The Cell Summit '25 Proceedings

Tools, Insights, and Strategies for CGT Advancement

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Preserving the Promise: Insights from The Cell Summit '25

On August 5–6, 2025, BioLife Solutions welcomed thought leaders from across the cell and gene therapy (CGT) industry to Indianapolis for the first-ever Cell Summit. The goal: bring together scientific and operational experts spanning therapy developers, tool providers, academics, and regulators to explore challenges and opportunities in biopreservation, closed system processing, and scale-up of advanced cell therapies. We asked the question: How do we work together to improve patient access, cell therapy enrollment and what can tool providers do specifically to help?

The result was two days of honest discussion, rich knowledge sharing, and a shared commitment to improving how we preserve, process, and deliver the living cells that power CGTs.

This proceedings eBook, developed in partnership with GEN (Genetic Engineering & Biotechnology News) and our meeting co-sponsors, Azenta Life Sciences and Entegris, captures key insights from the meeting and extends its reach to a global audience. Inside, you will find expert-authored articles on topics ranging from Biopreservation Best Practices and container selection to the biological impact of transient warming events. You'll also read real-world implementation stories, academic innovations, and emerging technologies poised to transform the field.

If one theme unified the Cell Summit, it was this: preserving the integrity of living cells is not a minor detail; it is the foundation of therapeutic success. Whether you are working on iPSCs, CAR-T cells, or allogeneic MSCs, understanding and optimizing biopreservation processes is essential to achieving consistent, scalable, and regulatory-compliant outcomes.

We hope these proceedings will serve as a practical reference and inspiration for researchers, manufacturers, and innovators across the CGT ecosystem. And if you are facing specific challenges or looking to refine your own cryopreservation strategy, we invite you to connect directly with our scientific team on our Ask the Scientists web page.

Thank you to our speakers, co-sponsors, attendees, and GEN collaborators who helped bring this content to life.

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The Critical Role of Biopreservation Best Practices in Cell and Gene Therapy Development

By Shea Vincent, Jennifer Shrider, Sean Werner
BioLife Solutions, Inc.

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As the cell and gene therapy (CGT) industry progresses toward wider clinical and commercial adoption, developers are increasingly focused on refining manufacturing, improving analytics, and simplifying delivery strategies. Yet amid these advancements, a foundational element continues to pose significant, if underestimated, risk: biopreservation.

Far from a peripheral logistics concern, biopreservation—and more specifically, cryopreservation—is a critical unit operation with direct impact on cell health, manufacturing consistency, and in many cases, cellular attributes that may contribute to therapeutic potency. This message was echoed repeatedly at The Cell Summit '25, where a cross-section of academic and industry leaders emphasized the scientific

complexity, operational nuance, and strategic importance of preserving the viability and functionality of living cells throughout the therapy lifecycle.

Reframing Biopreservation as Central to Maintaining Critical Quality Attributes

In many early-stage programs, cryopreservation is treated as a postscript, simply a method of extending shelf life or enabling transport. However, mounting evidence suggests it should instead be regarded as an essential process related to maintaining critical quality attributes (CQAs). The conditions under which cells are frozen, stored, and thawed can dramatically influence post-thaw phenotypic stability and cellular activity, key quality attributes required assure the cells perform as expected .[2]

Cells, by nature, are sensitive to environmental stress. The processes used to manufacture cell therapies are inherently stressful as the cells are exposed to conditions dissimilar to their normal environmental niche. Mostly, manufacturers attempt to limit the differences throughout the manufacturing process between the artificial, ex vivo environment and the normal physiology of the body. However, cryopreservation is, at least for mammalian cells, explicitly beyond tolerable physiological conditions. The freezing process introduces a range of physical and biochemical stressors including osmotic imbalance, ice crystal formation, cryoprotectant toxicity, and activation of stress-response and apoptotic pathways. Even when short-term viability appears acceptable, delayed onset cell death (DOCD) or sublethal injury may reduce downstream expansion potential or therapeutic efficacy.[6] Given the contemporaneous suspension of cellular activity at cryopreserved temperatures and the cryopreservation process itself, it should come as no surprise that the cellular response to the stress takes some time to evolve after the return to physiological temperatures.

How do developers address this, while also optimizing cryopreservation and thawing processes?

Scientific Variables and Process Levers

During the Cell Summit, speakers highlighted a wide range of process levers that can be optimized to mitigate these risks. These include:

1. **Cryoprotectant formulation:** Dimethyl sulfoxide (DMSO) remains widely used and is functionally a benchmark for cryoprotectant activity. Its concentration, carrier solution, and exposure time must be precisely controlled as it can have impacts on cells.
2. **Cooling and thawing profiles:** Controlled rate freezing (CRF) and appropriately matched thaw protocols are essential to minimize intracellular damage.
3. **Container selection:** The physical and thermal properties of cryogenic containers impact heat transfer, uniformity, and sample protection during freezing and warming.
4. **Transient Warming Events (TWEs):** Short-term temperature excursions, particularly during handling and shipment, were identified as a significant and often overlooked threat to product integrity.
5. **Post-thaw assessment:** Measuring viability immediately after thaw may not reflect the true health or functional potential of a sample. Extended recovery monitoring is increasingly recommended during the development phase.

Each of these variables can significantly affect the stability and consistency of a cell product. Importantly, they are not interchangeable. Optimal outcomes require systematic alignment across reagents, equipment, and handling protocols.

Impact of Poor Biopreservation Practices

The consequences of not implementing biopreservation best practices can be substantial, affecting a broad range of cell attributes. One of the most immediate and measurable impacts is a reduction in post-thaw cellular activity. This can often be identified in compromised cell viability or function. Following suboptimal freezing, thawing, or storage conditions, a cellular product may achieve some level of performance but, the reduction compared to pre-freeze performance inherently requires a higher number of cells to achieve a goal, increasing manufacturing time and costs. Even when initial viability metrics appear acceptable, sublethal injury or DOCD can diminish downstream performance and clinical efficacy.

Inconsistency between production lots is another common consequence, particularly in autologous workflows where variability is already high. Without standardized biopreservation protocols, batch-to-batch differences in cell yield, quality, or expansion potential can challenge comparability, complicate quality control, and hinder scale-up efforts. Furthermore, this variability can lead to inconsistent and difficul-to-interpret overall performance of the cellular product.

Manufacturing efficiency also suffers when cryopreservation is not well-controlled. Failed lots, poor post-thaw recovery, or reduced potency can lead to costly rework, schedule delays, and increased resource utilization. These operational inefficiencies may erode timelines and budgets, particularly in fast-moving clinical programs.

From a regulatory perspective, cryopreservation processes that are not adequately validated or reproducible introduce risk during inspections and submissions. Agencies increasingly expect biopreservation to be treated as a formal unit operation, with associated quality controls and documentation. Failure to meet these expectations can result in regulatory setbacks or delays in clinical advancement.

In the worst-case scenario, poor cryopreservation practices can undermine the entire development program, leading to product failures, missed endpoints, or inability to secure regulatory approval. For this reason, biopreservation must be prioritized as a foundational element of CGT process development—not a secondary consideration.

Recommendations for a Best Practice Framework

To mitigate these risks and improve the likelihood of clinical and commercial success, CGT developers are encouraged to adopt the following best practices:

1. Integrate biopreservation early in process development, not as a downstream consideration.
2. Use validated, chemically defined reagents that are GMP-manufactured and xeno-free when possible.
3. Standardize freezing and thawing protocols, ideally using closed or semi-automated systems to reduce variability.
4. Select appropriate storage containers that offer structural integrity, thermal uniformity, and compatibility with handling equipment.
5. Implement robust monitoring of temperature throughout the product journey, especially during shipment.
6. Validate processes at clinical scale, with special attention to post-thaw cell health, phenotype, and function.

Partnering for Biopreservation Success

BioLife Solutions is committed to supporting the CGT community in optimizing biopreservation workflows. Our scientists routinely collaborate with therapy developers, CDMOs, and academic centers to troubleshoot performance issues, guide protocol optimization, and ensure consistency across scale.

If your team is encountering challenges related to viability, recovery, or regulatory documentation, or if you simply want to ensure your approach is future-proof, our scientific team is available to help.

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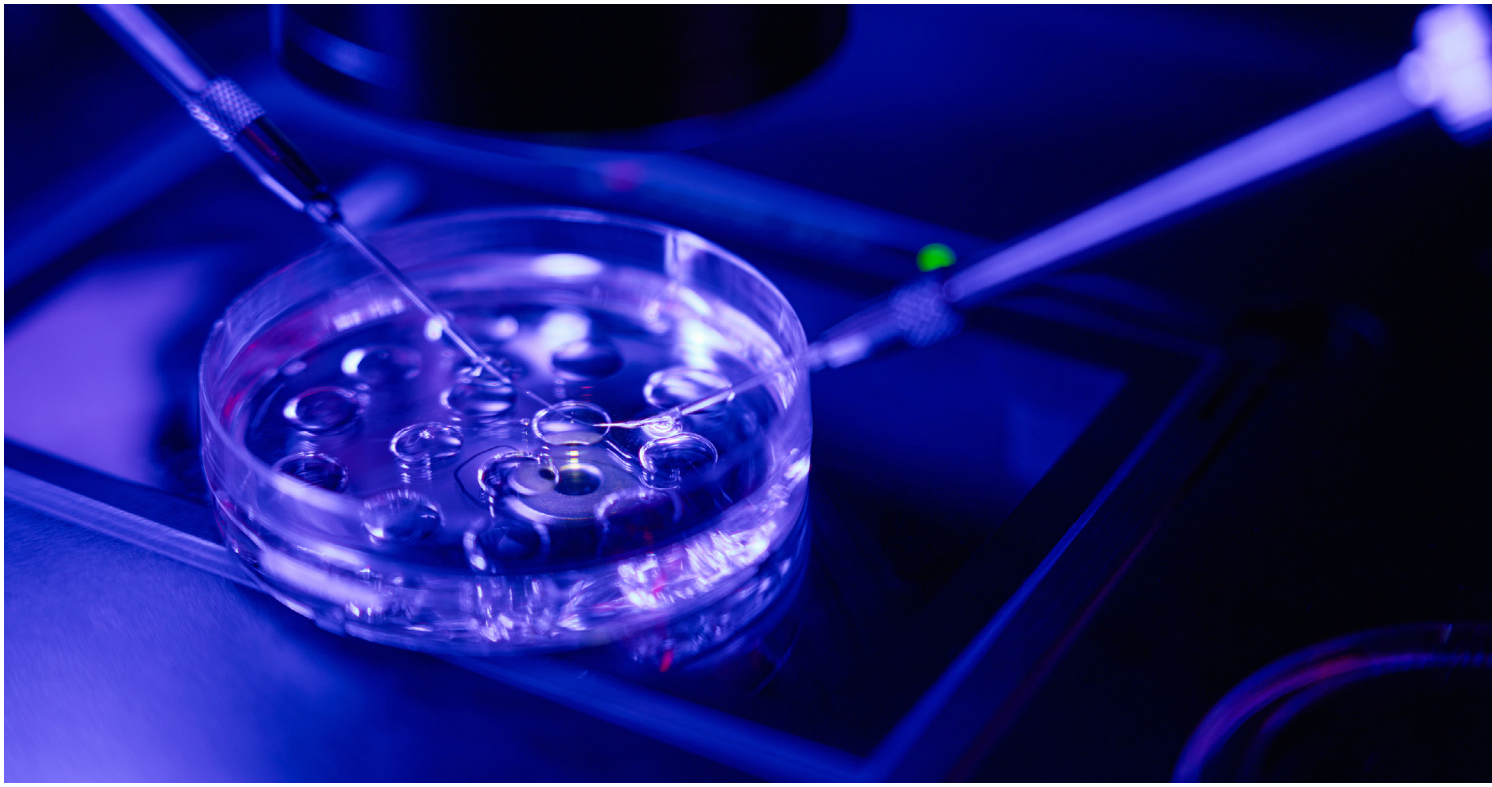
Scaling Cell Therapies: Why Closed Systems Must Precede Automation

By Donnie Beers, Entegris Inc.



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In the biopharmaceutical sector major socioeconomic forces have created a situation where transformative therapies for rare or ultra-rare diseases and curative medicines are seen as extremely risky for investors and developers alike. Many strategies have been attempted to risk-proof development including consolidation of development pipelines, elimination of projects with small patient populations and industry consolidation to fund expensive developments using blockbuster successes.

There are many challenges to scalability and processing for cell therapies that will need to be addressed to reduce production costs and ensure stable supply paving the way for future investment and development. Many believe that automation is the key to success by reducing labor costs, which account for nearly 50% of the COGS (cost of goods sold) for these therapies. However, automation is not the first and most important hurdle to clear for many development stages, as automating waste can lock in disastrous processes that are inflexible, hard to scale and end up costing millions in commercialization stages.

In this case it is important to remember to keep biology the most important, before jumping into an automated system, by implementing an end to

end completely closed system for the production process. Then you can pave the way for automating incrementally as you scale. There are challenges to conducting these cell-based processes in completely closed systems such as: availability of suitable tools, lack of knowledge, or inability to source critical raw materials in closed containers. In each of these situations, having reliable and experienced collaborative suppliers and partners – especially those that offer design services – is crucial to success. Additionally, while unit operations can be later automated, or you can have robots or co-bots to eliminate labor costs, taking a good look at your connection and disconnection points up front can save costly rework or revalidation when implementing automation. What if a platform technology has been adopted already? There are many ways to implement automation and closed systems even in situations where a part of the process is already locked in. Leveraging industry experts that have done it before is a good starting point to avoid pitfalls or wasteful, clunky processes that bake in waste and time in the process and are often costly and very difficult to change once implemented.

Entegris offers collaborative design services, training, and access to unit operation experts across filtration, upstream and downstream fluid handling, as well as cold chain.

One specific area that these services have benefited end users in both the production of viral vectors and for large scale cell therapies is the high weight percentage of solids in concentrated and process intensified bioreactors. As the titers increase, it is critical to reduce hold-up and utilize smaller surface area devices, achieving high flux and throughput to keep processing times short and eliminate loss.

Entegris, Inc.

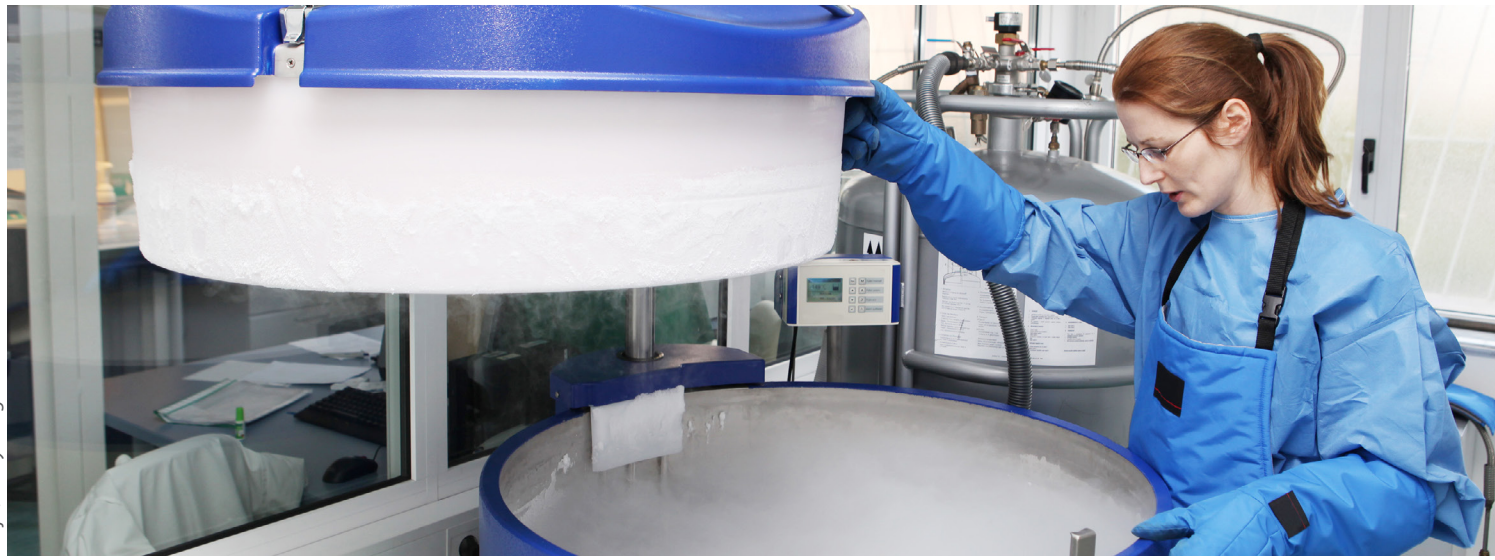
Entegris empowers you with expert advice and flexible products to accelerate your process development, addressing your biggest design and processing challenges. For more information connect with us here.

Applications personnel can help provide access to scale down tools for development and optimization of separation processes that are off the shelf, GMP ready, work well with leading edge third-party equipment and automation tools or can be configured and kitted to the specific use case.

Another area that Entegris applications experts specialize in is any process that requires optimization in freezing and storage of those cells or other drug substances once they are separated. Consider the conflicting need to keep individual containers small to reduce the risk of loss, with the need to freeze larger volumes of cells in a consistent and controlled manner. Similarly, it reflects on the necessity of businesses to keep initial costs low and move quickly, with the need to obtain optimized tools that are scalable to BLA (Biologics License Application) while still minimizing future capital costs. Again, implementing phase appropriate tools with collaborative suppliers and services providers can have substantive direct business impacts to cost and development timelines without requiring capex investment or automation.

Cryopreservation Considerations: From Hypothermia to Ice Recrystallization Inhibitors

By Sean Werner,[a] Jason Acker,[b] Robert N. Ben,[c] Shea Vincent,[a] Jennifer Shrider,[a] Alireza Abazari [a]



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Corralling the Complexity of Compliance

Early research on the impacts of hypothermic conditions on cells and tissues observed that lower temperatures could partially mitigate ischemic conditions. These observations led to the development of hypothermic surgery protocols.[1] Hypothermia was demonstrated to reduce metabolic activity, oxygen demand, and reactive oxygen species (ROS) generation. Prior to the development of extracorporeal oxygenation methods, the improved resistance to the impacts of ischemia was demonstrated to improve outcomes during organ transplantation and surgery. However, while hypothermia provides protection, it also induces stresses such as intracellular accumulation of protons and calcium, leading to cellular damage.[2] Over time, solutions such as EuroCollins, University of Wisconsin (UW) solution, and BioLife Solutions' HypoThermosol® FRS were developed to address ionic imbalance, acidosis, and oxidative stress to support temporary storage at reduced temperatures.[3] However, even under these improved conditions, stability of cells and tissues is relatively brief and not sufficient for long term storage as required for development of cell-based therapeutics. Cryopreservation is the only modality for long-term preservation of viable and functional cells and tissues that can fit the complicated manufacturing processes of cell and gene therapy (CGT), from collection of starting tissues and cells to delivery of a therapeutic product. While some autologous therapies may have been

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formulated based on delivering a fresh product with complicated logistics, cryopreservation remains the only viable option for biologic therapies.

Cryopreservation allows long term storage of cellular material with minimal biochemical activity once the cells reach storage temperatures below -150°C. Under cryopreservation conditions, cellular stability has been extended to 10 or more years. However, the process of cryopreservation leads to even more extreme changes in the critical intracellular and extracellular milieu. Ice crystal formation and the evolution of latent heat during the phase transition of water to ice can be damaging to cells when not appropriately controlled. The negative impacts of cryopreservation often are not observed immediately following thaw but show up after the cells respond to the insults through normal stress responses such as apoptosis. This response, termed delayed onset cell death (DOCD), can lead to false assumptions about the performance of the cryopreservation process if viability is only assessed immediately following thawing. Evaluation of the success or failure of cryopreservation should therefore be carried out on cell cultures that have been given the time to respond to the stress of cryopreservation. Generally, 24-48 hours provides a useful window. It was noted during The Cell Summit '25 event that evaluating the cryopreservation process should be considered as part of process validation to optimize cellular performance. Considering process optimization provides not only improved post-thaw performance but also reduces variability, improving the ability to interpret downstream outcomes.

Cryopreservation Fundamentals

Cryopreservation enables long-term storage of cells at ultra-low temperatures. The central challenge is avoiding damage caused by ice formation and osmotic stress. Mazur's Two-Factor Hypothesis[4] highlights two key risks: (1) intracellular ice formation when cooling is too fast, and (2) solute toxicity when cooling is too slow.

Cryoprotective agents (CPAs) such as dimethyl sulfoxide (DMSO) mitigate these effects by reducing ice formation and salt toxicity. DMSO is a particularly effective cryoprotective agent due to its colligative properties in formulation with buffers such as cell media or saline solution. To maximize the cryoprotection from colligative effects of salts and DMSO, parameters such as cooling rates, thawing rates, and CPA concentration must be optimized for each cell type. When appropriately balanced, the processes and formulation of cryomedia can result in high yield of viable, functional cells following cryopreservation.

Case Study: Induced Pluripotent Stem Cells (iPSCs)

During The Cell Summit '25 in Indianapolis this August, Dr. Alireza Abazari presented a case study demonstrating the potential impact of non-optimized processes and formulation. In the study, CryoStor® CS10 (containing 10% DMSO v/v) was compared to a commercial DMSO-free cryopreservation media, revealing the potential for undesirable, and possibly unanticipated impacts.

Both types of media preserved pluripotency markers (OCT4, SOX2, NANOG, SSEA4) post-thaw. However, DOCD was more prevalent in non-DMSO formulations. This was particularly impactful in extending the time to reach a targeted cell expansion. The impacts on expansion consistency were especially notable during early passages.

Unexpectedly, evaluation of the genetic profile of the cells indicated that different conditions may play a role in the stability of these cell types. Under some conditions and likely cell-line dependent, genetic drift was observed under the conditions of the study, with

abnormalities more frequent in older iPSC lines and with DMSO-free formulations.

The findings underscore the importance of assessing post-thaw behavior, not just immediate post-thaw recovery, in evaluating cryopreservation success.

Process Optimization Considerations

Successful biopreservation requires attention to the entire workflow, not just the freezing medium. Critical process parameters (CPPs) include:

- Timing and conditions of harvest
- Cryomedia formulation and cryoprotectant identity and concentration
- Cooling and thawing system parameters and performance capabilities
- Storage and transport conditions
- Thawing parameters and post-thaw processing (e.g. washing and recovery steps)

Even minor deviations in these parameters can significantly influence cell viability, genomic stability, and product consistency. BioLife Solutions emphasizes GMP-compliant, chemically defined, xeno-free formulations to support CGT manufacturing scalability and regulatory acceptance.

Emerging Advances in Cryopreservation

One aspect of cryopreservation that has recently gained attention is the phenomenon of ice recrystallization. This is a thermodynamically driven process that causes a dynamic reorganization (grain coarsening) of ice crystals while still in a nominal frozen state. During this reorganization, large ice crystals grow larger at the expense of smaller ones. Several factors are involved in this process although temperature fluctuations, cryoprotectant concentration, and storage at higher sub-zero temperatures are critical variables.

Long-term stability of frozen biologics is theoretically indefinite below -130°C, but real-world storage is challenged by operationally required handling of the cryopreserved materials. For example, transient warming events (TWEs) occur frequently during inventory management, shipping, and freezer cycling or liquid nitrogen consumption and replenishment.

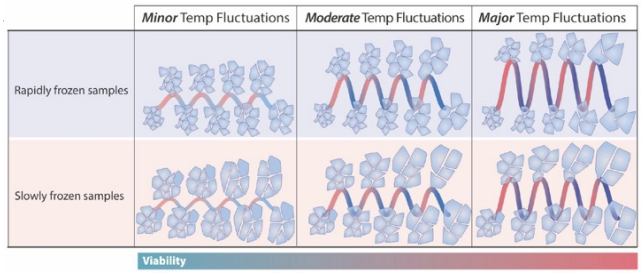

Figure 1. Transient warming alters the state of the system. Rapidly and slowly frozen samples exposed to minor, moderate, or major temperature fluctuations show decreasing cell viability as ice crystal size increases.[6]

Several mitigating steps were discussed. First, improved freezers should be considered. Modern freezer designs can minimize frequency and duration of openings or, in the case of automation enabled freezers, limit temperature fluctuations to the sample targeted for removal.

Second, implement robust packing, shipping, and monitoring strategies to reduce TWEs. Tracking and recording exposure to warming events can identify unexpected TWEs and enable improved control as part of quality-driven initiatives.

Lastly, novel small molecule ice recrystallization inhibitors (IRIs) have recently become available and are specifically designed to mitigate the cellular damage/death associated with reorganization of ice during cryopreservation.[5] The rational design of these IRIs was inspired by nature and have shown efficacy at improving post-thaw viability, recovery, and function in a growing list of cell types and tissues.

Key Takeaways for Process Development Scientists

- Hypothermia and cryopreservation are double-edged tools: protective but potentially harmful if not carefully controlled.
- DOCD is a major quality risk that requires post-thaw monitoring during process development and in quality assessment.
- When optimizing the process for minimized DOCD, DMSO remains the most effective CPA, though alternatives and DMSO-free formulations are under active exploration.
- Optimization extends beyond formulation—process control during freezing, thawing, and handling is equally critical.
- Continuous assessment of genetic stability is necessary, especially for pluripotent stem cell lines.
- Given that most cellular injury during cryopreservation is due to the uncontrolled growth of ice, novel strategies to control ice recrystallization should be considered early in process development.

In summary, cryopreservation is not merely a storage step but a multi-component, stress-inducing process that requires holistic optimization. For process development teams, incorporating best practices in cryobiology is essential to ensuring consistent, safe, and scalable manufacturing of advanced therapies.

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Performing Cryopreservation Correctly has the Potential to Expand the Scope of Life-Saving Cell and Gene Therapies

Biopreservation Best Practices for Cell & Gene Therapy Developers

Science Photo Library / Getty Images

By Shea Vincent, BioLife Solutions and MaryAnn Labant, GEN

Annually, an estimated 1.24 million blood cancer cases occur worldwide. Although many approved cell and gene therapies (CGTs) address these cancers, only about 40,000 patients have been treated to date. To advance the industry, therapy and tool developers need to work collaboratively.

In August 2025, BioLife Solutions hosted The Cell Summit '25, assembling an international roster of experts to discuss challenges facing the CGT industry, biopreservation best practices, and potential paths forward. "It is the community's responsibility to close the cryopreservation gap and educate practitioners, regulators, and inspectors," emphasized Erik J. Woods, PhD, Co-Founder & CSO, at Ossium Health.

The Crucial Role of Cryopreservation

A general consensus is that while fresh cells and tissues can offer many advantages, fresh also introduces massive operational hurdles. Yet, for early phase CGT studies, more flexibility is desired requiring qualification of both fresh and cryopreserved products in many cases.

When performed correctly biopreservation offers the consistency, scalability, and logistical control required to advance CGTs to market. The key phrase is "when done right." Poorly preserved cells translate into poor efficacy and poor therapy performance. "Garbage in, garbage out," Alex Sargent, PhD, Director of Process Development of Cell and Gene Therapy of Charles River Laboratories, succinctly stated.

Bruce Thompson, CTO of Kincell Bio, stated, "Cryopreservation and its associated freezing and thawing procedures are among the final steps in economically viable manufacturing and clinical application of diverse cellular therapeutics." Translation from preclinical proof-of-concept studies to larger clinical trials has indicated that these processes may potentially present an Achilles heel to optimal cell product safety and particularly efficacy in clinical trials and routine use."[1]

Best Practices

1. Define the Use Case and Cell Type

CGTs regulated as Advanced Therapy Medicinal Products (ATMP) present unique problems in terms of product stability, storage, and supply chain. Cryopreservation is a critical, but often overlooked, aspect of ensuring that starting, intermediate and final materials are optimal for manufacturing a given product.

But, cryopreservation is not a one-size-fits-all process. Starting material variability (PBMCs, TILs, CAR-Ts, iPSCs, etc.) demands customized strategies. Key factors to consider include dose requirements, immune compatibility, expansion potential, and susceptibility to cryoinjury.

Cryopreservation enables optimization of large segments of the CGT design space. In order to be accessible to larger populations, CGT products need better solutions, including a more comprehensive understanding of the biological impacts of formulation and freeze/thaw. As knowledge accumulates, it will continue to inform the technology used to characterize these products and advance their clinical use as well as to train AI/ML algorithms to better enable delivery of safe and efficacious products.

For example, establishing the shelf life of a cryopreserved cellular therapeutic can be controversial. Although many in the academic cryobiology community believe that cells at cryogenic temperatures can be stable indefinitely, those who apply cryobiology clinically find this is often not the case. Robust stability studies are imperative to determine the shelf life of a cellular product.

2. Use Purpose-Built, Chemically Defined Preservation Media

Validated, consistent preservation media are critical as serum-containing or under-defined formulations have limitations that may affect recovery rate. Purpose-built, chemically-defined cryopreservation media like CryoStor® and HypoThermosol® FRS reduce variability and risk. Defined media improves cell recovery, repeatability, viability, and long-term consistency.

It's also important to consider that standards have to be evaluated for specific applications. For instance, although dimethyl sulfoxide (DMSO) remains the most effective cryoprotective agent (CPA), toxicity, exposure time, and downstream compatibility must be managed. In addition, when it comes to more fragile cells like iPSCs or cone cells, custom formulation development may be needed.

"Frozen does not mean stable," advised Jason Acker, PhD, University of Alberta. "Controlling ice recrystallization is critical to quality." Although CPAs increase the amount of unfrozen water, which translates to less ice formation, less concentrated salt, less cell shrinkage, less osmotic stress and more viable cells, Ice Recrystallization Inhibitors (IRIs) keep ice crystals small and have the ability to protect cells from transient warming events (TWEs), enhancing cell quality and function.

3. Master Freezing Profiles and Cryogenic Processes

Controlled Rate Freezing (CRF) is essential. The importance of integrating CRF into scalable workflows and validation protocols cannot be overstated. Not only is passive freezing inconsistent, but it is also difficult to scale. Optimization of cooling rates, hold steps, and final temperature targets based on volume and container type is imperative.

Mazur's Two-Factor Hypothesis states that high salt concentrations and the formation of ice crystals within cells are two factors that can harm cells during cryopreservation. This hypothesis reinforces the crucial balance between ice formation and solution effects during freezing.[2]

4. Beware Transient Warming Events

Cell preparation, cryopreservation, and post-cryo processing are normally controlled but storage can be undefined and uncontrolled. Repeated cycling of freezing conditions has been shown to lead to a decrease in every parameter measured, demonstrating that TWEs should be considered when developing a stability study.

TWEs are harmful and happen during brief exposures of a product to temperatures above the critical storage temperature during routine operations like: freezer compressor cycling, filing events, inventory management, or removal of frozen segments for testing, packing and shipping.

Frequent, unmonitored TWEs during storage, handling, or transport can lead to cumulative damage and delayed onset cell death (DOCD). Monitoring systems and process controls should aim to minimize these events and ensure samples remain at cryogenic temperatures consistently.

5. Understand What is Happening to Cells at each Temperature

At hypothermic temps (+2°C to +8°C) cells experience oxidative stress, calcium influx, and mitochondrial dysfunction. At sub-zero temps (-20°C to -80°C) ice formation, osmotic stress, and CPA toxicity can occur, even though CPAs reduce salt toxicity.

At the frozen glass transition temperature, intracellular water shifts into a highly viscous, glass-like state that immobilizes biomolecules and slows degradative reactions. At cryogenic temperatures, molecular motion is essentially arrested, preserving cell structure and function over time.

After thaw, apoptotic pathways may be activated even if viability appears high immediately post-thaw—hence the importance of delayed analysis to assess DOCD.

A common mistake is to assume that potency and efficacy is the same thing and that a potency test that measures potency is also a measure of efficacy. This cannot be true since efficacy can only be measured by clinical response. Potency tests are laboratory assays which may or may not be a predictor of clinical response.[3]

6. Select the Right Container and Thaw Accordingly

A poor container or inconsistent thaw can undo months of manufacturing. Container closure integrity (CCI), visual inspection capability, and compatibility with automated thaw systems are key differentiators.

Thawing is a critical juncture in the CGT lifecycle. "Thawing is not the end. It's the final test," said Olga Bukatova, Associate Director Business Development CGT, Azenta Life Sciences. The process impacts viability, recovery, retention of therapeutic functionality as well as risk profiles and clinical consistency.

Ice recrystallization during suboptimal warming rates, osmotic stress, solute concentration spikes and CPA cytotoxicity can occur during thawing.

Controlled dry thawers such as the Barkey Varitherm or the ThawSTAR® CSV designed for CellSeal® Cryogenic vials are GMP compliant, control the risk of contamination, and are operator independent with heat transfer and agitation capabilities. Standardized, validated thawing protocols enhance reproducibility, reduce inter-site variability, and safeguard clinical outcomes.

7. Standardize, Automate, and Close Your System

Facilities need to plan for scaleup with the biopreservation process designed in from the start, rather than retrofitting it at the end.

Manufacturing CGTs involves complex manufacturing processes, which must be carried out in line with GMP requirements, following carefully controlled procedures to ensure maintenance of product safety and quality. Open handling increases contamination risk and variability. The adoption of closed-system processing, automated fill-finish, and closed thaw protocols is becoming the new standard.

The objective of a study designed by CTMC, a development accelerator formed through a joint venture between Resilience and MD Anderson Cancer Center, was to determine whether a closed-system process could produce similar results in terms of expansion and transduction compared to a standard process while eliminating the need for a biosafety cabinet.

The study demonstrated that CAR-T manufacturing can be fully enclosed when appropriate containers are used for both starting cells and viral vectors. CellSeal Connect vials provided a functionally equivalent alternative to conventional screw cap cryovials, with comparable cell viability, expansion, and transduction efficiency during culture. Additionally, CellSeal CryoCases are a closed-system option for filling of final drug product. Although the study authors note limitations that should be addressed, closed system innovations reduce contamination risks, streamline operations, and simplify facility design.[4]

The future of CGTs holds a great deal of promise for patients with no other treatment alternative. Optimizing cryopreservation of precious cells and tissues may make a difference, not only in the development and manufacturing process, but also in patient's lives.

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Securing Critical Quality Attributes in Advanced Therapies with Precision Cryogenic Management

By Olga Bukatova and Kathi Shea, Azenta Life Sciences
and Kelsey Musall, Ossium Health

Azenta Life Sciences

The commercial success and therapeutic reliability of cell and gene therapies (CGTs) hinge not just on upstream processing, but on the precise orchestration of cryogenic handling — from storage through thawing to final administration. Recent translational studies and operational data highlight that the cryogenic cold chain requires validated, harmonized, and controlled solutions across the product lifecycle.

This article draws from presentations at the 2025 Cell Summit hosted by BioLife Solutions to integrate operational experience, clinical data, and cryobiological fundamentals into a cohesive, data-driven perspective on cold chain management in CGT.

Cryopreservation imposes distinct biophysical stress on cells, including intracellular ice formation, osmotic imbalance, and cryoprotectant toxicity. Controlled-rate freezing (CRF), often targeting freezing rates of -1°C/min followed by plunge into vapor-phase liquid nitrogen (LN2), is now standard for minimizing lethal intracellular crystallization. However, cryopreservation

is not an endpoint — it is the beginning of a continuum that includes storage, transport, and ultimately thawing. An example of the impact of the elements of the cryogenic cold chain Critical Quality Attributes (CQAs) of the CGT product is represented in the table below.

CQA Category	Product Attribute	Example of Impact of Cryo Cold Chain
Safety	Sterility / bacterial endotoxins, mycoplasma	• Direct contact between cryopreserved product and LN_2 liquid phase can compromise container closure integrity, increasing risk of microbial contamination (e.g., mycoplasma) and introduce safety risks for personnel. • Use of open, wet thawing methods (e.g., water baths) poses a contamination risk.
General	pH, osmolality	• CO_2 sublimation during dry ice transport can permeate polymeric containers, acidifying the product and altering pH/osmolality—especially in small-volume formulations.
Purity/impurities	Dead cells	• Transient warming events during cryogenic storage or transport (especially if Tg of water -135°C is breached) can lead to intracellular ice recrystallization and membrane rupture. • Inadequate or overly rapid thawing can trigger osmotic shock or excessive CPA exposure, increasing apoptotic or necrotic cell fractions. • Inadequate cold chain controls can result in increased apoptosis rates, lowering total functional cell recovery post-thaw, affecting intended dose delivery • Variability in thaw-to-infusion timing may skew viability-based release criteria
Function	Functional response, biological activity	
Content	Total cell number, cell concentration, viability	

Fig 1. Table: Impact of the elements of the cryogenic cold chain CQAs of the CGT product

Transient Warming Events: Unseen, But Unforgiving

While liquid LN_2 vapor-phase storage is the industry standard for cryopreservation, not all storage practices ensure true thermal protection. Samples must remain below the glass transition temperature (Tg) of water, -135°C, to avoid devitrification and cellular damage.

A recurring concern in cryogenic workflows is the occurrence of transient warming — short, unintended increases in sample temperature during routine handling, storage access, or transport. Though often overlooked, these events can significantly compromise cellular quality, especially when they breach Tg.

The data demonstrated by Kathi Shea illustrates lower viability and recovery of MSCs observed for temperature cycled cells (20 exposures to -110°C) compared to control (-190°C), after three months storage:

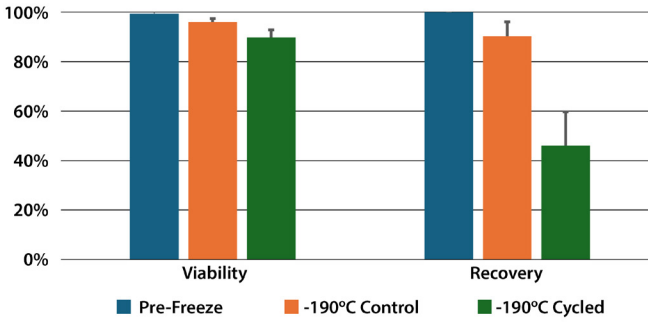

Fig. 2. Viability and recovery of mesenchymal stem cells pre-freeze and post-thaw

Examples of sources of transient warming:

- Manual lid opening during rack access
- Vial handling during identification or sorting
- Extended sample search time in crowded dewars
- During transfer on to thawing stations

Awareness and documentation of transient warming events must become part of Standard Operating Procedures (SOPs) and quality reviews, as they represent real deviations from intended storage protocols.

Storage: Is Automation an Answer?

Conventionally-used manual LN_2 freezers expose cell products to variability at every access point. Thermal mapping studies presented by Shea demonstrate that even brief rack exposure can trigger excursions above -135°C in neighboring samples — the threshold for devitrification and biochemical degradation.

In contrast, automated storage platforms (e.g., CryoArc™ from Azenta Life Sciences):

- Maintain uniform vapor-phase temperatures < -150°C
- Prevent transient warming via automated insulated retrieval
- Offer 21 CFR Part 11 traceability and real-time digital inventory

Automated storage systems allow significant stream-lining of the process of cryobox removal in both rapidity and warming rates of the samples.

Shea highlighted that it takes only 2 minutes to retrieve 1 cryovial from the CryoArc unit, while the manual process would require 11 minutes, subjecting the sample to higher transient warming risks: the automated retrieval demonstrates 70% slower

Fig 3. CryoArc Automated Cryogenic Storage Unit

warming rates in the first 30 seconds of exposure and a 51% slower crossing of Tg for innocent samples.

In-House Transport: Closing the Cryo Gaps

Cryopreserved materials routinely move between storage, manufacturing, and Quality Control (QC) areas. Shea presented data showing that dry ice-based transfer can allow sample cores to rise above Tg (-135°C) within minutes.

Fig 4. CryoPod Carrier for Biological Material Transport

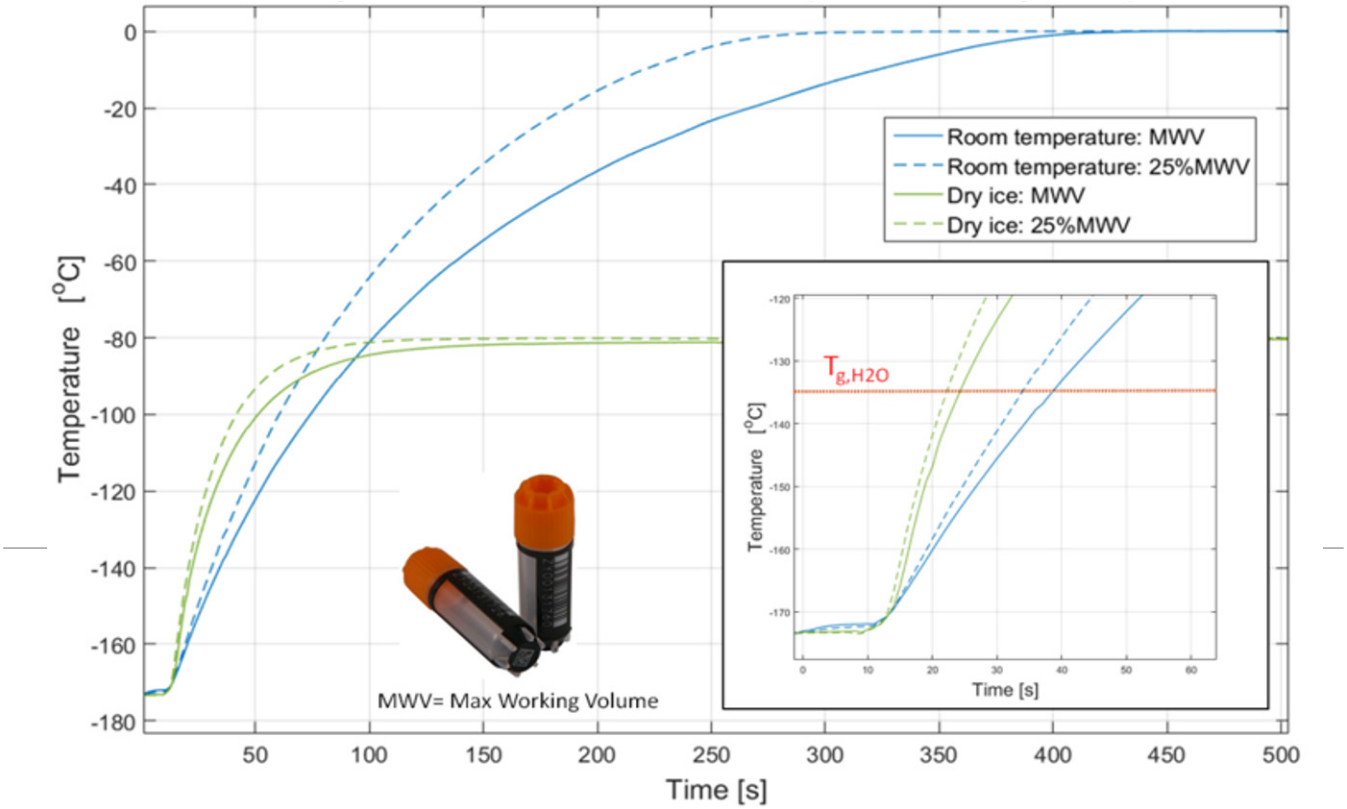

Fig 5. Sensitivity to Vial Filled Volume: Room Temperature and Dry Ice Exposure

In contrast, the CryoPod® Carrier maintained internal temperatures of < -150°C for over three hours, with onboard alerts and traceable excursion logs.

This minimizes the risk of unnoticed warming — which can lead to structural degradation or reduced functionality — especially during multi-room clinical manufacturing.

"Each transient warming event erodes material integrity, compliance, and ultimately discovery outcomes. Automation ensures consistent sample protection, real-time visibility, and audit-ready compliance — making the transition not just safer, but smarter." Kathi Shea.

Lessons Learned: Transitioning from Manual to Automated Cryogenic Systems

The shift from manual to automated cryogenic storage is not merely a technological upgrade, it requires comprehensive reengineering of infrastructure, digital integration, and operational workflows. Organizations that have successfully navigated this transition emphasize several key lessons:

1. Develop a Holistic Sample Management Strategy

Automated systems thrive on consistency and structure. Establishing clear protocols for sample identification, labeling, storage logic, and retrieval criteria is critical. This includes defining ownership, chain-of-custody controls, and lifecycle tracking aligned with regulatory expectations.

2. Standardize Container Types Early

For CDMOs and facilities managing diverse client requirements, early labware audits are essential. Incompatible tube sizes or container types can complicate validation and automation. Where feasible, labware should be consolidated and standardized,

particularly for QC samples, to streamline both physical storage and software logic.

3. Evaluate Physical Infrastructure

Automated storage platforms often require specific site conditions. Facilities must account for factors such as:

- Ceiling clearance for the insulated tower and lifting mechanisms (CryoArc can reach over 3 metres in maximal configuration)
- Floor loading capacity to support heavy, LN_2-filled systems
- Ventilation and oxygen monitoring for LN_2 safety compliance and avoiding frost build up

4. Prioritize Digital Integration

Automation is most effective when tightly linked with laboratory information management systems (LIMS). Robust integration supports:

- End-to-end sample lifecycle tracking
- Barcode/RFID SOPs (human- and machine-readable)
- Automated alerts and deviation logs
- Centralized dashboards for quality, compliance, and operational metrics

5. Build for Modularity and Scalability

Automated systems should support phased rollouts and scalable upgrades, including:

- Power and network bandwidth capacity
- Additional robotic modules or tank expansions
- Data storage and analytics flexibility for high-throughput environments

6. Establish Redundancy and Recovery Protocols

Business continuity planning is essential. Redundancy strategies may include:

- Dual-site storage or mirrored automated systems
- Backup LN_2 tanks or emergency manual access protocols
- Pre-validated recovery procedures in case of system malfunction or power loss

7. Enable Cross-Functional Implementation

Successful adoption of cryogenic automation involves collaboration across IT, Quality Assurance (QA), QC, engineering, and operations teams. Early alignment on objectives, validation plans, and data handling requirements accelerates deployment and ensures long-term sustainability.

When Warming Intentionally: Controlled Thawing

While thawing of cryopreserved cell-based therapies represents a critical step in the delivery of advanced therapeutics, it is often treated as a routine procedure rather than the scientifically governed, risk-sensitive operation. With increasing evidence that thaw conditions directly influence both viability and functional potency, controlled thawing must be viewed as a key quality and safety determinant.

Key thawing intervention points typically include thawing of cryopreserved starting material during GMP manufacturing for further processing or expansion, post-thaw handling of in-process or final product samples for QC testing and release assays, and preparation of the drug product for clinical infusion at the point-of-care setting.

Each instance of thawing introduces unique risks, but all demand the same principle: standardized, validated precision. Operational rigor, whether in cleanroom environments or at bedside, ensures that therapeutic potential is preserved across the product lifecycle.

Thawing is a biophysically dynamic phase that

exposes cells to multiple forms of stress. Ice recrystallization during slow or uneven warming, especially between -50°C and -10°C, can physically damage membranes and organelles. Simultaneously, solute concentration shifts create osmotic gradients that may cause swelling, dehydration, or lysis. Compounding these risks, cryoprotectants like DMSO become increasingly toxic as temperatures rise, with both dose and exposure time influencing the extent of cellular injury.

These combined insults can significantly reduce both viability and functional potential, the latter of which may not be immediately detectable with standard viability stains.

"Thawing is not the end of the process, it is the final gatekeeper of therapeutic integrity." Olga Bukatova.

Decades of cryobiology research have established best practices to counteract the risks of thaw-induced injury:

- **Rapid Warming is Critical:** Fast warming rates reduce the time spent in the high-risk zone of recrystallization, preserving cellular integrity. This principle is well-supported by Mazur's classical Two-Factor Hypothesis of cryoinjury.
- **Uniform Heat Transfer Minimizes Devitrification and Ice Growth:** Avoiding temperature gradients across the sample reduces the risk of localized damage due to uneven melting or ice nucleation.
- **Controlled CPA Exposure:** The cytotoxic effects of DMSO and other cryoprotectants increase with time and temperature. Efficient and timely dilution or removal post-thaw is critical for minimizing downstream functional loss.
- **Functionality ≠ Viability:** Cells may survive thawing but exhibit impaired performance, therefore, functional assays are essential complements to viability testing.

Olga Bukatova recapped the key considerations for the thawing process:

Parameter	Details	Operational Considerations
Duration	Passive warming and thawing duration Exposure to CPA, high osmolality Minimizing recrystallization	Transportation on dry ice: warming rates are higher than in ambient T
Formulation	CPA content Dilution strategy	Over 5 min thawing durations are common
Temperature	Thawing rate 50-100°C/min Target temperature 0-4° C, end-of-thaw criteria	Higher thawing rates can be applied to certain cell types (e.g. T cells at 39°C)
Packaging	Surface area to volume ratio (nominal volume vs fill volume) Overwraps	Container material and wall thickness (PP, COC, EVA)
Agitation	Minimizing thermal gradients	Should be delayed, avoiding potential damage to fragilized frozen container

Thawing and Mitigating Site-to-Site Variability in Clinical Settings

Presenting results from a recent study, Kelsey Musall explained how HLA-partially-matched cryopreserved allogeneic bone marrow from deceased donors was used to treat hematologic malignancies. To ensure product integrity at the point-of-care, a standardized, controlled thawing process using Barkey varitherm was implemented across bone marrow transplant units.

Core requirements included:

- Warming rate >100°C/min to prevent ice recrystallization
- Uniform thawing across the surface area of each bag
- Rapid temperature recovery between cycles for multi-bag workflows
- User-independent operation to reduce variability between technicians

Fig 6. Barkey varitherm, controlled dry thawing device

In her evaluation, Musall demonstrated that the Barkey varitherm device enables rapid and consistent warming, even when thawing multiple cryopreserved cell bags sequentially. The data presented showed that absolute viable cell counts and overall viability were comparable between the varitherm and conventional water bath thawing. However, water bath use was associated with greater variability in cell counts, suggesting that manual methods introduce inconsistencies, potentially due to operator technique or uncontrolled thermal gradients. These findings underscore the value of automated, precision-controlled thawing systems in achieving reproducible outcomes, particularly in clinical settings.

"Using a controlled thawing device ensures the last step of product delivery is held to the same high standard as the production process, guaranteeing the patient receives the best possible product." Kelsey Musall.

The point-of-care environment often lacks GMP controls, yet product integrity must remain uncompromised. Controlled thawing devices mitigate operator variability.

Key best practices for clinical thawing include:

- Defined thaw-to-infusion windows
- Training protocols for pharmacy and nursing staff
- Use of thawing logs and deviation flags

Musall emphasized that controlled thawing not only contributed to reduction of the inter-site variability, but also enabled bedside teams to handle products with confidence, improving compliance and patient safety.

Conclusion

Scientific data presented throughout The Cell Summit '25 confirmed that even brief excursions above the glass transition temperature of water (-135°C) during handling or transport can initiate irreversible biological damage, reducing both viability and cellular function. The automated and controlled solutions for storage and handling of cryopreserved material represent a critical evolution in ensuring product integrity, reproducibility, and compliance across the cell and gene therapy lifecycle. By minimizing human variability, preventing transient warming events, and maintaining samples consistently below critical thermal thresholds, these technologies form the foundation for a robust cryogenic cold chain—one that protects both therapeutic value and patient safety.

Likewise, thawing, historically treated as a routine end-point operation, was shown to be a critical control step. Studies from Ossium Health and others demonstrated that automated, precisely regulated thawing solutions reduce inter-operator variability and help maintain consistent functional recovery, particularly in decentralized clinical environments.

Ultimately, cold chain precision is quality by design. The future of cell-based therapies depends on our ability to maintain control not just in the lab, but in every link of the cryogenic lifecycle.

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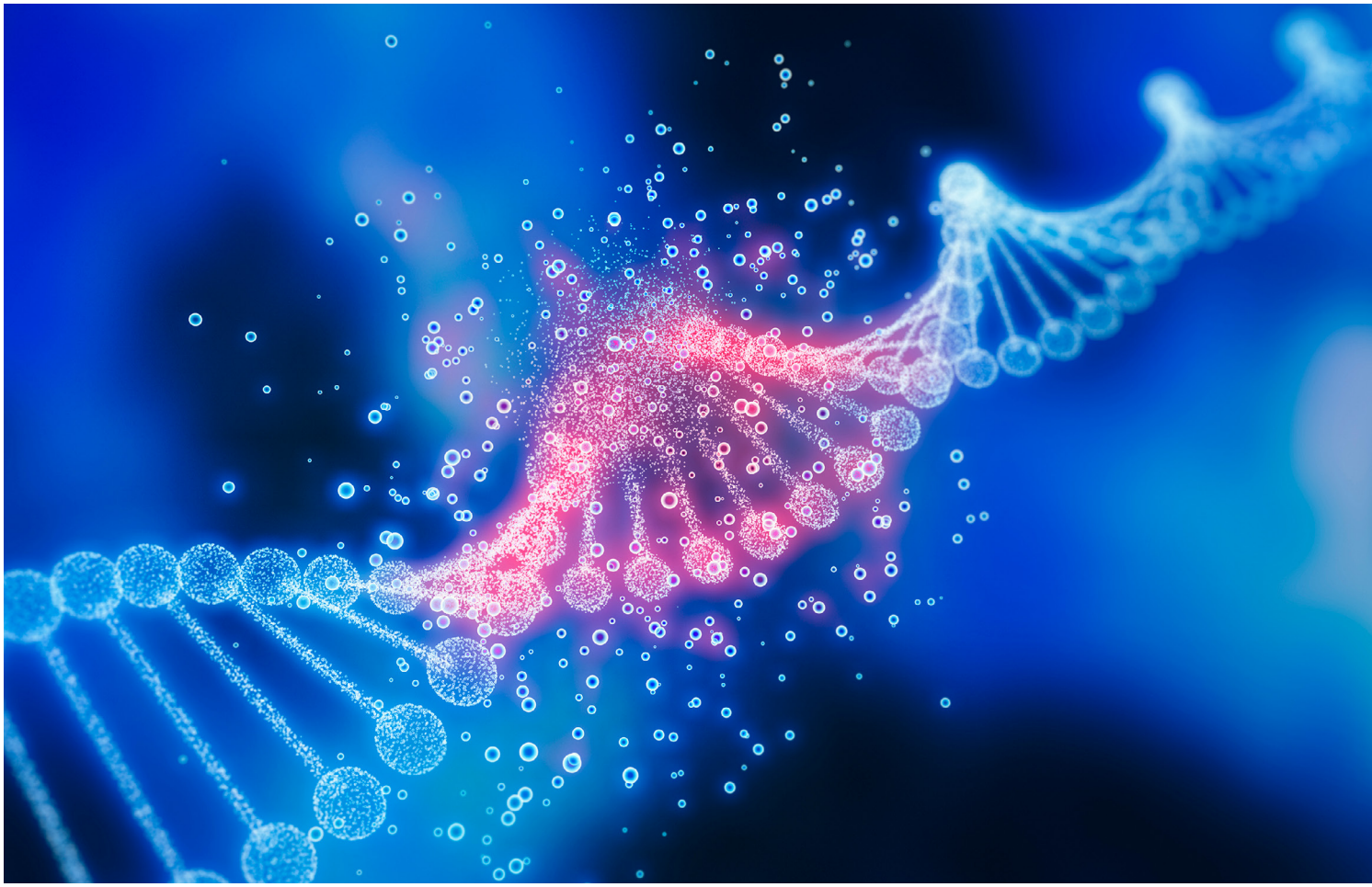
Closing Note: Preserving the Promise



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The insights captured throughout this eBook reinforce a simple truth: the future of cell and gene therapies depends not only on breakthrough science, but on how well we preserve, protect, and deliver the living cells that make these treatments possible. From implementing biopreservation best practices, to understanding the fundamentals of cryobiology, to applying cold chain precision and closed-system workflows, the collective expertise shared at The Cell Summit '25 demonstrates that progress is achievable when strategy, science, and practical application align.

If your team is navigating specific challenges or looking to refine your cryopreservation and cell processing strategies, we invite you to connect directly with our scientific team through our Ask the Scientists web page.

Together with our partners and the broader CGT community, BioLife Solutions remains committed to helping more therapies reach patients in need.

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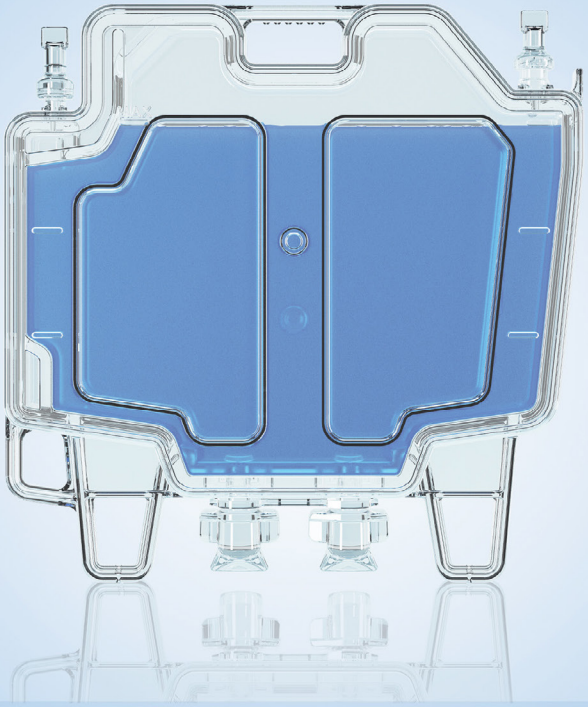

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