Preserving Functional Performance of Human T Cells with PentaHibe® Complete

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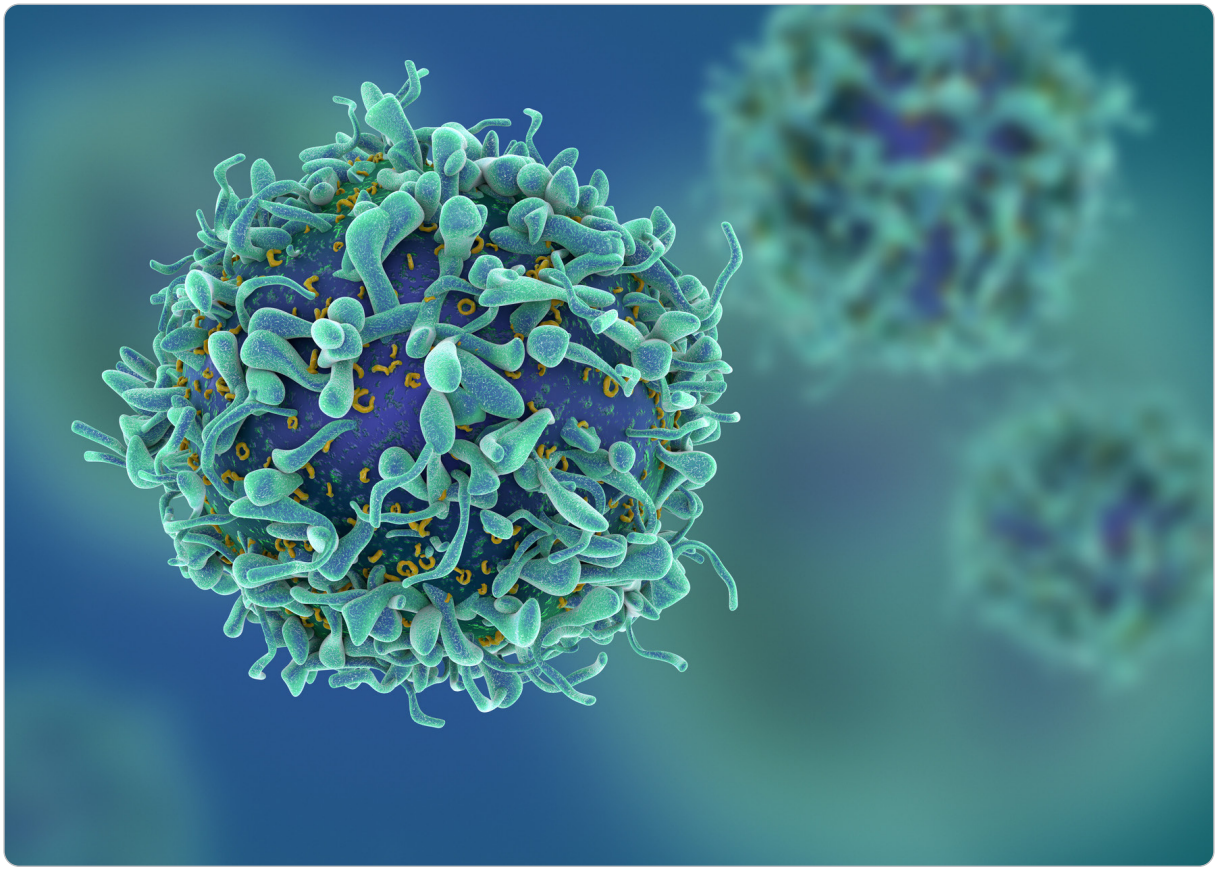

Executive Summary

Cryopreservation is a critical step in cell therapy manufacturing workflows. While post-thaw viability is often used as the primary performance indicator, viability alone does not ensure biological functionality. For T-cell–based therapies, cells must retain their ability to activate and execute effector functions in order to deliver clinical benefit.

Data generated on primary human T cells demonstrate that PentaHibe® Complete preserves not only viability and recovery, but also functional immune response as measured by interferon-γ (IFN-γ) secretion following activation.

These results position PentaHibe® Complete as a high-performance cryopreservation solution for advanced immunotherapy applications.

Preserving Function: The True Measure of Cryopreservation Performance

Cryopreservation enables flexibility and reliability in modern cell therapy workflows, but the freeze–thaw process challenges cellular integrity. Although many cells survive and appear viable after thawing, survival alone does not guarantee preserved biological performance.

Standard viability assays primarily assess membrane integrity. While essential, they provide limited insight into whether T cells retain their capacity to respond appropriately to activation. For therapies such as CAR-T and TCR-modified T cells, preserved functional responsiveness is a critical determinant of therapeutic efficacy (June et al. CAR T cell immunotherapy for human cancer. Science. 2018). Effective cryopreservation strategies must therefore protect not only cell survival, but also immune competence.

Study Overview

Primary human T cells were isolated from peripheral blood mononuclear cells, activated using CD3/CD28 activator and cultivated with IL-2 supplementation. Cells were cryopreserved in either PentaHibe® Complete or a leading commercial DMSO-based (10%) product, using a controlled-rate freezer followed by thawing on a 37°C water-bath. Post-thaw evaluation included assessment of viability, total recovery, and IFN-γ secretion as a key indicator of effector function.

This combined dataset allows comparison between conventional survival metrics and functional immune performance, providing a comprehensive view of post-thaw cellular quality.

Post-Thaw Viability and Recovery

Post-thaw viability measured immediately after thaw demonstrated mean values around 80% across both formulations. These levels are commonly regarded as acceptable benchmarks for high-quality T-cell preparations. Total recovery was similarly maintained at approximately 80%, supporting reliable downstream processing (Figure 1). These findings confirm that structural integrity and immediate survival are maintained. However, viability and recovery alone do not fully define the therapeutic fitness of T cells.

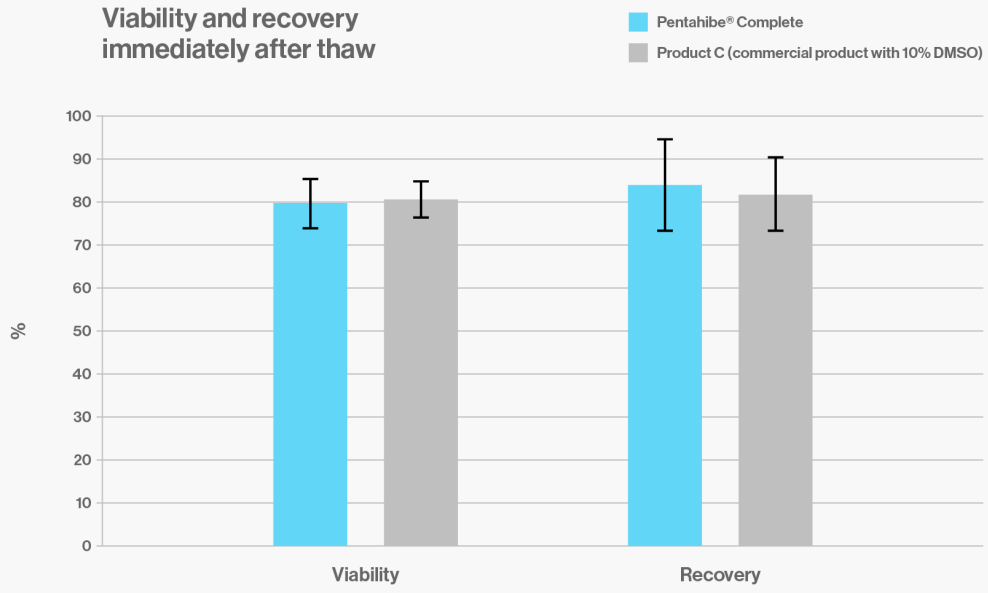

Figure 1: Immediate post-thaw viability and recovery for primary T cells cryopreserved in PentaHibe® Complete or a leading commercial 10% DMSO-based product. Values represent mean ± SD (n=9; 3 donors with triplicate measurements).

Preserved Functional Immune Response

Functional performance was assessed by measuring IFN-γ secretion following activation with phorbol 12-myristate 13-acetate (PMA) and ionomycin for 8 hours. IFN-γ production serves as a reliable indicator of retained activation capacity and immune responsiveness.

Activated T cells cryopreserved in PentaHibe® Complete demonstrated high IFN-γ secretion comparable to the commercial DMSO-based product (Figure 2). These results confirm that post-thaw cells retain their capacity to mount an effective immune response.

Unlike viability measurements, cytokine secretion reflects the ability of T cells to respond functionally to stimulation. It therefore provides a more meaningful assessment of therapeutic readiness.

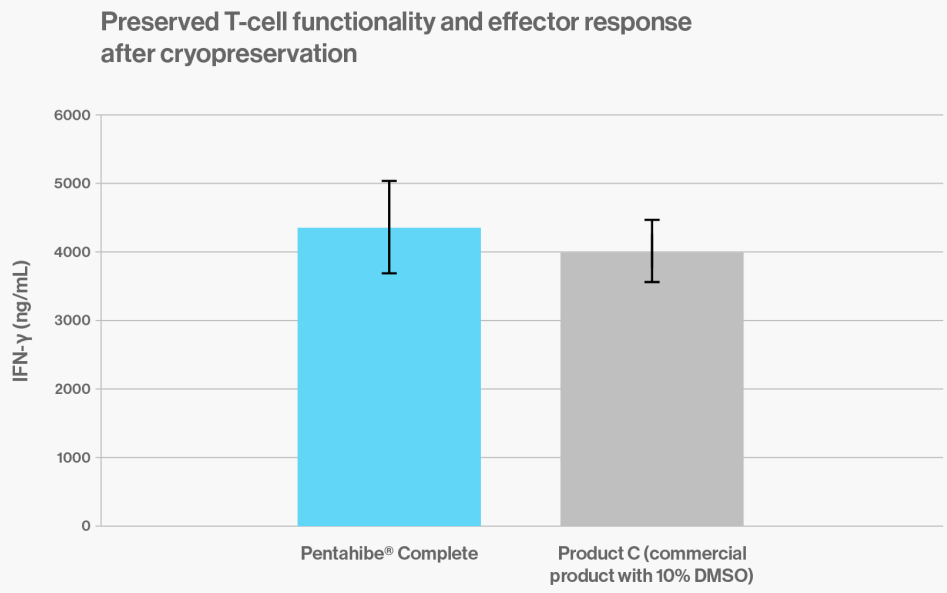

Figure 2: : Interferon-γ secretion after stimulation (PMA/ionomycin) immediately post-thaw. Values represent mean ± SD (n=9; 3 donors with triplicate measurements).

Implications for Advanced Cell Therapy Manufacturing

As cell therapies advance toward larger-scale manufacturing, defining appropriate quality attributes is increasingly important. While viability and recovery remain essential indicators of process robustness, functional assays provide critical confirmation of preserved potency.

Integrating functional evaluation into cryopreservation assessment frameworks strengthens confidence in manufacturing consistency and supports the delivery of clinically effective T-cell products.

Conclusion

Successful cryopreservation must safeguard more than cell survival. The preservation of immune function is central to therapeutic performance. Demonstrating maintained IFN-γ secretion alongside high viability confirms that functional performance can be preserved after cryopreservation.

Importantly, the comparator product evaluated in this study contains 10% DMSO, whereas PentaHibe® Complete contains only 2% DMSO. Despite this substantially reduced DMSO concentration, comparable viability, recovery, and functional IFN-γ γ secretion were achieved. These results demonstrate that high functional performance can be maintained with significantly lower DMSO exposure.

By protecting both survival and effector function – while reducing DMSO content – PentaHibe® Complete supports the demanding requirements of modern cell therapy workflows.