Simplifying Progress

Biopreservation in Cell Therapies — Streamlining Compliance with Pre-Clinical Safety Tests

June, 2024 | Maya Rotman, Katy McLaughlin

Keywords or Phrases:
Cold storage solution, Cryopreservation, Cell therapy, Clinical applications, Pre-clinical safety trials, GLP, Easy registration, High regulatory standards, DMF, NutriStor®, NutriFreez® D5

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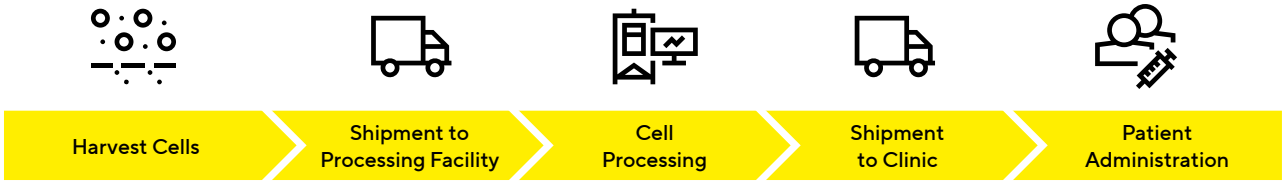
Introduction

As cell therapies continue to advance and the demand for treatments grows, the industry faces ongoing hurdles in ensuring their production processes deliver safe products, reproducible performance, scalability, and, ultimately, regulatory compliance. Ensuring product safety, demonstrating efficacy, and navigating complex and dynamic regulatory pathways that may lack harmonization consume valuable time and resources. With growing pressures to provide cost-effective medicines and cell therapies, developers need strategies to build efficient processes with tools and reagents that conform to the relevant standards.

Because the cells themselves are commonly the final product, effective cell therapy requires maintaining cell viability, cell recovery, and functionality along the complex supply chain (Figure 1). This relies heavily on the performance of reliable biopreservation solutions for short- and long-term storage and transport.

Figure 1: *Biopreservation in the Cell Therapy Supply Chain*



NutriFreez®	▪ Freeze the cells	▪ Liquid nitrogen	▪ Thaw the frozen cells ▪ Freeze before shipment	▪ Liquid nitrogen	▪ Thaw the frozen cells ▪ Remove NutriFreez*
NutriStor®	▪ Store in cold storage media	▪ 2 – 8 °C		▪ 2 – 8 °C	▪ Remove NutriStor*

In this white paper, we highlight the importance of selecting biopreservation media for cell therapy products as part of the regulatory strategy. We then discuss the value of pre-clinical safety assessments before showcasing the specific pre-clinical safety trials performed on our biopreservation media.

The Importance of Effective Biopreservation in Cell Therapies

Biopreservation media are critical products in cell therapy, as they ensure the timely transport of viable cell therapy products from the manufacturing site to the point of care. Moreover, because they interact directly with the final therapeutic product, their components are subject to critical quality and safety standards to avoid adverse patient reactions or compromised efficacy.

There are two options for cell preservation during cell therapies:

1. Cold | Hypothermic Storage

- Intended for short-term storage (several days)
- 2 – 8 °C
- Does not require the use of dimethyl sulfoxide (DMSO) or any other cryoprotectant agent (CPA)
- Does not require extra freeze | thaw stages during the cell preservation

2. Cryopreservation

- Intended for long-term and short-term storage
- Low and ultra-low temperatures, down to -196 °C
- Requires the use of a cryoprotectant agent, such as DMSO
- Requires freezing and thawing stages as part of the procedure

CPAs that help cells survive the freezing and thawing processes are often associated with cell toxicity, even at low concentrations, making them possibly unsuitable for some cell therapy applications. Nevertheless, there are immunotherapies currently on the market that contain DMSO. It is therefore crucial to select a biopreservation medium that not only preserves viability post-thawing but also ensures that cell functions and characteristics are maintained, while assuring patient safety.

Using products with proven pre-clinical safety study results helps reduce the failure of trials and avoid redundant steps during the development of clinical applications. Such a strategy will contribute to shorter timelines and more cost-effective development.

Biopreservation Solutions at Sartorius

Sartorius' portfolio of biopreservation solutions covers both options — cold storage and cryopreservation. NutriFreez® D5 and NutriFreez® D10 Cell Freezing Solutions and NutriStor® Cold Storage Solution are designed and validated for the preservation of cells, including sensitive cells such as primary cells, lymphocytes, stem cells, and T cells, making them suitable for cell therapy applications.

The animal component-free, chemically-defined formulations support consistent and reliable recovery after freezing and cold storage. NutriStor® and NutriFreez® users also benefit from extensive documentation to support their regulatory approval process, including drug master files (DMFs) and pre-clinical safety trial results.

Pre-Clinical Safety Tests

While the final drug product will be subject to rigorous safety testing, it is crucial for cell therapy developers to obtain preliminary safety data, particularly for excipients that will be present in the drug product. This is especially important given the often complex nature of the therapies being developed. As part of the stringent guidelines established by the U.S. Food and Drug Administration (FDA), drug developers must carry out pre-clinical studies before considering trials in humans. This is a preliminary step to investigational new drug (IND) submission and the early clinical trial stages. These studies are governed by FDA regulations, ensuring that the excipient meets the highest standards of safety and quality.

Both in vitro and in vivo systems are used to evaluate the safety of an excipient. This includes testing on different cell cultures (in vitro) to understand how the excipient might interact at the cellular level and conducting safety studies on animals in a controlled environment (in vivo).

The FDA defines the objectives of pre-clinical safety tests as:

1. Identify an initial safe dose and subsequent dose escalation schemes in humans
2. Identify potential target organs for toxicity and for the study of whether such toxicity is reversible
3. Identify safety parameters for clinical monitoring.

As such, pre-clinical safety tests provide valuable insights into the behavior of materials in relevant in vivo conditions. Reagents that have undergone pre-clinical safety testing come with a level of confidence that the products are safe and not toxic (under the test conditions) to an animal model. Through these studies, important insights are gained into critical factors like proper dosing, potential toxicity, and how the excipient might affect the patient.

This represents a huge step beyond establishing the reagent's efficiency and safety in in vitro models. The test results also provide an excellent starting point for further pre-clinical and clinical evaluations, as the results help inform decisions about moving forward with human trials, limiting the risk of failure.

A recommended first step in simplifying and shortening the approval process is to use highly regulated excipients that have already been evaluated during pre-clinical safety tests, such as our biopreservation solutions NutriFreez® D5 and NutriStor®. In the following sections, we outline our testing strategy and share findings from a range of tests that demonstrate that NutriFreez® D5 and NutriStor® revealed no signs of toxicity based on the criteria evaluated. We then present more detailed results for two example assessments: Acute systemic toxicity in mice and ISO systemic toxicity (14 days) in rats.

Pre-Clinical Safety Studies for NutriFreez® D5 and NutriStor®

Study Design
In our preclinical safety assessments, we carefully selected animal models based on species, sex, and age to ensure the validity of our results. The sample sizes and control (animals treated with 0.9% sodium chloride USP solution as opposed to the test article) were determined to provide enough data for meaningful scientific analysis.

We covered all possible administration methods for cell or gene therapy treatments and applied the most stringent case scenarios. The selected clinical dose was designed to exceed a simulation of administering 70 mL to a 70 kg human (1mL/kg), and the doses and concentrations administered were chosen to maximize our ability to detect any effects on the test system. The tests followed (and exceeded) the recommended maximal percentage according to the instructions for use (IFU).

While most safety studies involve administering a single dose, our studies include multiple dosage strategy models and one continuous study to assess systemic cumulative toxicity. This continuous study (2 weeks) involved 14 test article administrations over 14 days, ensuring thorough evaluation while minimizing discomfort to the animals. The tests are summarized in Table 1.

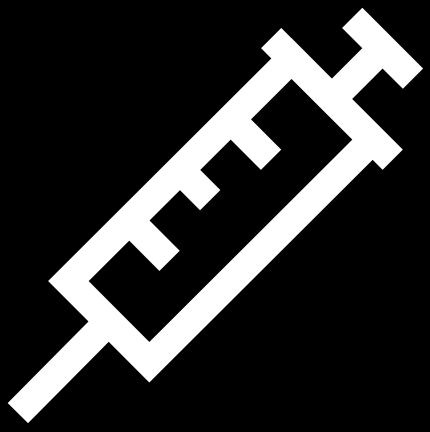
All tests were performed in compliance with the relevant ISO standards and were subject to the FDA regulations as mentioned under 21 CFR 58 known as GLP (Good Laboratoty Practice). Details are available upon request.

Results of Pre-Clinical Studies

Table 1: *Summary of Pre-Clinical Safety Studies for NutriStor® and NutriFreez® D5*

Study	Purpose	Group Size	Administration	Organism
ISO Modified Intracutaneous Study in Rabbits	Evaluating the potential of NutriStor® or NutriFreez® D5 to cause local dermal irritation following intracutaneous injections"	- 3 test - 3 control	Intracutaneous injection	Rabbit
ISO Guinea Pig Maximization Sensitization Test	Evaluating the potential of NutriStor® or NutriFreez® D5 to cause delayed dermal contact sensitization	- 10 test - 5 control	Intradermal injection and occlusive patch	Guinea pig
USP Rabbit Pyrogen Study	Evaluaing NutriStor® or NutriFreez® D5 for pyrogenicity and determining whether they induce a pyrogenic response following intravenous injection	- 3 test - Control: 2 temperature measurements were taken and served as baseline temperatures	Intravenous injection via the marginal ear vein	Rabbit
Acute Systemic Toxicity Study in Mice	Evaluating the acute systemic toxicity of NutriStor® or NutriFreez® D5 following injection in mice	- 5 test - 5 control	Intravenous injection	Mouse
ISO Systemic Toxicity Study in the Rat, Repeated Parenteral Administration of a Test Solution, 2 Weeks	Evaluating the systemic toxicity of NutriStor® or NutriFreez® D5 solution following repeated intravenous injections in rats for a period of 14 consecutive days	- 12 test - 12 control	Repeated intravenous injections	Rat

* Based on a human dose model of 70 kg human (1.0 mL/kg), the test article was administered at an approximately 10X safety factor.
** Based on a human dose model of 70 kg human (1.0 mL/kg), the test article was administered at an approximate 20X safety factor.
***All tests were administered separately with either NutriStor® or NutriFreez® D5. (10 tests altogether)



Procedure	Timeline	Result
▪ A 0.2 mL dose of the test article was injected intracutaneously into five separate sites on the right side of the back of each animal. ▪ The control was injected into five sites on the left side of the back of each animal	▪ Observations made immediately after injection, then at 24, 48, and 72 hours	▪ All animals appeared normal throughout the study and no erythema or edema were observed ▪ NutriStor® and NutriFreez® D5 met all requirements of the test
▪ Each animal received 6 injections of 0.1 mL test solution or control, undiluted or at different dilutions ▪ Following a recovery period, the test and control animals received challenge patches of the test solution and the vehicle control article	▪ All sites were scored for dermal reactions at 24 and 48 hours after patch removal	▪ Neither NutriStor® nor NutriFreez® D5 caused delayed dermal contact sensitization ▪ Both test articles were not considered sensitizers following this test
▪ 10 mL/kg of the test solution was intravenously injected into each animal via the marginal ear vein* ▪ Two control temperatures were taken at least 30 minutes apart. ▪ The second temperature was recorded no more than 30 minutes prior to the test article administration	▪ Temperatures were recorded at 30-minute intervals between 1 and 3 hours after injection	▪ No animal showed a temperature rise of 0.5 °C or more above its baseline temperature ▪ The total rise of the rabbits' temperature during the 3-hour observation was 0.1°C, which is within acceptable USP requirements ▪ No pyrogenicity was observed following NutriStor® or NutriFreez® D5 administration
▪ A single 20 mL/kg dose of the test article solution or control was injected into each animal**	▪ Animals were observed immediately and at 4 hours after dosing, and daily for 7 days ▪ Body weight was measured prior to dosing and daily for 7 days thereafter	▪ There were no mortality or evidence of acute systemic toxicity (clinical observations, weight loss) from the test article injected into mice ▪ NutriStore® and NutriFreez® D5 met the test requierments
▪ The test solution (10.0 mL/kg) or control were administered daily by intravenous injection at 10.0 mL/kg for 14 consecutive days ▪ Clinical observations, body weights, and more detailed health examinations were conducted	▪ Animals were observed immediately after injection for toxicity, general health observations were conducted daily, and detailed health examinations were conducted weekly ▪ Animals were weighed prior to the first dose and on days 8, 14, and 15	▪ According to clinical observations, body weights, hematology and clinical chemistry values, necropsy results, microscopic evaluation, organ weights, organ/body weight ratios and organ/brain weight ratios no evidence of chronic systemic toxicity was observed from NutriStore® or NutriFreez® D5 injected into animals

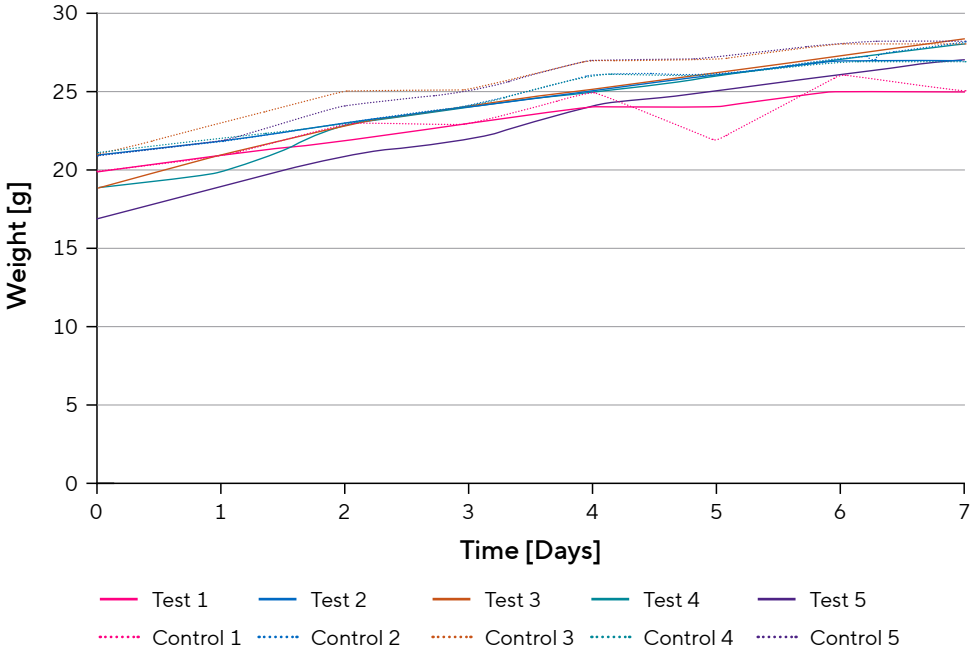
Visualization of Data From Two Example Tests

Acute Systemic Toxicity Study
The purpose of this test was to evaluate the acute systemic toxicity of NutriFreez® D5 Cryopreservation Solution, following injection in mice.

A single 20 mL/kg dose of the NutriFreez® D5 solution was injected into a group of five mice by the intravenous route. Similarly, a separate group of five mice was dosed with 0.9% sodium chloride USP solution as the control condition. The animals were observed immediately and at 4 hours after dosing and daily for 7 days. The animals were weighed prior to dosing and daily for 7 days thereafter. The animal weights were highly comparable between tests and controls. There was no mortality or evidence of systemic toxicity from the test article injected into the mice. NutriFreez® D5 Cryopreservation Solution met the test requirements.

Figure 2 shows the animal body weight during the study. Body weight remained consistent regardless of whether NutriFreez® D5 Solution (solid lines) or control (dotted lines) was administered.

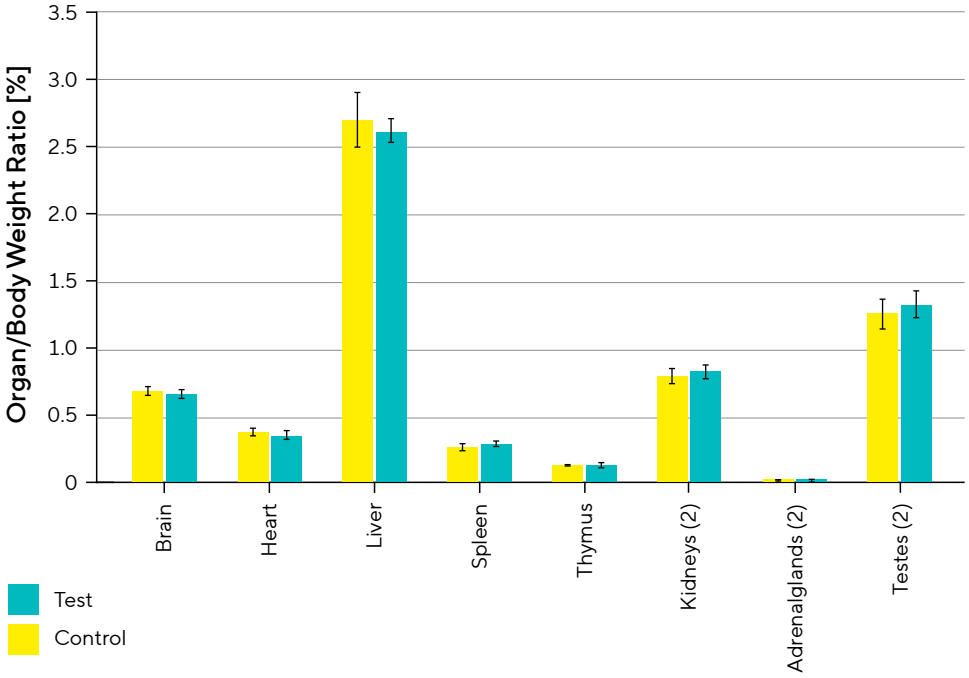
Figure 2: *NutriFreez® D5—Acute Systemic Toxicity Test Results*



ISO Systemic Toxicity Study (14 Days)
The purpose of this test was to evaluate the systemic toxicity of NutriFreez® D5 Cryopreservation Solution, following repeated intravenous injections in rats for a period of 14 consecutive days.

Twelve male and twelve female rats were randomly assigned to either the test or control group (six rats/sex/group). The animals received daily intravenous injections of the test solution at 10.0 mL/kg for 14 consecutive days. Control animals were similarly treated with 0.9% sodium chloride USP solution. Animals were observed immediately after injection for toxicity, general health observations were conducted daily, and detailed health examinations were conducted weekly. Animals were weighed prior to the first dose, and on days 8, 14, and 15. On day 15, blood samples were collected for hematology and clinical chemistry analysis, and the animals were euthanized. A necropsy was conducted, and selected organs were collected, weighed, and processed for microscopic evaluation (Figure 3).

Figure 3: *NutriFreez® D5 - ISO Systemic Toxicity Study (14 Days)*



Note. Summary of organ/body weight ratio in male rats following dosing for 14 consecutive days with NutriFreez® D5 (Test, Teal) or Control (Yellow)

Figure 3 shows that organ/body weight ratios in the male rats were similar between test and control groups. Clinical observations, body weights, hematology and clinical chemistry values, necropsy results, organ weights, and organ/brain weight ratios were similar between test and control groups. There were no microscopic changes considered to be related to NutriFreez® D5. Therefore, parenteral administration of NutriFreez® D5 did not produce systemic toxicity in rats.

Summary and Conclusion

Pre-clinical safety tests provide an excellent benchmark for further studies. They can reduce the need for time-consuming testing and optimization, and can streamline their regulatory approvals by enriching other safety data.

The results for NutriFreez® D5 and NutriStor® show an absence of toxicity in test animals. These insights can form part of a risk mitigation strategy, as they indicate that the excipients are well tolerated in animal models.

Therefore, Sartorius biopreservation solutions are bolstered with stringent clinical data, making them an excellent choice for simplifying interactions with regulatory authoriities during the development of cell and gene therapies.

For more information, visit
www.sartorius.com/en/products/cell-culture-media/cell-culture-reagents-supplements/cell-freezing-media

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Maya Holds an M.Sc in Medical Science from Bar-Ilan university and a B.Sc degree in Nutritional Science. She joined Biological Industries (later acquired by Sartorius) in 2015.

Her first position at BI was a QC analyst, and later she joined the advanced-Therapies team as a content-writer responsible for scientific materials for internal and market-oriented purposes. In 2021 Maya became a Product Manager and since 2023 has been responsible for Sartorius' Biopreservation Solutions.

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Katy is part of the Marketing Communications team at Sartorius, where she supports the creation of a variety of written pieces, from published articles to web content.

Before joining Sartorius in 2021, Katy was employed as a Post-Doctoral Research Associate at the University of Edinburgh, where she also completed her doctoral studies. Here, she carried out research in genetics and cellular biology and began taking on writing projects, eventually entering into a career as a freelance writer for various biotech companies and agencies.

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Status: 06 | 2024