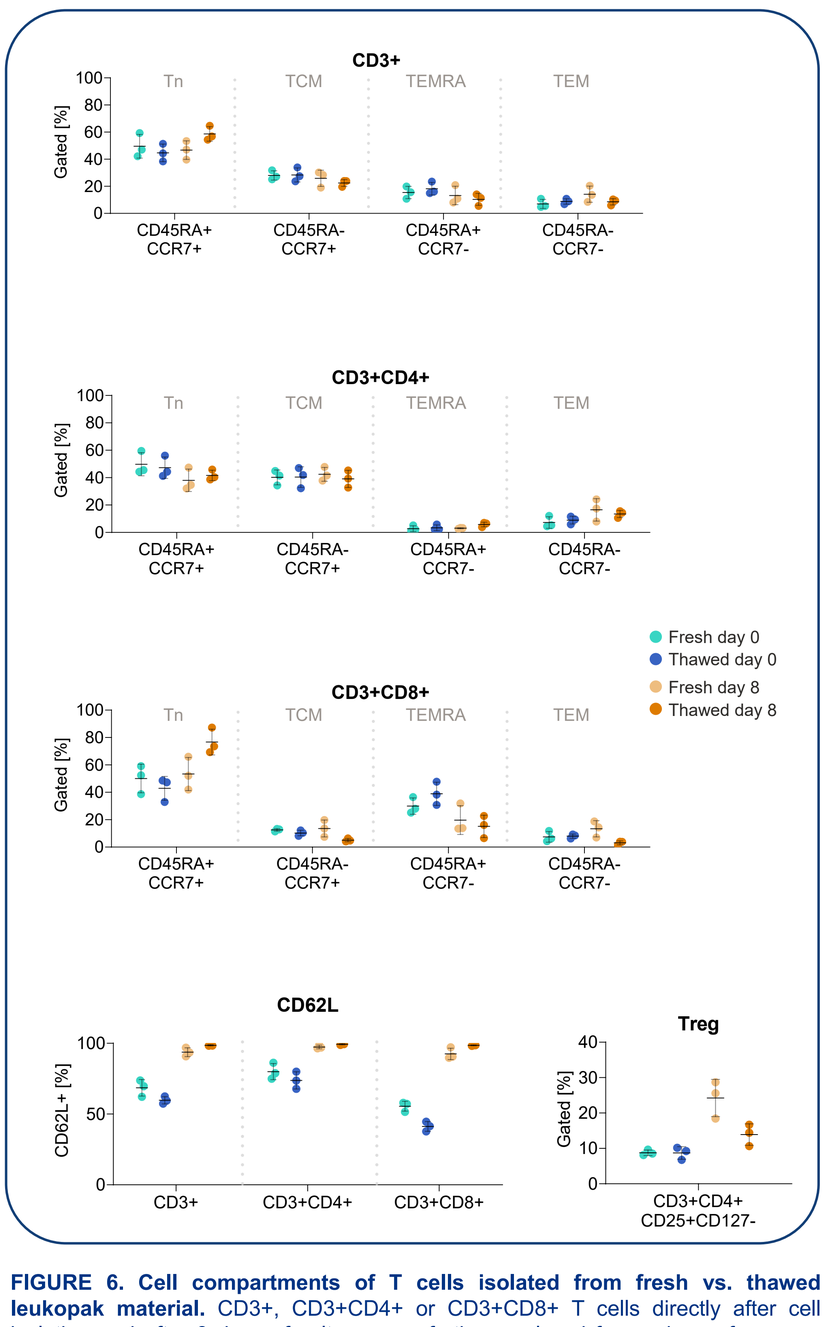

FIGURE 6. Cell compartments of T cells isolated from fresh vs. thawed leukopak material. CD3+, CD3+CD4+ or CD3+CD8+ T cells directly after cell isolation and after 8 days of culture were further analyzed for markers of memory compartments and Tregs using flow cytometry.

Minor shifts in memory subsets were observed immediately post-thaw but normalized after culture (**Fig. 6**). T_{reg} levels were preserved post-thaw but declined during expansion, indicating selective outgrowth of effector T cells.

Based on the results of this study, further investigations are planned as part of the SaxoCell collaboration.

REFERENCES

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DISCLOSURES
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