Optimizing Cryopreservation Performance in T-Cell Therapy Workflows

The Impact of Controlled-Rate Freezing Versus Passive Freezing

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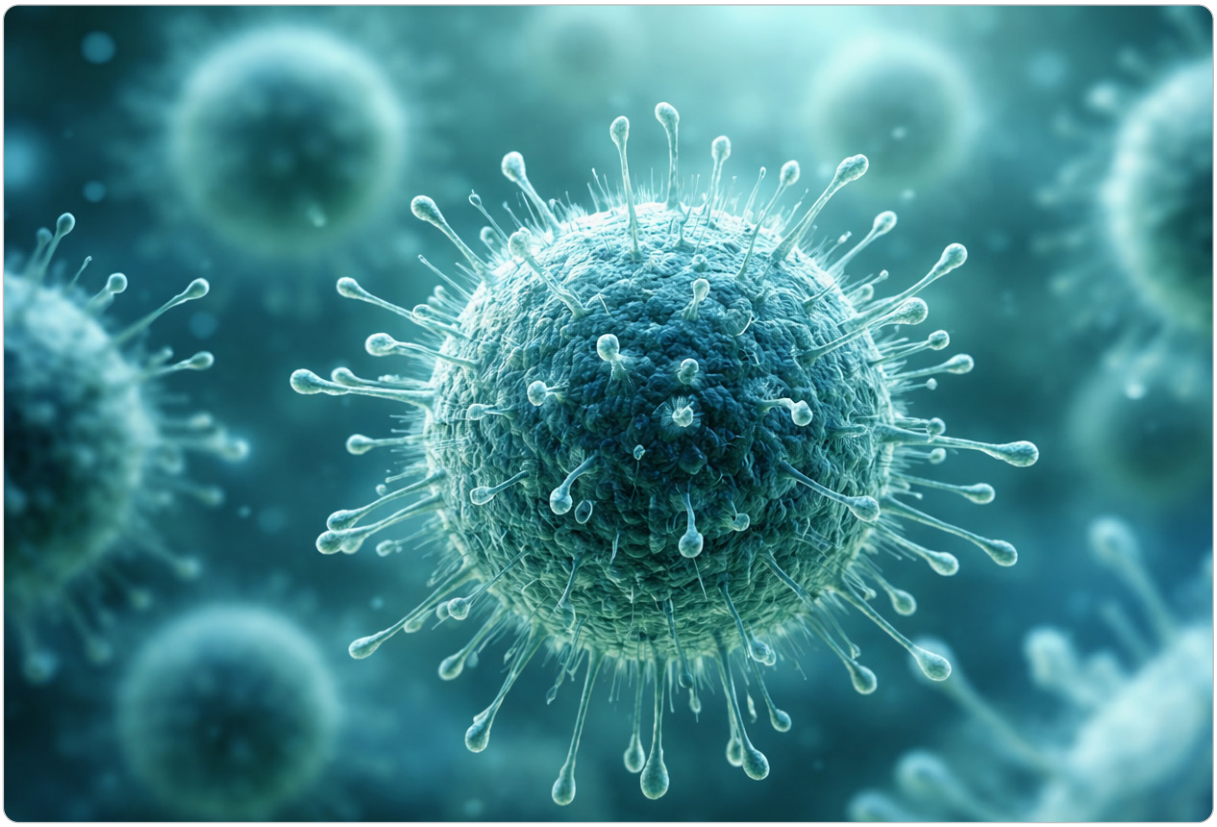

Executive Summary

Cryopreservation is widely used in T-cell therapy workflows to support manufacturing flexibility, storage, shipment, and controlled product handling. Passive freezing remains a common approach, while programmable controlled-rate freezing (CRF) offers greater control over cooling conditions.

In this comparative evaluation using activated primary human T cells, CRF and passive freezing produced similar immediate post-thaw viability. However, differences emerged at later timepoints. CRF was associated with higher viability at 24 and 48 hours post-thaw, along with lower variability across measurements. These findings suggest that immediate post-thaw viability alone may not fully capture cryopreservation performance and that 24-48 hour assessment can provide a more informative view of post-thaw cellular stability under the conditions tested.

These findings underscore the importance of evaluating sustained post-thaw recovery when comparing cryopreservation strategies and highlight the potential influence of cooling profile design on stability of outcome.

Why the Freezing Profile Matters in T-cell Therapies

T cells are sensitive to freeze-thaw stress, including osmotic imbalance, membrane damage, and downstream cellular stress responses. Not all cryopreservation-related damage is apparent immediately after thaw; some effects emerge over the following 24 to 48 hours. As a result, viability immediately post-thaw (T0) does not always reflect longer-term post-thaw stability.

Because cryopreservation performance depends on the interaction among cell type, cryoprotectant formulation, cell concentration, and freezing conditions, evaluation of the freezing profile can support improved reproducibility and consistency. Programmable CRF systems allow cooling parameters to be more precisely defined and reproduced across runs.

Comparative Evaluation Overview

Primary human T cells were isolated from peripheral blood mononuclear cells (PBMCs) using magnetic negative selection and activated using CD3/CD28 stimulation. Cells were cultured for five to six days in serum-free T-cell medium supplemented with IL-2 before cryopreservation. Pre-freeze viability exceeded 80%, and flow cytometry confirmed a predominantly CD3+ CD4+ T-cell population.

A side-by-side comparison was performed using PentaHibe® Complete cryopreservation formulation.

Samples from three independent donors were frozen at 10 × 10^6 cells/mL in 1.8 mL cryovials using either a defined controlled-rate freezing program or a passive freezing program (–1°C/min to -80°C). Following storage in liquid nitrogen, cells were thawed in a 37°C water bath. Viability was measured using Acridine Orange/Propidium Iodide staining with an automated cell counter immediately post-thaw (T0) and again at 24 and 48 hours. Each timepoint included triplicate measurements for each donor (n = 9 per condition).

Results

CRF and passive freezing produced comparable immediate post-thaw viability, indicating similar performance at T0. Differences became evident at later timepoints. At 24 hours post-thaw, viability was 28.7% higher with CRF, and at 48 hours, viability remained 15.0% higher than with passive freezing.

Variability was also lower with CRF at later timepoints. Coefficient of variation (CV%) values were 8.14% for CRF versus 12.99% for passive freezing at 24 hours, and 6.86% versus 11.22% at 48 hours. Together, these results suggest that freezing profile can influence sustained post-thaw viability and consistency even when immediate post-thaw measurements appear similar.

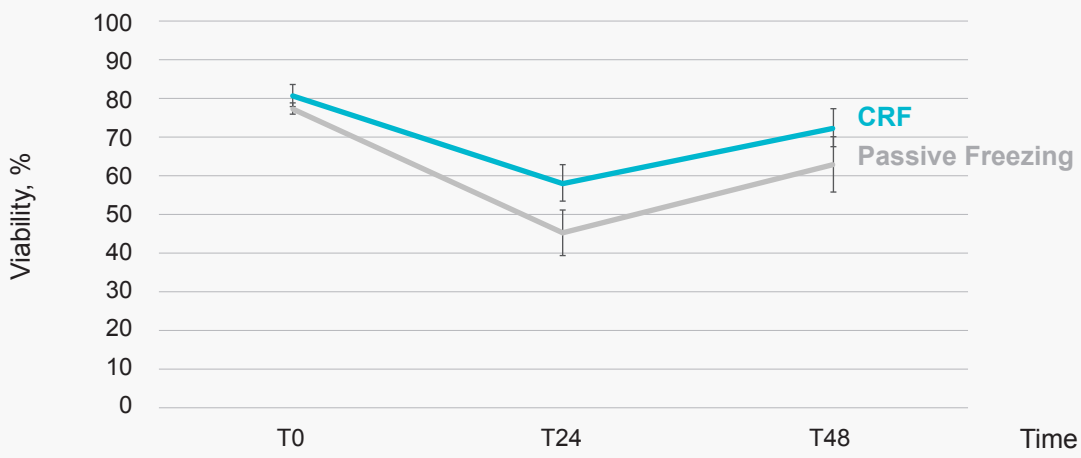

Figure 1: Post-thaw viability of activated primary T cells cryopreserved in PentaHibe® Complete using either a defined controlled-rate freezing program or a passive freezing program (-1°C/min to -80°C). Data represent the mean of three donors with triplicate measurements at each timepoint (n=9).

Relevance for T-cell Therapy Process development

In many T-cell therapy workflows, cells are expanded before cryopreservation and not further expanded after thawing. Immediate post-thaw viability is therefore often used as a practical indicator of product recovery. However, comparable T0 values do not necessarily indicate equivalent biological stability.

For process development and characterization, adding a 24–48 hour post-thaw assessment may help distinguish freezing strategies that appear equivalent at thaw but diverge during early post-thaw recovery. Passive freezing remains a well-established and widely applied approach, while CRF may offer an advantage when tighter control of cooling dynamics and more consistent post-thaw outcomes are priorities.

Conclusion

In this comparative evaluation of activated primary T cells, controlled-rate freezing and passive freezing produced similar immediate post-thaw viability, but CRF was associated with higher viability and lower variability at 24 and 48 hours post-thaw. These findings support evaluating cryopreservation performance beyond T0 when comparing freezing strategies during process development. Under the conditions tested, freezing profile optimization appears to be a practical lever for improving post-thaw consistency without changing formulation components.

Technical Support

For additional information regarding the evaluated freezing protocols, please contact Pharmacosmos technical support at pentahibe@pharmacosmos.com