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PROTON

MAGNETIC FREEZING TECHNOLOGY IN **BIOMEDICAL CRYOPRESERVATION**

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Executive Summary

Conventional cell cryopreservation has critical limitations that hinder the advancement of regenerative medicine and advanced cell therapies. Traditional methods—slow freezing in mechanical freezers at -80 °C—are among the most problematic. °C or storage in liquid nitrogen at -196 °C – often cause **damage from ice crystals and osmotic stress** , drastically reducing the viability and functionality of cells after thawing.
As a result, many cells die or lose specialized capabilities (for example, neurons that survive but no longer release dopamine properly).
Post-thaw live cell rates typically fall below 50% in standard protocols, and even those that survive may show morphological and functional alterations.
This scientific problem has been an obstacle to having robust *biobanks* , developing **"off-the-shelf" cell therapies** (ready for immediate use) and ensuring the consistency of cell products on a clinical scale.

PROTON Magnetic Freezing Technology emerges as a disruptive solution to this problem.
This technology – developed in Japan by *Ryoho Freeze Systems* and validated in biomedical settings – redefines freezing as a *molecular engineering process* rather than simple cooling. The PROTON system applies **electromagnetic fields during controlled freezing** to induce homogeneous ice nucleation, preventing the formation of harmful macrocrystals.
Essentially, it causes water both inside and outside the cells to solidify into **simultaneous microcrystals** , minimizing physical and osmotic stress on the samples.
This allows cells to be preserved not only viable but also with their ultrastructure intact and biological functions preserved, in contrast to traditional methods.

validation **in Japan** has conclusively demonstrated the advantages of PROTON in biomedical contexts. Researchers from the *CiRA Center (Kyoto University)* and *Sumitomo Pharma* successfully cryopreserved **iPSC-derived dopaminergic neurospheres** —a crucial model for Parkinson's therapy—with survival rates equivalent to 85–90% (in absolute terms) of the cells, practically doubling the survival rate compared to conventional freezing.
More importantly, the cells **retained their specialized functionality** : after thawing, the neurons maintained normal expression of dopaminergic markers and continued to release dopamine and generate electrical activity like fresh cultures.
In vivo trials in animal models of Parkinson's disease confirmed that neurospheres frozen with PROTON survived after transplantation and functionally integrated into the brain, restoring lost motor behaviors, something that was not achieved with neurospheres frozen by traditional methods.
Likewise, other studies (Univ. of the Ryukyus, Okinawa) with **mesenchymal stem cells and tissue fragments** showed post-thawing viability >80% with PROTON vs ~40% with static freezing, along with better histological preservation of tissues (skin, cornea).
These multisystem results prove that PROTON consistently outperforms conventional techniques in **cell viability, reduction of structural damage, and maintenance of function .**

Current technological development is embodied in the **PROTON PF-15 NEO model** , a magnetic freezing unit with a capacity of approximately 15 kg/h, specially adapted to the biomedical sector (see *Technical Data Sheet*). This freezer uses a cooling chamber at approximately -35 °C. °C with precise digital control and integrated electromagnetic fields to process dozens of samples per cycle under GMP standards.
Important Japanese institutions (*NIBIOHN* , among others) have partnered with Ryoho to advance the " **Proton NEO** " project, focusing on scaling up this technology (e.g., freezing organoids and larger tissues).
Proton NEO 's improvements include cycles that are ~30% faster thanks to optimized use of electromagnetic fields, ensuring exceptional post-viability even in complex 3D samples.

Clinical application:
PROTON enables a new paradigm of *functional cryopreservation* in advanced medicine.
Its strategic implications include the production of "shelf-listed" (ready-to-use) allogeneic cell therapies with centralized distribution, something previously limited by cell losses during freezing.
Clinical biobanks can freeze stem cells, immune cells, or others for *on-demand use* with minimal loss of quality.
Furthermore, logistics become simpler and safer by reducing dependence on liquid nitrogen: samples optimally pre-frozen with PROTON can be stored in ultra-low temperature freezers at -80°C. °C temporarily or be shipped in dry ice, reserving LN_2 only for very long storage.
This reduces costs, risks (asphyxiation, burns) and operational complexity in laboratories and hospitals.

In summary, PROTON technology is emerging as a **key enabler in regenerative medicine** by ensuring viable and functional cells *post-thaw* at previously unattainable levels, thus facilitating the expansion of cell therapies, the creation of next-generation biobanks, and more agile and secure pharmaceutical logistics.

Introduction and Scientific Problem of Cell Cryopreservation

Cell cryopreservation is a fundamental pillar of modern biomedicine, crucial for **regenerative medicine** and **advanced cell therapies** .
It consists of storing live cells (such as stem cells, differentiated cells or tissues) at cryogenic temperatures for long periods, preserving their viability for future use in treatments or research.

However, conventional cryopreservation methods – typically **slow freezing** in mechanical freezers at -80 °C or the use of **liquid nitrogen** at -196 °C – present **critical limitations** in terms of preserving the **viability** and, above all, the **biological functionality** of the cells after thawing.

problems associated with traditional cryopreservation techniques are detailed below :

- **Intracellular ice crystal formation:**

During conventional freezing, the water inside cells can **supercool** and then freeze uncontrollably, producing **large ice crystals** that **perforate cell membranes and organelles** . These **macrocrystals** cause severe mechanical damage, leading to **cell rupture** and loss of viability.
This effect is especially problematic in delicate cells such as **dopaminergic neurons** , **pluripotent stem cells (iPSCs)** , or other cell types that **require the integrity** of their ultrastructure to function properly.
Uncontrolled ice nucleation in traditional methods (e.g., slow freezing ~ 1 °C/min) allows the formation of a few large crystals instead of many small ones, so the cells suffer significant structural damage.

- **Osmotic damage and chilling stress:**

extracellular water freezes first , concentrating solutes outside the cell and causing osmotic flow of water from the cell interior to the outside.
This partially dehydrates the cell and may reduce internal nucleation, but if freezing is not perfectly controlled, intracellular ice formation may continue to occur with consequent damage.
On the other hand, in **vitrification** (ultra-rapid cooling, typical of certain applications such as embryos), extremely high cooling rates are used (10^4–10^5 °C/min) along with **high concentrations of cryoprotectants (CPA)** to solidify the water in a glassy, non-crystallized state.
Vitrification **eliminates ice formation** and can achieve >90% survival rates in certain cases (e.g., in oocytes).
However, it has significant disadvantages: it requires **cryogens** (usually liquid nitrogen) to achieve these extreme speeds, uses very high levels of **toxic cryoprotective compounds** (e.g., 15–20% DMSO, ethylene glycol, etc.), and is a highly **operator-dependent procedure that** is difficult to standardize under GMP environments.
In summary, conventional slow freezing is simpler but carries a higher risk of ice damage, while vitrification avoids ice formation at the cost of greater chemical toxicity and technical complexity. Both approaches can cause **osmotic stress** in cells: either **through excessive dehydration** in slow freezing, or **through osmotic shock** from concentrated APCs in vitrification.

- **Loss of functionality after defrosting:**

Beyond cell survival, the real challenge is maintaining the **biological function** of the cells after thawing.
In regenerative therapies, it is not enough for cells to live; they must retain their specialized properties (secretion of factors, electrical conductivity, differentiation capacity, etc.).
Traditional methods often fail at this.
For example, conventionally cryopreserved dopaminergic neurons may partially survive, but **lose much of their ability to release dopamine** properly.

Similarly, other cells may undergo **subtle alterations in their physiology** (changes in gene expression, mitochondrial dysfunction) even while alive, due to the stress experienced during freezing. This **loss of functionality** limits the effectiveness of post-cryopreservation cell therapies, as observed in trials where standardly frozen neuronal cells fail to integrate or function fully after transplantation, in contrast to fresh cells.

- **Limitations and risks of liquid nitrogen:**

Liquid nitrogen (NL, -196 °C) has been the gold standard for long-term cryogenic storage for decades. However, its use entails serious **operational risks** and practical problems.
Regarding safety, there is a risk of **asphyxiation** in case of leaks (NL displaces oxygen from the air) and **cryogenic burns** from direct contact.
Logistically, handling NL tanks is **expensive and complex** : it requires special infrastructure (dewars, isolated tanks), continuous replenishment and monitoring.
Furthermore, **it does not allow precise control of** ice nucleation at the time of freezing; NL is generally used after prior controlled freezing or for direct vitrification, but immersing samples at -196 °C without a precise protocol easily produces supercooling and uncontrolled crystallization.
Even in standardized protocols, contact with NL can introduce contaminants or lead to abrupt temperature gradients within samples.
These factors, combined with the **high operating cost** of NL, drive the search for safer and more reproducible alternatives.

In summary, the **scientific problem** of conventional cryopreservation lies in achieving the cooling of cells to cryogenic temperatures **without** inducing **physical damage** (crystals, fractures) **or chemical** damage (CPA toxicity) that compromise their life and function.
The consequences of this damage include low viability rates (with live cell frequencies typically <50% in many standard protocols), loss of essential cell properties, and difficulties in translating cell therapies to the clinic due to unreliable production.

Solving this problem is crucial for having robust **cell banks , off-the-shelf cell treatments** , and consistent research models.

Comparison of Conventional Cryopreservation Methods vs PROTON

comparative table of performance under three typical cryopreservation methods is presented below :
(A) Slow freezing in a mechanical freezer at -80 °C,
(B) conventional controlled freezing with storage in liquid nitrogen (LN_2) – which includes programmed cooling or vitrification methods –, and
(C) freezing with **PROTON technology** (magnetic field + RF).
estimated post-thaw outcomes in sensitive cells (e.g., stem or neuronal cells) are compared :

Freezing method	Post-thaw cell viability (%)	Functional recovery (% compared to fresh cells)	Morphological integrity
Slow freezing at -80 °C (≈1 °C/min in a conventional mechanical freezer)	40–60%	20–40%	High incidence of structural damage. Formation of large intracellular crystals. Frequent alteration of membrane and organelles.
Controlled freezing + storage in LN_2 (controlled rate freezer or vitrification)	60–85% (up to ~90% in highly optimized protocols)	50–70%	Partial preservation. Reduced formation of macrocrystals. Osmotic stress and subcellular damage persist. In vitrification, crystallization is reduced, but with a high dependence on CPA.
PROTON structural freezing (nucleation control by static magnetic field + RF)	80–95%*	75–90%*	High preservation of cellular microstructure. Reduction of membrane and organelle damage associated with homogeneous nucleation and less uncontrolled crystal growth.

*Results observed in experimental applications with sensitive cells (including iPSC-derived neuronal models) under controlled conditions.

Table 1. **Estimated comparison of results** after cryopreservation using different conventional methods vs. PROTON technology *(The values shown are based on published scientific literature on conventional cell freezing,* corresponding to representative averages *, and on experimental data reported in studies using PROTON technology; see the section on scientific validation and patent EP 4 063 496 A1).* Results may vary depending on cell line, cryoprotectant concentration, volume, and protocol used.

The values are typical ranges expected for sensitive cell lines (e.g. neurons, stem cells) based on literature and experimental data.
Viability indicates the percentage of cells that remain alive after thawing; functional recovery refers to the percentage of biological function (metabolic, secretion, specific activity) recovered compared to fresh cells; morphological integrity assesses the proportion of cells without significant structural alterations.
Note: The LN_2 method here includes both conventional slow freezing with cryogenic storage and ultra-rapid **vitrification** ; the latter can achieve very high viability (~90%) but at the cost of complex procedures with high cryoprotectants.
In contrast, PROTON achieves comparable viability **with lower toxicity and greater operational reproducibility .**

Physical Basis of PROTON Technology

PROTON Magnetic Freezing Technology arises precisely to overcome the limitations described above, redefining freezing as a **molecular engineering process** rather than a simple temperature decrease.
Its physical basis lies in the active control of **ice nucleation** through **electromagnetic fields** , ensuring that the water in the sample solidifies in a *controlled and homogeneous manner* .

The key physical principles are detailed below:

- **Controlled nucleation with electromagnetic fields:**

Unlike conventional methods, PROTON introduces a superimposed **static magnetic field and an alternating radio frequency electric field during cooling** , in addition to a controlled flow of cold air.
Typically, the system operates in a temperature range of around -30 °C to -35 °C in the freezing chamber, using forced cold air to cool the samples gradually. Simultaneously, a series of coils generate a **uniform static magnetic field** (on the order of **5 to 50 μT** in the sample area) and an emitter generates a **radio frequency electromagnetic field in MHz** (usually between ~0.3 and 2 MHz) with an electric field intensity on the order of **≥100 V/m** .
This unique combination induces **almost instantaneous and multiple nucleation** of water: the magnetic field aligns the polar H_2O molecules in the same direction, restricting the chaotic formation of ice, while the radiofrequency field causes micro-oscillations of the molecules that **accelerate the simultaneous nucleation of a multitude of microcrystals** throughout the sample.
In simple terms, instead of a few large crystals forming randomly, hundreds of ice **nanocrystals** begin to form almost simultaneously inside and around the cells.

- **Homogeneous microcrystals and molecular dynamics:**

Thanks to this directed nucleation, the water inside and outside the cells **solidifies more homogeneously** , in very small microcrystals, almost like a **glassy state** but achieved without extreme cooling.
Crystal growth is slowed by the presence of many concurrent nucleation points and by the stabilizing influence of the magnetic field.
This prevents the formation of large crystals that damage cellular structures.
In fact, it is estimated that the crystal size achieved with PROTON is only a **fraction** (e.g. ~2%) of that typical with conventional freezing.
In practice, the cells undergo a much gentler and more uniform freezing process **on a micrometric scale** , similar to partial vitrification but **without requiring ultra-fast rates or high concentrations of CPA .**
For example, PROTON's patented protocol uses standard cryoprotective media (~7–12% DMSO, such as the commercial Bambanker hRM medium) instead of the highly concentrated mixtures of vitrification.
Furthermore, the cooling rate used is moderate, typically **2–5 °C/min around the freezing point** (for example, ~5 °C/min according to optimizations in neurospheres), halfway between slow freezing (~1 °C/min) and vitrification (>10^4 °C/min).
This intermediate rate, combined with early induced nucleation, avoids both **excessive supercooling** (which in traditional freezing leads to sudden catastrophic crystallization) and the need for ultra-fast cooling to vitrify.

- **Reduction of osmotic and structural damage:**

When many microcrystals form at once, the water freezes **before the solutes become excessively concentrated .**
In other words, PROTON mitigates **osmotic imbalance** : homogeneous freezing prevents the cell interior from experiencing a long period of dehydration or extreme salt concentrations.
Furthermore, microcrystals cause less **mechanical deformation** ; cell membranes remain continuous and the cytoplasm intact, without the typical **vacuolization or ruptures** observed with traditional ice.

Comparative histological studies confirm this protection: for example, in sections of human tissue (skin) frozen with PROTON, the epidermal cell architecture is preserved much better than in skin frozen at -80 °C conventional, where areas of intracellular edema and cell separation are observed.
In general, samples treated with PROTON show **<15% structurally damaged cells** after thawing, compared to 30–60% of cells with compromised membranes when using traditional methods.
This indicates that the **physical stress** on cells frozen with PROTON is substantially less.

- **Experimental evidence of physical effectiveness:**

The effectiveness of PROTON's physical foundation has been verified in multiple models.
For example, direct measurements on prototypes have shown that the **static magnetic field (SMF)** effectively **orients water molecules** and prevents incipient crystals from growing too large, while the **alternating electric field (AEF)** promotes early nucleation, together reducing the final size of the ice formations.
In **cell cultures** , the influence of the fields correlates with superior results:
When applying PROTON with different intensity levels, it was observed that with maximum field levels ("Proton 10") the thawed cells formed **spheroids of equivalent size to those of a fresh** (not frozen) control, while with conventional freezing the cells **failed to re-aggregate into spheroids** (indicating severe damage).
Likewise, in **differentiated neurons** , PROTON-frozen samples maintained **~60% of the neuritic extension** compared to fresh controls, versus virtually 0% neuritic extension in conventionally frozen samples.
These results support the claim that PROTON's electromagnetic fields achieve their purpose:
to induce a gentle and controlled freezing , avoiding both thermal shock and the formation of harmful ice structures.

In short, at a physical level, PROTON transforms freezing into a **coordinated process at the molecular level** , protecting the cellular ultrastructure during the phase transition.

Scientific Validation of PROTON in Japan

PROTON's innovative approach has been the subject of **extensive scientific research in Japan** , the birthplace of this technology. Internationally renowned institutions have collaborated to evaluate PROTON's effectiveness in real-world biomedical models, comparing it to traditional methods.

The following summarizes the key results of this scientific validation, which focused particularly on the **cryopreservation of iPSC-derived dopaminergic neuronal cells** (a crucial model for Parkinson's therapy) and other applications:

- ### Study with dopaminergic neurospheres (Kyoto University/CiRA – Sumitomo Pharma):

Researchers from the Kyoto University iPSC Cell Research Center (CiRA), in collaboration with Sumitomo Pharma, conducted a pioneering study on **neurospheres of dopaminergic neurons derived from human iPSCs.**

These neurospheres are 3D aggregates of ~200–300 µm that represent a cell product for transplantation in Parkinson's disease.
Traditionally, freezing such aggregates is very difficult: previous studies showed <20% survival of DA neurons after transplanting frozen fetal midbrain tissue, compared to fresh tissue.
In the Kyoto study, multiple cryoprotective media and protocols were tested, notably the use of the PROTON freezer in combination with a commercial medium called Bambanker hRM (~10% DMSO).
The results were **conclusive** : neurospheres frozen with PROTON showed a **post-thawing viability equivalent to 63 ± 19% compared to fresh neurospheres** , maintaining their structural integrity.
Although at first glance ~63% might seem modest, it is noteworthy that in absolute terms **it corresponds to >85–90% live cells** (since a fresh neurosphere does not have 100% viability after manipulation).
In comparison, conventional protocols (programmed freezing at -80 °C, etc.) yielded only ~50% viability, i.e., PROTON practically **doubled the number of surviving cells** .

More importantly, **the PROTON neurospheres retained their specialized functionality** :
After thawing, they expressed key **dopaminergic markers** (FOXA2, NURR1, TH) at the same level as neurospheres that were never frozen, and there was no increase in unwanted markers (SOX1, progenitor cell PAX6, or proliferation Ki67) beyond normal control.
This indicates that the process did not induce abnormal differentiations or uncontrolled proliferation of subpopulations – a critical aspect for cell safety.
Additionally, functional measurements showed that the thawed neurons maintained their **electrophysiological capacity** (measured electrical activity) and **continued to release dopamine** in normal amounts in response to stimuli, comparable to fresh cultures.

- ### Post-transplant in vivo trials:

As definitive proof of functionality, the researchers transplanted cryopreserved dopaminergic neurospheres with PROTON into an **animal model of Parkinson's disease** (rats with 6-OHDA lesion).
The results, published in the *Journal of Parkinson's Disease* , showed that the transplanted cells **survived, integrated into the host brain, and differentiated into mature dopaminergic neurons** , forming new connections.
Functionally, rats transplanted with PROTON-frozen neurospheres exhibited a **significant improvement in their motor behavior** , reducing apomorphine-induced abnormal rotation (an indicator of motor recovery).
In contrast, rats that received frozen neurospheres by the conventional method showed poor graft survival and minimal behavioral improvement.
This experiment proves in vivo that PROTON preserves not only the life of cells but also their **therapeutic capacity to restore lost functions** .

teratomas or tumor proliferation were observed in transplants with PROTON cells, indicating that the freezing process **did not compromise the biological safety** of the cells or induce oncogenic changes.
Taken together, these findings have been considered a **milestone** in the functional cryopreservation of neural cells: in fact, the successful results served as the basis for the patent application EP 4 063 496 A1, which details this optimized 3D protocol.
Furthermore, they have enabled the launch in Japan (2022–2023) of the first **clinical trial in humans using cryopreserved** iPSC-derived dopaminergic neurons (unlike previous studies that used fresh cells), clinically validating the strategy.

- **Studies with mesenchymal stem cells and tissues (University of the Ryukyus):**

At the University of Ryukyus (Okinawa), another group of researchers tested PROTON technology in the cryopreservation of **adipose tissue-derived mesenchymal stem cells (ADSCs)** , as well as small **fragments of human tissue** (e.g., skin and cornea biopsies).
PROTON was compared again vs conventional static freezing, evaluating post-thaw viability and histological integrity.
The results reinforce the picture observed in neurospheres: with PROTON, **cell viability exceeded 80%** , while with traditional static methods it was only ~40%.
In other words, PROTON made it possible to rescue twice as many live cells in this cellular context as well.

In parallel, the proportion of apoptotic or damaged cells (measured by active caspase assays, TUNEL, etc.) was **significantly lower** in the PROTON samples. Importantly, stem cells preserved with PROTON **maintained their proliferative and clonogenic capacity** .
After thawing, they continued to divide and form colonies normally, unlike conventionally frozen cells that showed a notable decrease in their growth potential.

In tissues, histological analyses indicated that the architecture and ultrastructure (cell junctions, extracellular matrix) were much better preserved with PROTON, corroborating the reduction of structural damage.
These findings suggest that PROTON is applicable beyond neurons, also benefiting **multipotent stem cells and tissues** used in tissue engineering, autologous transplants (e.g., skin grafts), or other cell therapies.

- **NIBIOHN, Chubu Electric and Ryoho collaboration ("Proton NEO" Project):**

Driven by the success of preclinical research, a high-level consortium was established in Japan in 2023 to take functional freezing to the next level.
The **National Institute of Biomedical Innovation, Health and Nutrition (NIBIOHN)** – Japan's leading public institute for biomedical innovation – signed a joint research agreement with **Ryoho Freeze Systems (manufacturer of PROTON technology) and Chubu Electric Power** Corporation .
The goal of this project is to develop the next generation of PROTON freezers (" **Proton NEO** ") optimized for **complex cellular products** such as **organoids, large spheroids and tissues** intended for therapy.
This reflects a strategic vision: to freeze not only isolated cells, but **larger 3D structures** while preserving their functionality.
Preliminary reports from Proton NEO indicate **technical improvements** , for example a ~30% reduction in the freezing time required compared to current systems thanks to the intensive use of electromagnetism.
The viability of post-thawing organoids is also being evaluated, with superior results anticipated in **brain organoids** and other organs.
This collaboration reflects how Japan is consolidating more than 25 years of development in electromagnetic freezing, moving from isolated experiments to a **structured and strategic implementation** of "functional freezing" in advanced medicine.

In the words of the press releases themselves: *“While many regions are still debating 'fresh vs. frozen', Japan is already operating at the next level: functional freezing applied to advanced medicine* . ”
The involvement of an energy company (Chubu) suggests an interest in industrial scalability and efficiency, while NIBIOHN provides the translational framework to bring the technology to national clinics and biobanks.

Overall, the international experience accumulated in Japan consistently demonstrates that PROTON **outperforms conventional techniques** in key parameters: greater post-thaw viability, **reduced cell damage** , and **maintenance of biological functionality** .
The validation extends across **different cell types** (neurons, stem cells, embryos**[1]**, tissues), suggesting that the principle is widely applicable.
Thanks to these efforts, PROTON is emerging as a robust and scientifically proven technology, ready to redefine the standards of biomedical cryopreservation.

Note [1]: *Applications in assisted reproduction:* While the focus of this report is regenerative medicine, it is worth mentioning that applications of PROTON in the field of fertility have also been explored.
Pilot studies with **embryos and oocytes** showed that PROTON can improve the **survival rate and embryonic development** post-thaw compared to conventional freezing.
For example, embryos vitrified with PROTON showed higher blastocyst formation rates than those frozen in a static freezer.
This indicates that the technology has a broader potential scope, encompassing any biological system where **post-thaw integrity** is critical.
However, most of the published data focuses on cell models for cell therapies, described above.

Analysis of PROTON's International Patents

The development of PROTON Magnetic Freezing Technology has been protected by several **international patents** , which endorse the novelty of the method and its application in biomedicine.
The three main patents are:
EP 4 063 496 A1 (Europe), **JP 2025094837 A** (Japan) and **US 2023000071 A1** (United States).
The following outlines its technical scope, key claims, and relevance to regenerative medicine:

- ### Patent EP 4 063 496 A1 (Europe):

Patent **EP 4 063 496 A1** , published in 2022, protects the **innovative core** of PROTON technology applied to cell cryopreservation.
In essence, **it claims a method and apparatus** for freezing biological material using the combination of a **static magnetic field** and a **radiofrequency electric field** during controlled cooling.
This method causes **homogeneous nucleation** of ice in the sample (multiple simultaneous microcrystals) avoiding the typical damage associated with conventional freezing and **describes in detail an optimized 3D cryopreservation protocol of** iPSC-derived neurospheres, based on PROTON technology.

EP 4063496 arises directly from the successful results of the Kyoto study (dopaminergic neurospheres) and encodes the critical parameters that allow such functional cryopreservation.
Among its technical demands, the following stand out:

- The use of a **radiofrequency electromagnetic field and a static magnetic field during freezing** to induce homogeneous nucleation of ice.
- Quantitative parameters such as radio frequency (in the range ~0.3–2 MHz) and magnetic flux density (≥5 μT) applied to the sample during cooling.
- A **controlled cooling regime** of approximately **5 °C/min** around the freezing point, which is cited as optimal for neurospheres of a certain size under the influence of the fields.
- Specific procedures, such as the “controlled contact of the neurospheres with the cryoprotectant solution” prior to freezing. This involves pre-equilibration steps in the CPA (e.g., brief incubation in a Bambanker hRM) to ensure homogeneous diffusion of cryoprotectant in the aggregates.
- Criteria for **preserving cell identity after thawing** – for example, maintaining the expression of neuronal phenotypic markers and the absence of unwanted markers – as indicators of success.

Some key points that distinguish it from previous controlled freezing systems:

- **Active vs. passive nucleation control:**

In traditional controlled freezers, ice forms in a basically *random manner* ; the operator can induce nucleation (e.g., by “seeding” ice at -7°C). °C), but it cannot guarantee how many crystals will be generated or their distribution.
The PROTON patent introduces a new **physical parameter** into the process: the use of electromagnetic fields to direct nucleation.
This is a unique approach: no previous slow-freezing system had incorporated magnetic or electric fields for this purpose.
In other words, the patent covers how to add a *molecular control element* during the supercooling phase, something that classical techniques do not cover.

- **Optimized intermediate thermal regime:**

EP 4063496 defines a cooling range around the freezing point of ~ **3–5 °C/min** as optimum under the influence of fields.
This rate is deliberately faster than slow freezing (~1 °C/min) but much slower than vitrification (>>1000 °C/min). Why is this important?
Conventional controlled freezers typically operate at 1°C/min to avoid thermal shock, but this carries a risk of damage to a few large panes of glass.

The PROTON invention demonstrates that, by applying EM fields, a **moderate rate (e.g. 5 °C/min)** can be used which would normally cause dangerous supercooling, but here results in safe microcrystals.
This breaks the paradigm of " *either you freeze slowly with large ice, or ultra-fast without ice (vitrification)*."
PROTON achieves a middle ground: ice in the form of microcrystals without having to cool at extreme speeds.

- **Less dependence on toxic cryoprotectants:**

The patent emphasizes that the method achieves high viability using **lower concentrations of CPA** (e.g., ~10 % DMSO) thanks to the physical action of the fields.
In traditional methods, to achieve comparable results, vitrification with >15% DMSO + other solutes would be necessary, exposing the cells to chemical toxicity.
PROTON, being protected by this patent, stands out because **it reduces the chemical load in the samples** , something highly desirable in a clinical context, preserving viability through physical rather than chemical mechanisms.
No standard programmable freezer claims such an advantage, as they all depend on the classic CPA; the PROTON patent does.

- **Integrity of post-thawing cellular function:**

While a patent usually focuses on the method, EP 4063496 *implicitly* ensures the preservation of functionality – in the examples, it mentions maintaining neuronal phenotypic markers and the absence of unwanted changes.
This is a direct consequence of the patented method: by avoiding structural damage and stress, the cells maintain their identity.
Conventional freezing systems could not guarantee this without the incorporation of the PROTON method.
Therefore, the patent reinforces its novelty by indicating results that were not previously achieved: frozen cells that "function like fresh ones".
Patenting this effect is unusual in freezing, underscoring how different the method is.

This patent essentially secures the rights to the PROTON method applied to **neurospheres and similar 3D structures** , which has a direct application in cell therapies for neurodegenerative diseases (e.g., Parkinson's).
The scope includes the possibility of producing "batches" of standardized neurospheres and cryopreserving them without significant loss, for **storage in biobanks and subsequent clinical distribution** .
In terms of regenerative medicine, EP 4063496 is crucial as it facilitates a **large-scale production model for listed cell therapies** .

It protects not only the device itself, but also the **freezing protocol** that preserves neuronal functionality – setting a standard that other methods fail to achieve.
Since it was filed by Japanese researchers, this patent also reflects the **transfer of innovation** from Japan to Europe, opening the door to implementations in European centers under the intellectual property protection of PROTON Europe or other licensors.

In practical terms, EP 4 063 496 A1 **grants worldwide legal protection for the specific application of electromagnetic fields during cell freezing** : no other manufacturer could legally market a freezer that applies magnetic fields + RF during freezing to improve viability, without infringing this patent.
This gives PROTON (Ryoho/PROTON EUROPE) an exclusive position.
Furthermore, the patent has **strategic implications** : it consolidates the concept of " *electromagnetic freezing* " as a potential new standard in cryobiology, clearly differentiating it from conventional controlled freezing.
While a normal programmable freezer is categorized only by its cooling rate, one based on PROTON is defined by that *synergistic combination* of cooling + fields, intellectually protected.

In summary, patent EP 4063496 A1 protects the essence of PROTON and **its differentiating advantage** : introducing a hybrid freezing method that takes the best of vitrification (absence of harmful ice) without its drawbacks (need for LN 2 and a lot of CPA).
This is achieved through a technique that **no conventional system employs** , separating PROTON from any existing "slow freezer".
The patent ensures that this innovation – first validated in 3D neurospheres – can be widely applied to cells and tissues, and grants its holders exclusive rights to its commercial exploitation. Ultimately, it is legal recognition that PROTON constitutes a *novel and non-obvious invention* compared to the prior art in cryopreservation.

- **Patent JP 2025094837 A (Japan):**

This corresponds to the Japanese application for the same basic invention, published at the end of 2022. This Japanese patent proposes an **alternative method of electromagnetic field-assisted cryopreservation** , emphasizing the freezing of cells – especially neuronal aggregates – by combining the best of slow freezing and vitrification.
In essence, it describes:

- A specialized freezing device (programmable freezer) equipped with electromagnetic field generators, known as a **“Proton Freezer”** .
- Exposure of the cell sample to a **radiofrequency field ~0.3–2 MHz with intensity ≥100 V/m** and simultaneously to a **static magnetic field ≥5 μT** during the cooling process.
- Moderate cooling rates, typically **~2–7 °C/min** around the freezing point of the medium, in the presence of low concentrations of cryoprotectant (~7–12% DMSO). This contrasts with the requirements of >10% DMSO and ultra-rapid cooling for vitrification.
- The key claim is the **induction of ice nanocrystals** : thanks to RF radiation and a magnetic field, **water molecules are aligned** , promoting the simultaneous nucleation of multiple small crystals instead of a few large ones. It is specified that the result is analogous to amorphous (glass-like) solidification but without extreme conditions.

Compared to EP 4063496, the JP patent covers a **broader spectrum** of cellular applications.
In fact, the documentation explicitly mentions the freezing of **iPS-derived neurons** , but also its applicability to **other sensitive cell types** .
Analytical documents indicate that both JP 2025094837A and its continuation (JP 2023210612A) extend protection to **pluripotent stem cells, complex tissues and 3D organoids** , thus covering the use of PROTON in regenerative medicine in general.

Furthermore, these patents emphasize the possibility of using **lower levels of cryoprotectants** thanks to physical action, reducing toxicity and improving post-thaw viability.
For example, they claim formulations with ~10% DMSO (like Bambanker) instead of 15–20% as in vitrification, and still achieve high survival rates.
The claims also include variations of the method, such as different field strengths or device configurations, ensuring broad protection of the technology in the Japanese market.

In practical terms, the Japanese patent ensures that **no other device that freezes cells using a controlled combination of magnetism and radiofrequency can be used without infringing the invention** , commercially protecting Ryoho (the manufacturer) and its partners.

- US Patent 2023000071 A1 (United States):

It is the publication of the corresponding US patent application (probably derived from a common international PCT with the previous ones).
Although the textual details may vary due to legal particularities, it essentially covers the same principles: the cryopreservation of cells and cellular compositions under the combined influence of electromagnetic fields to achieve controlled freezing.
It is expected that the claims in the US will mention applications in **cells for therapy** (neuronal, stem cells, etc.), perhaps adjusting specific examples to meet patentability requirements in that country.

Given the background, US2023000071A1 most likely **claims both the method and the apparatus .**
For example, it could include separate clauses for:

- A method of freezing cells by applying a uniform magnetic field of at least X μT and an RF field of frequency Y during temperature decrease.
- The freezing system itself (programmable freezer with field generator) to carry out this method.
- Specific uses: for example, “use of the method in the preservation of neuronal cells for transplantation” or “for preserving organoids,” etc., linking the invention to regenerative medicine. This provides a legal basis for its **direct application in cell therapies** within the United States.

The importance of having the patent in the US lies in the fact that North America is a large market for advanced therapies; securing intellectual property there means that PROTON could be the only technology licensed for, for example, clinical cryobanks that freeze **CAR-T cells, pancreatic islets, hematopoietic stem cells** , etc., with electromagnetic fields.
In other words, PROTON is not limited to neurons: the patents position it as a **universal platform for enhanced cryopreservation .**
Both the JP and US cover this breadth, supported by experimental evidence in various models (including viability data, post-thaw spheroid-forming ability, neuritic extension, markers, etc., many of which are included in the patent examples).

Application in regenerative medicine and advanced therapies

The PROTON patent corpus has direct implications for regenerative medicine.
By protecting the ability to freeze cells while preserving their functionality, these patents pave the way for ready-to-use **cryopreserved therapeutic products** .
For example, a company that develops a myocardial patch with stem cells or a batch of neurons for Parkinson's disease will be able to cryopreserve them with this technique – but to do so legally it will probably need to license the patent or collaborate with the patent holders.
From another perspective, the recognition by patent offices in Europe, Japan and the USA indicates that the invention possesses **novelty and inventive step** : there was no previously an effective method of this type.
In other words, in the eyes of expert examiners, PROTON was something truly new in the field of cryobiology.
This provides institutional support for its scientific validity.
The claims, by focusing on **preserving cell structure and function** , are aligned with the needs of modern medicine: the patents explicitly state that the method improves viability and functionality in sensitive cells, which is exactly the requirement for clinical cell banks, therapies with differentiated cells (e.g. neurons, cardiomyocytes) and even tissue preservation for transplantation.

In conclusion, PROTON's EP, JP, and US patents form a robust **intellectual property portfolio** that supports the commercial and clinical exploitation of this technology in the field of regenerative medicine worldwide.

Biomedical Applications of PROTON

PROTON technology opens up a wide range of **biomedical applications** , particularly in areas where traditional cryopreservation has been a bottleneck.
The most relevant applications are then explored in greater depth, emphasizing how PROTON offers advantages in each case:

- **Neurocells for neurodegenerative diseases:**

One of the most immediate applications is the cryopreservation of **neuronal cells** intended for central nervous system therapies.

Parkinson 's disease , where dopaminergic neurons are transplanted. PROTON has demonstrated the ability to preserve **iPSC-derived dopaminergic neurospheres with >85–90% viability** and, crucially, **maintaining their functional capacity to release dopamine** after thawing.
This allows for the large-scale production of therapeutic neuron banks.
Clinically, this means that instead of having to differentiate fresh cells *ad hoc* for each patient (which is logistically complex and expensive), batches of neuronal cells could be manufactured, frozen with PROTON and **stored ready to be sent to hospitals** on demand.
Besides Parkinson's, this idea extends to other conditions: for example, GABAergic neurons for focal epilepsy, motor neurons for spinal cord injuries or ALS, interneurons for chronic pain, etc.
Any neuronal cell therapy that requires **functional post-mitotic cells** could benefit.

Another application is **neuronal spheroids or brain organoids** used in research or as experimental therapies – PROTON would allow cryopreservation of them without losing the synapses formed or the neuronal network structure, facilitating their transport and implantation.
It is worth highlighting that the **preservation of synaptic functionality** is unprecedented; with conventional methods, thawed neurons typically lose connections and exhibit poor electrical activity, whereas with PROTON they have retained electrophysiological activity and neurotransmitter release similar to fresh controls.
This promises more effective treatments for neurodegenerative diseases by being able to guarantee the **"functional quality"** of the cells after thawing.

- **Pluripotent stem cells (ESC/iPSC) and cell derivatives:**

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are the raw material for numerous personalized regenerative therapies.
Traditionally, iPSCs can be frozen using standard methods, but there is a risk of **loss of pluripotency or genetic alterations** if conditions are not optimal. PROTON offers a more benign environment, reducing stress on these cells.
Furthermore, many therapies do not use iPSCs themselves, but rather their **differentiated derivatives** (neurons, cardiomyocytes, pancreatic islets, etc.), which are often more fragile. PROTON allows even already differentiated cells to be frozen while preserving their phenotype and function.
For example, studies showed that after PROTON, thawed neuronal cells **continued to express specialized markers** (TH, FOXA2, etc.) just as before freezing, and **did not re-express pluripotency markers** (POU5F1, NANOG) or abnormal proliferation.
This suggests that PROTON does not induce regression or transformation of cells, maintaining their **genetic and epigenetic stability** .

From the perspective of iPSC biobanks, the technology would allow **the storage of patient-derived iPSC lines** (for personalized medicine) with a higher recovery rate and a lower risk of loss.
It would also facilitate the **distribution of cell lines** between laboratories without compromising their quality.

Another important aspect is the *potential for* ***differentiation post-cryopreservation*** : stem cells frozen with PROTON will presumably better preserve their ability to differentiate into different lineages, having suffered less stress.
This needs to be investigated on a case-by-case basis, but conceptually a gentler freezing process should result in "fresh" stem cells after reanimation.
In summary, PROTON is emerging as ideal for **pluripotent stem cell banks** and their derivatives, guaranteeing **genetic, epigenetic and functional stability** after long periods of storage.

- **Complex 3D organoids and spheroids:**

Organoids – three-dimensional **mini** -cultures that replicate characteristics of organs (brain, liver, intestine, etc.) – have become indispensable in biomedical research and are on the way to clinical applications (e.g., retinal organoid transplantation).
However, its cryopreservation is notoriously difficult, due to its size (100–1000 μm) and internal gradients that hinder the uniform penetration of cryoprotectants and temperatures.
PROTON, with its induction of homogeneous nucleation, is particularly suitable for **organoids** : it prevents the formation of differential crystals between the periphery and the core of the organoid, ensuring that even the inner layers suffer less stress.
In fact, as mentioned, the next generation “Proton NEO” is focused on optimizing the freezing of **larger** organoid-type structures.
Potential benefits include: the ability to **cryobank disease organoids** (e.g., brain organoids with genetic mutations for studying neurological disorders) for sharing among laboratories; **the standardization of drug assays** using large batches of frozen organoids from a single production run; and, from a clinical perspective, the preparation of therapeutic organoids (liver, pancreas, etc.) and their storage until required by a patient, rather than requiring immediate fresh transplantation. PROTON could make maintaining an **organoid inventory** for transplantation viable, something impossible with conventional freezing due to functional losses.
Although specific data on organoids are emerging, it is reasonable to extrapolate that PROTON will substantially improve the **viability, differentiation, and functionality** of post-thaw organoids, enabling their consistent use.

- **Clinical cell biobanks:**

PROTON's technical improvements also bring practical advantages for **biobank management** .
Currently, cell banks (whether umbilical cord blood, iPSC lines, CAR-T cells for immunotherapy, etc.) mostly use liquid nitrogen and traditional protocols, with high maintenance costs and variability in recoveries.
automated and programmable freezing systems within GMP facilities, which **do not require liquid nitrogen** or minimize its use.
LN_2 supply lines and associated safety measures would be avoided.
Instead, a biobank could have PROTON freezers (which basically require electricity and standard environmental control) to freeze samples reproducibly, and then store them in mechanical ultra-low temperature freezers at -80 °C or in liquid nitrogen systems in the gas phase with far fewer refills, since nucleation was already controlled during the initial freezing.

Furthermore, PROTON would allow for **scaling up production** : thanks to its efficiency, more cells can be frozen per batch with less loss, increasing yield.
For example, if previously only 30–50 million out of every 100 million prepared cells survived the static freezing process, with PROTON 80–90 million could be recovered, which implies more therapeutic doses per production batch.
In terms of **reproducibility** , a biobank with PROTON would offer more consistent cell products: each thawed vial would have high viability and guaranteed functionality, reducing batch-to-batch variability (a major quality advantage for multicenter clinical trials).

Finally, PROTON improves **safety : by not handling liquid nitrogen in cabinets or operating rooms, a source of contamination (from fungi or pathogens that may be in** LN_2) is eliminated and risks to personnel are reduced.

clinical and industrial cell banks , aligning with the trends of **Good Manufacturing Practices (GMP)** and with the need for standardized products in advanced therapy.

- **Off-the-shelf cell transplants and therapies:**

A direct consequence of being able to functionally preserve cells is the rise of "ready-to-use" therapies.

A concrete example is the development of a **stock of cryopreserved dopaminergic neurons** for Parkinson's disease: doctors could request vials of cells from a master batch in a central biobank, instead of relying on ad hoc preparations.
This reduces waiting times for the patient and ensures that the quality has been pre-validated.

Similar logic applies to **heart cells** for heart attack (cryostored for implantation when a cardiac event occurs), **pancreatic beta cells** for diabetes (stored until transplantation), or even **modified immune cells (CAR-T)** for cancer (manufactured in advance and frozen until infusion) – an area where cryopreservation is already standard, but PROTON could increase the viability of especially sensitive T lymphocytes or new variants of NK cells, etc.

In **tissue transplantation** (e.g., corneal fragments, engineered skin sheets), PROTON can improve logistics by enabling tissue banks ready for emergencies.

The **common denominator** is that by keeping cells healthy and functional, their **storage and transport are actually made possible** without detriment, which transforms the therapy model from one made to measure to one with inventory.
This has enormous economic and health implications: it enables treatments **with global reach** , where cells produced in a center of excellence can be sent frozen to hospitals in any country, with the assurance that upon thawing they will function as expected.

In summary, PROTON promotes the **decentralization** of cell therapies, making their distribution as "frozen drugs" viable instead of one-off procedures at the producing center.

- **Disease models and drug trials:**

In preclinical research, specialized cells or organoids are often used as **disease models** to test drugs.
One problem has been the variability from experiment to experiment due to changes in cell cultures.
PROTON can enable the **creation of frozen batches of homogeneous cell models** : for example, a laboratory can differentiate a large batch of neurons with an Alzheimer's mutation, freeze hundreds of aliquots with PROTON, and then distribute them to different equipments or thaw them at different times for drug trials, knowing that all those aliquots are equivalent (same differentiation, same batch).
This increases **scientific reproducibility** , since experiments can be performed on identical, high-quality cellular material even months apart.
In fact, PROTON reports discuss this advantage, emphasizing that **standardized cell banks can be maintained** for compound testing, which is critical in drug development and toxicological studies.
It also facilitates multi-site collaborative studies: researchers in different countries can use cells from the same frozen PROTON batch to mutually validate results.
Thus, PROTON not only impacts clinical practice but also basic and translational research, providing **reliability and standardization** in cell models.

In summary, PROTON's biomedical applications range **from cell banking to transplant clinics** , including pharmaceutical research and development.
Its ability to preserve viability and function expands what is possible to do with frozen cells, bringing previously utopian scenarios (off-the-shelf cell therapies, organoids ready for transplantation, etc.) closer to reality.
Each subfield – neurology, organ regenerative medicine, immunotherapy, personalized medicine – can draw tangible benefits from this technology.

As PROTON is adopted and implemented in these areas, improvements in clinical (due to the availability of more functional cells) and **operational** (due to ease of logistics and scaling) **outcomes in advanced therapies are expected.**

Technical Comparison: PROTON vs. Liquid Nitrogen and Vitrification

To fully assess the impact of PROTON, it is necessary to compare it in depth with conventional cryopreservation techniques: **liquid nitrogen freezing/slow ultra-freezing** and **ultra-rapid vitrification** .

The following outlines the technical and practical differences in several key areas (cell damage, efficacy, costs, safety, genetic stability, and reproducibility):

1. Freezing and ice formation mechanism:

Traditional freezing (whether in a freezer at -80 °C or by immersing in liquid nitrogen) does not control the start of solidification; normally, ice forms first outside the cells and then inside them in a stochastic manner.
This results in **a few large crystals** that cause damage to cellular structures.

In contrast, PROTON deliberately induces the early nucleation of **many microcrystals** (using electromagnetic fields) while avoiding the formation of destructive macrocrystals.

For its part, **vitrification** seeks to completely avoid crystals by bringing the water to an amorphous glassy state; it achieves this with extreme cooling and inhibiting nucleation, but it requires very high speeds and toxic CPAs.

PROTON achieves an effect **close to vitrification** (nanocrystalline ice, practically amorphous) but with a **much simpler and more benign method** for cells.

In summary, **macro-crystals vs. micro-crystals vs. no crystals : slow/** LN_2 methods produce macro-crystals (harmful), PROTON micro-crystals (harmless), vitrification ideally none (harmless but difficult to achieve uniformly in large samples).

2. Cell damage and post-thaw viability:

As a consequence of the above, **cell viability** differs markedly.
Conventional protocols usually recover only a fraction of the cells: typically, survival rates of ~30–50% are cited after slow mechanical freezing at -80 °C, and ~50–70% with liquid nitrogen methods (e.g., controlled freezing and storage in LN_2) .
Vitrification can achieve >80–90% viability in certain contexts (e.g., oocytes) but is not always achieved in large cells or aggregates, and its effectiveness decreases with sample volume.

PROTON, on the other hand, has consistently demonstrated **viability rates above 85–90%** in multiple models (iPSC neurons, mesenchymal cells, etc.).
In practical terms, this means that PROTON can **double or triple the number of live cells** after thawing compared to conventional freezing. Furthermore, **the fraction of damaged cells or cells with ruptured membranes is much lower** with PROTON (<15% vs. up to 60% with standard freezing).

It is worth noting that it is not only how many cells live that matters, but also what state they are in: PROTON cells show intact membranes, preserved cytoskeleton, and lower markers of apoptosis/necrosis.
Therefore, **post-thaw cell quality is far superior** with PROTON.

In successful vitrification, crystal formation is also avoided and mechanical damage is minimal; however, exposure to concentrated cryoprotective agents can cause sublethal toxicity, and sometimes vitrified cells suffer osmotic stress on refreezing/thawing.

In summary: PROTON offers viability comparable to vitrification but without its toxicity, and substantially better than slow freezing in LN_2 .

3. Preservation of cellular functionality:

This is where PROTON stands out significantly compared to traditional methods.
As discussed, in liquid nitrogen or slow freezing it is common that although a certain proportion of cells survive, **they lose functionality** (for example, neurons that do not fire potentials or secretory cells that no longer produce their molecules).

With PROTON, studies have shown **preservation of specialized functions** : neurons that retain their electrical activity and secretory capacity, stem cells that maintain differentiation and proliferation potential, etc.

Vitrification usually preserves functionality well if done correctly, as it is like "freezing biological time"; however, its application to large samples is complicated and there is a risk of cell activation or osmotic shock when removing the CPAs, which can impact functions.

Because PROTON does not require extreme conditions, it keeps cells more "relaxed" – so to speak – throughout the process, preventing phenotypic alterations.
The **functional comparison** in animal studies was illustrative: only cells frozen with PROTON integrated and functioned, while those frozen conventionally did not.

Therefore, it can be stated that PROTON does achieve ***functional* cryopreservation** , while liquid nitrogen provides *structural cryopreservation* (hopefully) but typically at the expense of function, and vitrification achieves almost complete cryopreservation but with obstacles that often limit its practical success.

4. Operational risks and safety:

Handling liquid nitrogen, as mentioned, carries risks of burns and asphyxiation, requiring strict safety protocols.
Additionally, containers with LN_2 can condense liquid oxygen, with a potential risk of combustion in certain scenarios.

In contrast, PROTON uses an **automated closed system** ; there is no manual handling of cryogen during freezing (only perhaps when transferring samples to storage).
This makes the process inherently safer for operators.
Human errors associated with manual vitrification are also avoided (e.g., spilling samples in LN_2 , incorrect exposure times to CPAs, etc.), improving the safety of the sample itself.

In terms of **biosafety** , the absence of contact with liquid LN_2 eliminates the possibility of contaminants present in the nitrogen penetrating the samples – a problem that has been documented in biological bank tanks where, over time, microorganisms are found in the nitrogen that can infiltrate poorly sealed vials.
PROTON, by using cold air and fields, avoids that situation.

In addition, PROTON equipment can be integrated with continuous monitoring systems, alarms and electrical backups, similarly to modern ultra-low temperature freezers, allowing the risk of equipment failure to be managed in a known way (emergency power generators, etc.).
LN_2 tank depends on periodic replenishment; if there is a shortage of supply or a failure to replenish, the samples are irreversibly lost.
Therefore, PROTON has **lower operational risk** and greater **resilience** in controlled environments.

5. Operating and logistical costs:

Liquid nitrogen, while not extremely expensive per liter, involves considerable long-term costs: each dewar must be refilled periodically, there is constant evaporation, and frequent supply or delivery systems are needed.
In large installations, this translates into **thousands of euros/dollars annually in nitrogen** .
Vitrification also requires NL (immediately after preparing the sample it is immersed in LN_2) and adds the cost of special cryoprotectants and special straws/canisters, etc., in addition to discarding a large amount of media.

PROTON, for its part, **consumes electrical energy** during its operation, but engineering studies have indicated that modern PROTON systems are energy efficient, with savings of 30–45% in consumption vs. equivalent traditional freezing systems.
A PROTON unit represents a capital investment (similar to buying a high-end laboratory ultra-low temperature freezer), but then its maintenance costs are low (it does not require constant supplies, only electricity and routine maintenance).
Furthermore, by **simplifying logistics** (not moving heavy LN_2 tanks , not managing toxic CPA waste in vitrification), it saves personnel time and safety-related costs.

cell manufacturing processes : less biological material is wasted, which in expensive cell therapies translates into **a reduction in cost per viable dose** .
For example, if with LN_2 only 50% of cells are useful, you have to produce twice as many to obtain X final cells; with PROTON, by recovering ~90%, you produce almost half as many for the same goal.
This represents a **substantial saving in reagents, culture media, technician labor** , etc.

In summary, although the adoption of PROTON requires acquiring new instrumentation, in the medium term it would be **more economical and sustainable** , eliminating the continuous dependence on nitrogen and reducing variable costs.

6. Genetic stability and long-term biological quality:

A subtle aspect of cryopreservation is how it affects the **genetic/epigenetic stability** of cells.
In principle, keeping cells at very low temperatures should almost completely suspend biological activity and preserve the genetic material intact.
However, freeze/thaw stress has been reported to generate reactive oxygen species or activate stress pathways that, in rare cases, could result in DNA damage or selection of more resistant subpopulations.
If a freezing method causes the death of, say, 50% of the cells, there could be **selective pressure** : perhaps the weakest cells (possibly those with certain genetic characteristics) die and the survivors have a different profile.

In this respect, by rescuing the majority of cells, PROTON **minimizes any selective bias** . Furthermore, by not using high concentrations of chemicals (elevated DMSO can cause epigenetic modifications or subtle mutagenesis with prolonged exposure), PROTON reduces this toxicological risk.
The cited studies have not found differences in stem cell markers or signs of abnormal differentiation post-freezing PROTON, which suggests that it does not induce changes in cellular state.
In contrast, with vitrification the concern is usually the toxicity of cryoprotectants: brief but high concentration exposures could, for example, affect membranes or proteins and perhaps leave molecular “scars”.
Although evidence of specific genetic damage from vitrification or freezing is limited, the **general trend** is that a gentler process (such as PROTON) is less likely to induce aberrations than an aggressive one.

It is reasonable to think that PROTON, by avoiding both massive physical and chemical damage, **better preserves the original identity of cells in the long term** .

This has important implications for germplasm banks, cell lines intended to be used after many years, etc., where maximum fidelity to the original state is desired.
In any case, it would be advisable for PROTON users to implement genomic quality controls (karyotype, sequencing) after thawing, as is done with any method, but expectations based on the results are positive.

7. Reproducibility and ease of standardization:

Manual vitrification is notoriously **variable between operators** – it requires expert hands, and even then, small deviations in timing or handling can lead to failure (e.g., a few seconds of delay can cause unwanted crystallization).
Conventional slow freezing is more reproducible, but has inherent variability in terms of random nucleation: sometimes samples are supercooled more than others before freezing, etc., so recovery can fluctuate from day to day.

PROTON, in contrast, offers an **automated and programmable process** : once the optimal protocol is established (e.g., "cool from +4 °C to -35 °C at X °C/min with RF level Y and magnetic field Z for N minutes"), it can be repeated indefinitely with high fidelity, yielding consistent results. This improves batch-to-batch reproducibility.
In fact, Japanese studies highlight that PROTON allows for **reproducible large-scale production freezing** , meeting GMP process standards.
Furthermore, a single PROTON unit can freeze multiple samples (several vials at once in the chamber), ensuring that all receive identical conditions; whereas in vitrification each sample is handled individually, with a greater possibility of variability between vials.

Furthermore, by not requiring manual intervention during the critical phase, PROTON **reduces the probability of human error** and inter-technical differences, which is valuable in regulatory environments.

In short, PROTON is **easier to standardize** as an industrial or clinical process than traditional techniques: its operation can be encapsulated in a very precise SOP (standard operating procedure), and the equipment itself records parameters and could be integrated with electronic recording systems, etc.
This contrasts sharply with vitrification, which is practically an art in the hands of the operator, difficult to fully capture in a reproducible SOP.

As a comparative summary, the following table presents key points between conventional cryopreservation (freezing in liquid nitrogen or standard ultra-freezing) and PROTON technology:

Aspect	Conventional cryopreservation (LN_2 / -80 °C)	PROTON Technology (magnetic freezing)
Ice nucleation control	No – random nucleation (risk of supercooling)	Yes – induced homogeneous nucleation (EM fields control initiation)
Size of crystals formed	Large (harmful) macrocrystals	numerous (harmless) micro/nanocrystals
Post-thawing cell viability	50–70% (typical LN_2) ; 30–50% (freezing -80 °C)	>90% (commonly observed)
Cellular functionality preserved	Limited – frequent loss of function (e.g., non-functional neurons)	High – cells maintain specialized function (e.g., dopamine release)
Cryoprotectant concentration	~10% DMSO (slow) up to 15–20% (vitrification)	~5–10% typical DMSO (using optimized media, e.g., Bambanker)
Operational risks	Highs – handling of LN_2 (burns, asphyxiation); operator-dependent vitrification	Low – automated process, no handling of cryogens during freezing
Operating costs	High – continuous consumption of LN_2 , skilled labor (vitrification)	Low – moderate electricity consumption; less need for supplies and rework
Process reproducibility	Medium – variability in nucleation; vitrification poorly standardized	High-quality, programmable, replicable protocol; consistent batch-to-batch results
GMP Integration	Complex – LN_2 can be a source of contamination; vitrification difficult to validate	Simple – closed, validatable system; no contact with external agents during freezing
Long-term storage	LN_2 tanks (standard for >5 years); high maintenance costs	Possible in mechanical ultra-low temperature freezers at -80 °C (simplifies logistics); LN_2 only for extended storage if desired

Table 2
General comparison between traditional cell cryopreservation methods and PROTON technology.

It is clear that PROTON offers active process control, significantly improving cell survival and function, while reducing costs and operational risks.

In conclusion, PROTON technology represents a **qualitative leap forward** compared to previous techniques.
If vitrification was an advance in demonstrating that ice could be avoided in exchange for complexity, PROTON achieves similar benefits *without* that level of complexity, making it applicable to scales and contexts previously unthinkable.
The comparison shows that PROTON combines **the best of both worlds** : the safety of a controlled method and the effectiveness in cell preservation, with the practicality of an automated and scalable process.
Therefore, it is foreseeable that as this technology spreads, it will progressively replace traditional freezing in critical biomedical applications, just as vitrification once replaced certain uses of slow freezing in assisted reproduction, but now extended to regenerative medicine in general.

Implementation of PROTON in Clinical and Research Centers

Adopting PROTON Magnetic Freezing Technology in clinical and research settings requires planning, but is feasible thanks to its design intended for **simple and safe integration** .

Infrastructure and technical requirements:

Fortunately, PROTON does not require complex cryogenic infrastructure.
A **typical PROTON system** is similar in size to a commercial laboratory freezer (there are compact models with, e.g., 3 kg/h to 15 kg/h freezing capacity, which correspond to units the size of a medium-sized cupboard).
It requires a suitable (three-phase) electrical supply and a place with normal ventilation to dissipate heat.
It does not require liquid nitrogen piping or special cold rooms beyond the standard of an air-conditioned laboratory.
Since it operates at around -30 °C, it does not generate the massive heat load of a boiling LN_2 tank ; its system is a closed-loop mechanical cooling system, so the installation environment is a common laboratory or even a GMP cleanroom.

Space must be provided for the **subsequent storage** of the samples: depending on the strategy, this may involve keeping the vials in ultra-low temperature freezers at -80 °C (for medium-term storage) or transferring them to liquid nitrogen vapor-phase tanks for very long-term storage.
There is flexibility here: many centers would opt for -80°C if internal studies show that the cells remain stable at that temperature for the required time (e.g., 1-2 years).
For longer periods (decades), LN_2 can still be used, but the advantage is that with PROTON the critical phase (freezing) has already occurred under control, so **storing in the vapor phase** of LN_2 is sufficient (avoiding direct contact with liquid LN_2) .
In either case, the cryogenic storage infrastructure would be similar to the existing one, only **the number of LN_2 tanks could be reduced since** part of the stock could be kept at -80 °C.
cryoprotectants is also necessary : for example, it is recommended to use media such as Bambanker hRM or other synthetic cryoprotectants already validated with PROTON.
These media are commercially available and meet clinical grade standards.
No additional humidity control equipment or other components are required – the PROTON Freezer comes with integrated field generators, temperature sensors, controllers, etc. For validation purposes, the facility will need to qualify the equipment (IQ/OQ/PQ – Installation, Operation, Performance Qualification) as it would for a programmable freezer.
PROTON EUROPE will provide documentation for this, including calibrations of magnetic field strength and RF.
In summary, the infrastructure for implementing PROTON is **relatively plug-and-play** : locate the equipment, provide power, ensure electrical backup systems if the application is critical, and establish SOPs for its use.

Success metrics and quality control:

When incorporating PROTON, it is essential to define how to measure its impact.
The most relevant **success metrics** include:

- **Post-thawing viability (% of live cells):**

measured by standard methods (trypan blue, AO/PI, etc.) immediately after defrosting.
A significant increase would be expected compared to the previous method (ideally >20 percentage points improvement, for example from 60% to 85%).

- **Specific post-thawing functionality:**

Here, each application defines its own trial.
In neurons, it may be the density of neurites and the release of neurotransmitters; in secretory cells, the amount of hormone secreted vs. control; in stem cells, the efficiency of forming colonies or differentiating; in lymphocytes, their ability to lyse target cells, etc.
These comparative functional tests will confirm that PROTON maintains the expected properties.
The aim would be, for example, to ensure that "90% of functional activity is preserved" compared to a fresh control, in contrast to perhaps <50% previously.

- **Cell recovery rate per batch:**

It relates the number of frozen cells to the number that are recovered viable.
PROTON should show a greater absolute recovery of usable cells.
This impacts productive efficiency.

- **Reproducibility of results:**

Consistency between batches will be evaluated.
Ideally, after validating the process, each batch cryopreserved with PROTON should yield very similar metrics (low standard deviation), better than the historical variability with LN_2 .

- **Additional quality indicators:**

membrane integrity (e.g., levels of released LDH), apoptosis markers (active caspase 3), morphological analysis (microscopy to see if there are vacuoles or intracellular fractures), and stress markers (HSPs, etc.).
Overall, PROTON should reduce signs of damage on all these fronts.

- **Operating parameters:**

total process time (was freezing achieved faster or with fewer manual steps?), ease of use (number of incidents or errors), and direct costs (nitrogen consumption before vs electricity consumption now, etc.).

- **Regulatory compliance:**

An intangible success metric is achieving approval of the process before regulatory bodies (e.g., including PROTON in a dossier of a cell product under investigation or commercial).
Having an agency approve your cell therapy being frozen with PROTON would validate that GMP standards were met.
To this end, the validation of the PROTON cycle, the stability of the cells in storage, sterility tests after thawing, etc. will be documented.

These metrics should be monitored during an initial adoption phase.
A typical plan would be to freeze, for example, 3 batches of cells using both methods and compare them, documenting improvements.
If PROTON meets predefined criteria (e.g., “viability >80% and function retention >70%),” then it is decided to fully transition to PROTON for that application.

Transition from research to industrial commercialization of IPS therapies

Japan has just taken a historic step by conditionally approving **iPS-derived cell therapies for Parkinson's and other diseases** (March 2026).
This is not research, but the **beginning of the industrial phase of regenerative medicine** .
With the approval, **cryopreservation** ceases to be an experimental stage and becomes a **critical quality variable** (CPP) in the manufacture of the final product.
In turn, the industry must now comply with specific GMP standards for ATMP: standardized batches, master cell banks, controlled storage and validated transport, always ensuring **post-thaw reproducibility** .
In other words, freezing technologies become part of the **critical infrastructure** for these new drugs.

In strictly regulatory terms, this step consolidates three **transformations** :
First, the cell product ceases to be a “trial” or “hospital protocol” and becomes a **regenerative drug/product** with dossier, manufacturing and surveillance obligations, aligned with the CTL spirit : early access conditioned on incremental evidence and reproducible quality control.
Second, the public conversation changes: CTL inevitably includes aspects of **manufacturing, distribution, price, and coverage** , because the therapy enters the real healthcare circuit (Journalistic and financial coverage explicitly highlights subsequent debates on price/coverage and manufacturing/distribution readiness.)
Third, the system “forces” **industrialization** : the CTL is granted for a finite period (up to 7 years) and continuation depends on confirming effectiveness and sustaining quality; this encourages developers to adopt robust industrial strategies from the start: scaling, standardization, cold chains and power/function metrics.

In parallel, there is peer-reviewed evidence, in a context directly connected to the Kyoto/CiRA–industry clinical ecosystem, that **cryopreservation of iPSC-derived dopaminergic neurospheres using PROTON FREEZER** , maintaining **viability, dopaminergic markers and functional properties post-thaw** , and demonstrating in vivo performance in animal models.
This positions **“functional** ” cryopreservation as an **industrial bottleneck** and, at the same time, as a **vector of technological differentiation** with clinical and regulatory relevance.
PROTON NEO , validated in relevant dopaminergic models, fits into this scenario as a **structural crystallization control platform** , designed for batch reproducibility and adaptable to GMP processes.

The following outlines the key elements that make this chapter a strategic contribution:

- **Japanese regulatory context** :

Japan created a unique legal framework for regenerative therapies.
On the one hand, the **Act on the Safety of Regenerative Medicine** regulates the clinical use and safe provision of regenerative medicine technologies, with safety and bioethical measures; on the other hand, the **PMD The Act** (Pharmaceuticals and Devices Act) establishes the fit of **“regenerative medicine products”** as a distinct category and enables authorization pathways.
and the one that explains much of Japan’s “regulatory leadership”—is the Conditional and Time- Limited **approval scheme** Approval , CTL). This scheme allows the Ministry (MHLW) to grant conditional authorization, **for a period not exceeding 7 years** , when:
(i) the heterogeneity of the product is an inherent fact,
(ii) efficacy can **be predicted** with the available data and
(iii) The benefit-risk balance does not indicate a lack of clinical value.
This mechanism requires the manufacturer to submit a rigorous plan for confirming efficacy and quality after launch, to prevent the approval from expiring.
The first use of CTL for **iPS in Parkinson's** emphasizes that the consistency of the process (including cryopreservation) will be continually reviewed.

Historical relevance :

The key news in 2026 is that an expert committee of the MHLW endorsed the conditional and temporary approval of **two iPS-derived regenerative medicine products** , including the Parkinson's candidate (AMCHEPRY) developed by Sumitomo Pharma with RACTHERA, and a heart failure product (ReHeart) from Cuorips .

The Japanese coverage describes it as the **world's first case of "practical implementation" (実用) of drugs based on this technology** , and notes that the committee's decision is sent to the MHLW for final approval, with conditions regarding duration and additional data requirements.
Although other countries are conducting trials with iPS, **none** have yet granted market authorization.
This **regulatory leadership** makes Japan a global benchmark and encourages other agencies to observe how safety and quality are managed in practice (which involves sharing standards and reports with the international community).

- **From regulation to industrialization** :

Once conditional approval is granted, the emphasis shifts to **GMP-scale production** .
Standardized batches, full traceability, and tracking data are required (all-patient). registry) as did previous Japanese ATMPs (e.g., HeartSheet).
Cryopreservation of mature cells becomes critical to enable storage/distribution logistics, opening of CDMO lines and establishment of cell banks.
In this phase, the process design must integrate cryopreservation as a control parameter: the freezing/thawing rate, the prevention of osmotic concentration, and post-thawing functional viability are now product quality factors.
In cell therapies, **cryopreservation** is not passive “storage”: it is a **process step** that can modify biological attributes (viability, phenotype, potency, integrity), and therefore must be treated as a critical variable.

- **PROTON as a technological platform** :

PROTON NEO fits into this scenario as *a strategic infrastructure* in cryopreservation.
The claim—aligned with the regulatory testing standard—is not that the approved commercial product is necessarily manufactured with PROTON, but that there is published evidence, in a context linked to the Kyoto/CiRA clinical line—where PROTON FREEZER was used as a cryopreservation method for a Parkinson's relevant product.
Specifically, a peer-reviewed study on cryopreservation of **iPSC-derived dopaminergic neurospheres** reports that, when cryopreserved using **PROTON FREEZER in Bambanker hRM (** BBK) medium , they show **favorable post-thaw viability** and **equivalent expression of dopaminergic markers** , as well as **dopamine secretion and electrophysiological activity** comparable to fresh spheres; and that, after transplantation into 6-OHDA-injured rats, they survive and differentiate into mature dopaminergic neurons with functional improvement in the rotation model.
This point is especially relevant because the article itself positions **cryopreservation** of the final product as desirable for logistics and manufacturing scale in ESC/iPSC therapies, recognizing that the in vivo survival of cryopreserved cells is usually lower than that of fresh cells.
Furthermore, the same work describes the physical/engineering rationale of the system (SMF/AEF fields) and shows comparisons in feasibility and neuritic extension, identifying **PROTON FREEZER** as a robust condition in the evaluated set.
Its combination of a cold chamber with electromagnetic modulation (static and alternating fields) allows for the control of ice formation and the minimization of intracellular damage, which has proven useful in studies of iPS dopaminergic neurospheres.
These data support the use and functional validation of the method in a context scientifically connected with the translational development of iPSC-derived dopaminergic therapies.
Thus, it is not presented as a simple "freezer", but as a **molecular alignment system to preserve cellular functionality .**

Its use in preclinical studies (in collaboration with CiRA–Sumitomo) effectively anticipates its applicability at GMP scale: thermal reproducibility, cell load management and consistency between batches.
The dossier emphasizes that "in published studies... PROTON FREEZER was used as a cryopreservation method, demonstrating maintenance of dopaminergic markers and post-thaw functionality."

- **Industrial impact and recommendations** :

The shift in Japanese regulations necessitates integrating PROTON NEO into an advanced manufacturing strategy. The following is recommended:
(a) qualify the machine in a GMP environment (IQ/OQ/PQ, thermal validations),
(b) develop "post-thawing" protocols that measure critical factors (viability, dopamine secretion, neuronal phenotype),
(c) install pilots with a CDMO and a hospital-manufacturer to demonstrate traceability and consistency, and (d) prepare the pharmacovigilance plan required by the Japanese CTL .
The goal is to position PROTON NEO not only as laboratory equipment, but as part of the **cell therapy value chain** : from the master cell bank to delivery to the patient.

What it means for Japan and for the future: medical, industrial and geopolitical implications

The Japanese approval sets an international regulatory precedent that will reduce uncertainty in other markets (EMA/FDA), accelerating the industrial adoption of iPS therapies and, therefore, the need for robust cryopreservation infrastructures.
In this new regulatory environment, cryopreservation goes from being a secondary technical parameter to being integrated within the **quality assurance architecture** of the cellular product.
From a medical point of view, the change is that dopaminergic cell transplantation ceases to be an academic “promise” and becomes subject to real conditions of health practice: reproducible manufacturing, safety control on scale, and evaluation of benefit in real life under post-marketing requirements.
This introduces positive pressure towards the design of clinical endpoints and follow-up schemes that can support a subsequent step towards full approval or re-examination.
From an industrial point of view, it opens up a **new category** of demand: infrastructure for cell banks, distribution logistics, cold chain monitoring and control of "freezing-thawing" as a quality component.
GMP guidelines for ATMPs insist that storage and transport conditions must be defined, and that in cryopreservation attention must be paid to the rate of temperature change during freezing/thawing.
In other words: the regulator is saying, indirectly but explicitly, that **the physics of freezing is part of the quality dossier .**

From the perspective of the Japanese state, this move has three implications:

- It validates and materializes a two-decade-long public strategy: Japan continuously invested in iPS and built a legal framework for “ swift and smooth” implementation ” without losing the anchor in security, with early approval and post-marketing measures.
- International pressure for regulatory convergence is increasing: other regulators will be watching the performance of the Japanese CTL , including its ability to withdraw/deny if effectiveness is not confirmed (exemplified by reassessment experiences).
This makes the Japanese market a regulatory “testing ground” with a high reputational impact.
- It shifts the competitive focus towards industrial capabilities (CDMOs , quality control, scaling): the Japanese CTL explicitly states that quality reproducibility must be improved and sustained. In terms of industrial policy, this favors technologies that reduce variability in critical steps (such as cryopreservation) and manufacturing/service networks capable of ensuring traceability, storage, and transportation.

Integration with GMP flows and large-scale production:

One benefit of PROTON is that it has already been used in regulated contexts – recall that **Sumitomo Pharma produced iPSC lines under GMP that were then frozen with PROTON** for the Kyoto study.
This indicates that it is feasible to integrate PROTON into a manufacturing flow while adhering to good practice guidelines.

The PROTON equipment itself will need to be qualified (IQ/OQ/PQ as stated) and then each freezing run can be considered a process batch, with parameter records (the machine can provide temperature vs time records, perhaps field intensity, etc., which could be attached to the batch record).

In practice:

- Before freezing, the cells are prepared with their cryoprotective medium as usual (e.g., centrifuge, resuspend in medium with 10% DMSO at established concentrations).
- Then, instead of putting the vials in a slow-freezing box or an isopropanol cooling container, they are placed in the **Proton Freezer** .
  The cooling protocol starts (automatically programmed).
- Once the program is completed (say 60 min down to -35 °C), the vials are transferred to final storage (either at -80 °C or LN 2 steam).
  This step can be done within a laminar flow if asepsis is required, or in the GMP room itself if the PROTON FREEZER is there.
  Since the vials remain hermetically sealed at all times, the risk of contamination is minimal.
- Since PROTON does not require adding or removing media during the process (unlike vitrification, which requires making CPA dilutions very quickly upon removal), it is easier to **encapsulate in a reproducible SOP** .
  And it eliminates human variability, which regulatory agencies like because it reduces the chances of error.
- It is also important that PROTON does not introduce new “non-GMP” materials: electromagnetic fields do not leave any residue or contaminant in the cell.
  The medium used (Bambanker or other) must be approved for clinical use; there are *Research Use Only versions* and *GMP-compliant versions* of these media, which should be used according to the case.
- Regarding scalability: models such as the **PF-15 NEO (Proton Medical)** have the capacity to freeze ~15 kg of material per hour, which in terms of cell vials can be equivalent to tens or hundreds of 1-2 mL vials per run.
  This is sufficient for many clinical-scale productions (e.g., manufacturing 100 vials of cell therapy in one campaign).
  If more is needed, larger models are available, or multiple runs can be performed. PROTON's scalability is linear: more machines running in parallel increase output, similar to how multiple freezers are used in a bank.
- For GMP purposes, each PROTON run can be considered a special freezing process; post-thawing cell potency retention validations would be performed after X months, etc., to ensure that the procedure does not cause changes over time.
  This is already routine for any cryopreservation, only now **better stability results would be expected** thanks to the reduction of initial damage.

In summary, the implementation of PROTON in clinical/research centers follows similar steps to introducing any new equipment: proof of concept, validation, training, and then *go live* in operations.
The advantages it offers – simplicity (no LN_2) , safety, reproducibility and better biological results – facilitate its adoption, because it solves problems for both the scientific equipment (better cell quality) and the operations equipment (fewer risks and costs).
With a well-structured plan, a center can migrate its cell freezing processes to PROTON technology in a matter of months, and soon begin to reap the rewards: more live cells, safer procedures, and patients/experiments with better outcomes.

Considerations on the Regulatory Framework (GMP and ATMP Environment in Europe)

Adopting innovative equipment like PROTON in clinical and advanced therapy manufacturing settings requires compliance with the applicable **European regulatory framework , particularly** *Good Manufacturing Practices* (GMP) for Advanced Therapy Medicinal Products (ATMP). The key points are summarized below:

- **Equipment ranking:**

The PROTON freezer **is not currently considered a "medical device"** under the definition of EU regulations (MDR), as it does not act directly on patients or diagnose/treat on its own, but is a laboratory processing equipment.
It is marketed with CE marking but it refers to electrical safety requirements and industrial machinery, not under a medical device CE marking.
This means that its manufacturer did not have to obtain MDR certification to sell it as a laboratory freezer.
However, in some EU countries, it may be possible to voluntarily seek classification as a Class I device (manufactured accessory) for greater acceptance in clinical settings – Ryoho/PROTON Europe are exploring such certifications depending on the jurisdiction.

- **Integration into a GMP process:**

When PROTON is used to manufacture a cell product that will be administered to patients (e.g., a batch of CAR-T cells, a cell culture for therapy), **that process falls under** ATMP drug GMP regulation.
There is no specific "GMP approval" for a device (the device is not certified in the abstract); rather, **it is the responsibility of the therapy manufacturer to validate and qualify the device** within their process. This involves performing the **Installation/Operation/Performance (IQ/OQ/PQ) qualification** of the PROTON device on-site, documenting that:

- The equipment is installed correctly and meets specifications (IQ).
- It operates consistently according to its parameters (OQ, e.g., it verifies that it achieves -35) °C ± tolerance, that the fields reach the expected intensity, etc.).
- It performs its function in the context of the process with acceptable results (PQ, e.g., freezing X type of cells with reproducible viability results).

ATMP manufacturers must notify the health authority of this during inspections; **CE marking alone is not sufficient evidence** of suitability for a specific process.
It is necessary to demonstrate through internal documentation that the PROTON is suitable for its purpose in the manufacture of the product.

- **Equipment not classified as medical devices:**

Authorities understand that much of the equipment used in GMP laboratories (bioreactors, freezers, flow cytometers) is not a medical device and therefore **does not have a "medical" CE marking** . This does not prevent their use, but it places the burden on the user (the company/manufacturer of the medicine) to ensure that the equipment does not compromise product quality.
The EU *GMP Guideline for ATMPs explicitly states that simply having a general CE mark "is not enough to demonstrate suitability" in the process.*
A risk assessment is expected: e.g., ensuring that the materials inside do not contaminate the cells, that cleaning is effective, that electromagnetic fields do not alter unwanted cell properties, etc.
All these aspects must be covered in the facility's SOPs and validation records.

- **Cleanroom installation requirements:**

Under GMP, any equipment entering a clean area must be cleanable and preferably sterilizable or have sanitary surfaces. PROTON complies with this (stainless steel, sterilizable parts).

Before its first use with products intended for humans, a **cleaning qualification must be carried out** : demonstrating that after the cleaning/disinfection cycle there are no bioburdens or chemical residues left on the equipment.

Also, since PROTON generates an internal airflow, it must be integrated into the room's environmental rating: verify that it does not increase particles beyond limits in the room (which is expected not to happen, due to its internal filter).
Additionally, any associated *consumables* (e.g. trays, racks) must have material traceability and, if reusable, be cleaned/sterilized between uses and stored properly.

- **Records and traceability:**

In GMP operation, every use of the PROTON must be recorded. Ideally, the equipment produces **electronic records** (curves, etc.) that can be integrated into the *batch record* .
If it weren't electronic, times, temperatures, etc. would have to be recorded manually, but PROTON makes this easier with its digital logger.
These records will demonstrate in each batch that the validated freezing profile was followed.
Any deviation (temperature alarm, inadvertent door opening) should be investigated as a possible quality deviation.
It is also important to include the PROTON in the **periodic calibration program** (e.g., calibrating thermometers and field sensors perhaps annually, and checking alarms).

- **ATMP vs device:**

It should be noted that if PROTON were used to freeze a cell product considered a medicinal product (ATMP), the resulting *product* must be evaluated in its **regulatory dossier** with the authority (EMA or national agency).
In other words, in the application for authorization of an ATMP, the company will describe its cryopreservation process (indicating that they use PROTON, with what parameters, etc.) and will provide comparative data demonstrating that the product frozen in this way is equivalent to or better than fresh, etc. In this sense, PROTON is part of the drug's "manufacturing process".
Regulatory agencies will focus on the quality/safety of the resulting product, not on approving the equipment itself; but indirectly, if the data shows that the equipment improves quality, this will be welcome.
Example: If a process modification is requested to use PROTON instead of slow freezing, a comparative package (feasibility, product potency, stability) must be submitted to the EMA to justify the change. This is part of the ongoing regulatory management of an ATMP.

- **Clinical trials and academic research:**

In clinical research contexts (academic Phase I/II trials), it is possible to introduce PROTON under *investigational GMP* .
Similar principles apply here as well, although there may be some more flexibility.
Even so, the authorities will require that the equipment be qualified and that patients give informed consent knowing that their cells were processed with a new technology, if applicable.
It is advisable to contact the local authority (e.g., AEMPS in Spain) to notify the use of innovative equipment in the manufacture of the investigational medicinal product, even if a formal equipment approval process is not mandatory.

In summary, **implementing PROTON in a GMP laboratory is viable** and does not conflict with regulations, provided that the appropriate process qualification and validation steps are followed.
Many cell therapy facilities already use programmable freezers; PROTON would be integrated in a similar way, with the exception of its electromagnetic fields (an aspect that would have to be documented as controlled and safe for the product).
Since it is not an end-use medical device, **it does not require additional certifications for internal use** , but the quality of the product obtained with it will be under scrutiny.
Complying with GMP in this context means documenting everything: that the equipment works as expected and that it improves (or at least maintains) the quality of the cellular product.

Limitations and scope of use of PROTON technology

While PROTON technology represents a significant advance in cryopreservation, it is important to outline its **technical limitations** and *appropriate scope of use* in order to have realistic expectations:

- **It does not completely replace ultra-cold cryogenic storage:**

The PROTON system typically operates down to around **-35 °C** . This means that, after the initial freezing, the samples must still be transferred to a **long-term storage medium** (ultra-low temperature freezers at -80°C). °C, liquid nitrogen tanks at -150 °C, etc.) for indefinite preservation.
In other words, PROTON solves the **controlled freezing phase** (preventing damage during solidification), but **does not eliminate the need** for conventional cryogenic equipment to maintain samples for extended periods.
Even so, by minimizing damage during the critical freezing stage, **it reduces the frequency of** LN_2 intervention and allows part of the cold chain (transport, intermediate storage) to be handled at -80°C. °C with lower risk.

- **It requires cryoprotectant (CPA), although at a lower concentration:** PROTON **does not eliminate the need to use** chemical cryoprotectants.

While in some resistant materials a “CPA-free” freezing could be explored, to date the documented PROTON protocols always use media with DMSO, glycerol or other CPAs in **moderate concentrations (5–10%)** .
The advantage is that these concentrations are lower than those used in classic vitrification (e.g., 15–20% DMSO), reducing toxicity.
However, PROTON *should not be interpreted* as allowing cells to be frozen in pure saline solution without protection: a cryoprotectant is still necessary to prevent osmotic damage and stabilize the structures.
This dependence could be mitigated in the future with non-toxic cryoprotectants or combinations of pressure cooling, but for now, **the PROTON method complements, not replaces, the chemistry of cryoprotectants** .

- **It does not, by itself, solve the problem of defrosting (reheating):**

Most cell damage occurs during freezing, but in large samples there is also a risk of crystal formation during **thawing** if it is not done quickly. PROTON focuses on the **beginning of the process (freezing)** , addressing the main bottleneck of cryopreservation.
However, for large samples (e.g., vitrified whole organs) advanced reheating techniques (such as magnetic particle *nanoheating) will still be needed to avoid thermal fractures.*
small to medium- sized samples (vials up to a few mL, spheroids, thin tissue sheets).
Freezing larger structures (>1–2 cm) might require combined strategies: for example, cooling with PROTON to -35 °C and then vitrifying the interior with cryoprotectants, as some authors suggest.
Therefore, **the use of PROTON in cryopreservation of whole organs is still experimental** and not an immediate application.

- **Infrastructure and cost:**

Unlike a conventional freezer, a PROTON unit involves a slightly higher investment cost and greater space requirements.
Furthermore, the equipment weighs ~500 kg and generates heat, which means it is neither portable nor trivial to relocate once installed.
technical training is required for staff, although routine use is then simple.

- **Emerging scientific evidence in some cell types:**

Although the reported results are very promising, most of the studies so far come from specific collaborations in Japan (iPSC neurospheres, mesenchymal stem cells, embryos).
There are relatively **few independent studies** published in international journals outside the developer circle.
While there is no indication that PROTON fails in other systems, further third-party validation in different cell types (CAR-T immunotherapies, endothelial cells, etc.) would be desirable. This will likely occur in

the coming years given the growing interest, but for now, the **body of evidence** is concentrated in certain models.
The effectiveness of the system will depend on the correct optimization of the protocol for each cell type, so it should not be considered a universal plug-and-play system without specific validation.
Institutions that are early adopters of PROTON should be prepared to generate their own backup data and protocol optimizations.

- **Current scope of use:**

PROTON is designed as **laboratory process equipment** .
It is not a device intended for *direct use on the patient* or for environments outside the laboratory (e.g., it would not be used in the operating room to freeze grafts "live").
Their role is in the **production or preservation phase** of biological materials: cell banks, GMP cell therapy laboratories, fertility units (embryos), etc.
In that respect, it offers great benefit.
in situ preservation of organs for transplantation, special adaptations might be required (it is not trivial to put a solid organ into the chamber as is; it would have to be perfused with CPA and possibly the dimensions of the equipment would have to be adapted).
Thus, **the optimal use of PROTON today is in the biomedical supply chain** , improving the quality of preserved cells/tissues before their clinical use, rather than in clinical procedures *per se* .

In summary, while PROTON **expands the limits** of cryopreservation by dramatically reducing freezing damage, **it does not eliminate all the difficulties** inherent in cryogenic preservation.
Its implementation must be planned with the understanding that it complements existing methods: for example, after freezing with PROTON it will still be stored in LN_2 banks (although with less frequent access), and DMSO will continue to be used (although in a smaller proportion).
With this understanding, institutions can maximize the benefit of PROTON in those scenarios where it truly makes a difference (valuable samples, difficult to freeze, where post-thaw functionality is critical), while continuing to use conventional methods in more routine applications where the increased efficiency may not justify the change.

PROTON represents a **powerful but specific tool** , whose value is best appreciated in high-level cryopreservation (advanced cells and tissues for therapy, national biobanks, cutting-edge research) rather than in basic uses where cells tolerate traditional freezing well.

PROTON Implementation Model – Three-Phase Plan

To maximize success when adopting PROTON technology in a biomedical organization, a **three-phase phased implementation model is proposed .**
This approach minimizes risks, generates comparative data, and facilitates clinical acceptance:

Phase I: Comparative Validation (Initial Pilot)

Aim :
To demonstrate, under the specific conditions of the institution, the **advantages of PROTON** over current methods.

Actions:
Select one or two critical cell types (e.g., the most used cell line in the bank, or the one most sensitive to freezing).
Perform **comparative trials** by freezing with the traditional technique vs. PROTON, measuring viability and functionality post-thawing (following a protocol as described above).
Include sufficient replicates for statistical analysis.
Also document operating times, ease of use, etc.

Expected results:
Data showing **significant improvements** with PROTON (e.g., doubled viability, greater functional recovery, better batch homogeneity).
Identification of any initial setbacks (e.g. need to adjust CPA concentration).
This phase ideally culminates in an **internal validation report** and, if possible, a publication or presentation of results that supports the technology. *(In Japan, this approach was key: institutions such as CiRA published their successful findings, lending credibility to the method.)*

Decision to move forward:
If the results confirm benefits, form an internal committee (scientific and quality) to plan the next phase.
If there are mixed results, identify causes (e.g., program optimization) and repeat focused tests.

Phase II: Optimization and Protocolization

Aim :
Integrate PROTON into the institution's **standard processes** , fine-tuning all parameters for each specific application.

Actions:
Based on Phase I, optimize details: for example, adjust the ideal cooling ramp for each cell type (3 °C/min or 5 °C/min?), the best DMSO concentration that balances viability and functionality, the maximum sample size per batch without losing uniformity, etc. Establish **standard operating procedures (SOPs)** : from sample preparation and equipment loading to thawing.
Include equipment maintenance plans and staff training (make sure multiple technicians know how to operate it correctly).
If it is a GMP environment, proceed with **formal** equipment qualification (IQ/OQ/PQ) and process validation: run controlled runs that confirm reproducibility of results within predefined specifications (e.g., "≥80% post-thaw viability in all tests"). During this phase, also **optimize internal logistics** : where to physically locate the PROTON (cleanrooms, material flow), and how to synchronize it with other stages (cell culture, formulation, storage).

Expected results:
Protocols ready for routine use, validated and approved by internal quality control/regulator.
Practical details will have been worked out (for example, it is defined that the trays are sterilized in an autoclave before each use; that the equipment is preheated 30 min before loading samples, etc.).
The institution will be prepared to use PROTON without improvisation.
An indicator of Phase II completion is that **test batches** intended for actual use (e.g., master stem cell banks, therapy doses for clinical trials) can now be frozen, meeting all release criteria.

Phase III: Full Clinical and Operational Adoption

Aim:
Incorporate PROTON into regular operation and/or clinical production, replacing or complementing older methods as appropriate, and strategically leverage its capabilities.

Actions:
Initiate the use of PROTON in the routine: for example, **cryopreserve with PROTON all new batches** of cells intended for patients or research, using validated SOPs.
Monitor during the first iterations that the results are maintained (do *trend analysis* of viability, etc., in case batch-to-batch variations arise).
At the same time, **communicate and train clinical staff** about the change: for example, if cells are given to doctors, explain that they now come from an improved process, with expectations of greater effectiveness, but stress that thawing must follow protocols (if something has changed, e.g., perhaps a different thawing time given that the viable cells are more concentrated).
Establish a **contingency plan** : maintain the ability to revert to the previous method for any batch if an unexpected failure occurs in PROTON (although unlikely, it is good risk management practice).
As confidence is gained, **extend its use** : incorporate more cell types or products to be frozen with PROTON, gradually eliminate dependence on LN_2 in intermediate steps (if it can now be transported dry).
This phase can also involve seeking **strategic alliances** : for example, participating in consortia or multicenter studies that use PROTON, positioning the institution as a leader in advanced cryopreservation.

Expected results:
PROTON technology functioning as an integrated part of the process, with tangible benefits: less cell loss, easier logistics (perhaps fewer LN_2 refills) , standardization of batch-to-batch results.
In the clinical context, improvements in outcomes could begin to be observed: for example, cell therapies with greater potency than before, thanks to the cells better preserving their function.
This feeds back into the virtuous cycle: more adoption, more positive data.
paradigm shift will have been completed : cryopreservation will cease to be a bottleneck and will become a strength of production.

Summary of milestones:
Phase I – internal proof of concept achieved;
Phase II – optimized and validated protocol;
Phase III – routine use and expansion of capabilities.
Each phase should be documented and, ideally, disseminated (internally or in publications) to capitalize on the knowledge generated.
This phased model mitigates risks (it is not released for clinical use without data), convinces stakeholders with local evidence, and allows the institution to adapt organizationally to change.

Conclusions

PROTON magnetic freezing technology represents a **disruptive advance** in the field of cell cryopreservation.
Throughout this technical report, its scientific foundations, experimental validation, clinical implications, and practical implementation considerations have been examined, leading to the following main conclusions:

- **Solution to the problem of traditional cryopreservation:**

PROTON addresses the root cause of the main obstacle to conventional cell freezing – intracellular damage from uncontrolled ice formation – by inducing homogeneous ice nucleation using electromagnetic fields.
This eliminates the cause of mechanical injury to cells and organoids, preserving their structural and functional integrity in a way that traditional techniques (slow cooling to -80°C or liquid nitrogen without nucleation control) cannot achieve.
In this way, PROTON overcomes the historical limitations of post-thaw viability and functionality that have plagued regenerative medicine for decades.

- **Proven and reproducible effectiveness:**

Several collaborative studies in Japan – supported by leading institutions such as Kyoto University (CiRA), NIBIOHN, University of Ryukyus and pharmaceutical companies – have consistently shown that PROTON **dramatically improves cell survival** (typically achieving >85–90% viable cells vs <50% with standard methods), while **maintaining critical cellular functions** (neuronal activity, secretory capacity, proliferative potential) that are otherwise lost.
These results are not isolated cases, but have been reproduced in multiple models (dopaminergic neurons, mesenchymal stem cells, tissues, etc.), which validates the robustness and generality of the technology.
The existence of **international patents** granted or pending for this method (EP, JP, US) underlines its novelty and usefulness, also providing a legal umbrella for its exploitation in advanced medical applications.

- **Impact on regenerative medicine and advanced therapies:**

PROTON enables for the first time the **truly functional cryopreservation** of highly specialized cells, allowing things like: clinically ready therapeutic neurosphere banks for Parkinson's, storable 3D organoids for transplantation or research, and pluripotent stem cell lines and their derivatives safeguarded without loss of quality during their global distribution.
This has transformative implications: cell therapies that previously had to be administered "fresh" (limiting their geographical and temporal scope) can now be produced centrally, cryopreserved with minimal loss, and **distributed** on demand to different centers.
Furthermore, the improvement in reproducibility and stability of cell models will boost the development of new drugs by providing standardized and reliable samples.
In summary, PROTON is emerging as a **key facilitator** for scaling up and globalizing regenerative medicine, bringing it closer to the conventional medicine model in logistical terms (stock, distribution, shelving).

- **Operational and safety advantages:**

From the user center's point of view, PROTON provides tangible advantages: it largely eliminates dependence on liquid nitrogen (and its associated risks and costs), is compatible with automation and good manufacturing practices (GMP), and reduces process variability, making results more predictable. Handling the cells is safer, both for them (less stress) and for the staff (less exposure to dangerous cryogens).
Furthermore, in a sustainability context, eliminating tons of LN_2 that are constantly evaporating also represents a long-term environmental and economic benefit.

- **Viable implementation through collaboration:**

The adoption of PROTON in new environments is facilitated by collaborative pilot validation strategies, such as those already employed in Japan.
There is a clear path for hospitals and laboratories to integrate this technology, with technical support from the manufacturer, minimizing risks.
Experience so far suggests that once a center tests PROTON and confirms the improvement (e.g., doubling cell viability), the complete transition is natural.
It is expected that a **global network of pioneering centers will emerge** , sharing protocols and results, accelerating the community learning curve.
In conclusion, PROTON Magnetic Freezing Technology represents a **crucial shift** in applied cryobiology: it reimagines cell freezing as a physically orchestrated process to protect life in suspension, rather than as mere passive cooling with harmful consequences.
The scientific data collected suggests that this technology allows **life and function to be preserved where they were previously lost** , with profound implications for the storage of biological material, the manufacture of cell therapies, biomedical research and, ultimately, for patients who will benefit from higher quality cell products.

With scientific validation in hand and a clear added value over existing methods, PROTON is positioned to become a **standard tool** in biomedicine in the coming years, raising the potential of regenerative medicine to new heights and contributing to making the dream of effective, safe and widely available cell therapies a reality.

Strategic Impact on Advanced Medicine

The incorporation of PROTON technology in cryopreservation marks a **strategic turning point** in the field of advanced medicine.
Beyond the positive experimental results, its long-term implications encompass multiple aspects:

- **Enabling “off-the-shelf” cell therapies:**

One of the major challenges in regenerative medicine has been to develop cell-based products that are ready for immediate use, have a long shelf life in biobanks, and maintain their efficacy. PROTON directly contributes to this vision by enabling the freezing of cells and tissues while preserving their *therapeutic potency* .
allogeneic (donor) therapies is envisioned : for example, batches of CAR-T cells, mesenchymal stem cells or iPSC-derived neurons produced in specialized facilities, frozen with PROTON and shipped on demand to hospitals.
This reduces time and costs (patients will not have to wait for "fresh" preparation of each dose) and improves safety (less ad-hoc handling).
Off-the-shelf cell banks could become as common as blood banks are today, but with more complex cells, thanks to the confidence that frozen stock maintains near-fresh culture quality.

- **Strengthening biobanks and biological storage institutions:**

Large national or regional biobanks – which store stem cells, tissues, cell lines for research and therapy – will be able to update their protocols to incorporate PROTON in the freezing phase.
This **raises the quality** of the stored samples at the population level.
For example, a country's induced pluripotent stem cell (iPSC) bank could distribute cell lines to laboratories knowing that, upon thawing, >85–90% will survive and function properly, thus standardizing research results.
Furthermore, the reduced use of liquid nitrogen in routine operations decreases the risk of accidents and simplifies the internal logistics of these biobanks.
Strategically, those who adopt PROTON first in their biobanks will gain an advantage: they will offer “premium” samples in terms of viability, attracting international collaborators and projects.

- **Reducing dependence on liquid nitrogen and modernizing cryogenic logistics:**

For decades, LN_2 has been synonymous with cryopreservation, but it comes with safety and handling drawbacks.
The possibility of minimizing **LN_2 use** in certain stages thanks to PROTON represents an operational paradigm shift. We can envision a logistics flow where cells are frozen with PROTON and **initially stored at -80°C. °C** , allowing them to be transported in dry ice containers (solid CO_2) instead of nitrogen tanks.
Many international shipments of cells could be made simpler, as dry ice is widely available and regulated for transport, while LN_2 presents more obstacles (dangerousness, customs).
LN_2 is ultimately required , the **volume and frequency** of its use would decrease drastically (for example, a bank could keep most samples in an ultra-freezer and only transfer to LN_2 those that need to be stored for >1 year).
This implies significant long-term **cost savings (fewer** LN_2 refills , less cryotank maintenance) and contributes to *greener* and safer operations in hospitals and laboratories.

- **Pushing towards new frontiers in cryomedicine :**

The availability of a method that preserves functionality expands the horizon of what can be frozen. Emerging fields such as the **cryopreservation of 3D-printed or bioengineered organs** , or complex tissues for reconstructive medicine, could benefit from PROTON or its derivatives. Japan is already investing in the "Proton NEO" project for freezing large organoids and tissues, indicating that this approach is considered viable.
In assisted reproduction, early studies with embryos and oocytes suggest improvements in post-thaw development rates using PROTON, which could increase the success of fertility treatments.
All these advances open up **new clinical possibilities** : for example, having tumor organoid banks from patients to test drugs (precision oncology) would be more viable if the frozen organoids retain high fidelity to the original upon thawing, as PROTON aims to achieve.

- **Competitive advantage and technological leadership:**

Institutions that adopt PROTON early will position themselves at the forefront.
This can translate into **attracting funds and projects** .
For example, international clinical trials of cell therapies could select sites with advanced cryopreservation capabilities to ensure a stable cell supply chain. At the industrial level, advanced therapy companies can differentiate their products by guaranteeing superior post-thaw quality (supported by PROTON data), which in a future saturated market could be a decisive factor.
Even in terms of intellectual property and derivative patents: knowing and handling this technology allows innovation in its applications (specific PROTON protocols for a certain cell type could be patented, for example), contributing to the **innovation ecosystem** around cryopreservation.

In conclusion, PROTON technology is not simply a **new** freezer, but an **enabler of systemic changes** in translational medicine.
Its ability to preserve "live and ready to function" cells after thawing addresses one of the historical problems that limited bringing discoveries from the laboratory to the clinic on a large scale.
By ensuring greater uniformity and quality in frozen biological materials, PROTON allows many processes to be scaled up with confidence: from cell therapy manufacturing to global distribution of biological materials.
This results in treatments that are more accessible (as they can be stocked and transported more easily), safer (less last-minute manipulation), and potentially more effective (cells that work better in the patient).
The adoption of PROTON, supported by robust scientific evidence and protected by global patents, indicates that **functional cryopreservation** is emerging as the new gold standard. We can anticipate that, just as the advent of the incubator or the perfusion reactor marked milestones in biomedicine, the widespread adoption of magnetic freezing will mark a turning point in how we conceive of *cryopreservation* : no longer as a necessary evil that reduces efficacy, but as a *controllable and optimizable phase* that maintains the quality of the biological product.
In summary, PROTON provides a **strategic advantage** to 21st-century biomedicine, accelerating the arrival of advanced therapies to society and consolidating the infrastructure for a truly industrial and global regenerative medicine.

Technical and Bibliographic Annexes

Annex A – Scientific Articles and Key Studies
Annex B – PROTON Patents (original documents)
Annex C – Technical specifications of the PROTON P-15 NEO equipment
Annex D – References to cited academic publications
Annex E – Biomedical Validation Protocol (Example for implementation)
Annex F – Institutional recognition and international dissemination
Annex G – International Coverage of Regulatory Approval of iPS Therapies in Japan (2026)
Annex H – Institutional Contact Information (PROTON EUROPE SL)