The Cold Truth:

Cryopreservation of Final Product in Cell Therapy Manufacturing

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Many nascent cell therapies arise from a hospital or academic setting. Because of this, **the developer initiates clinical trials with therapies that have a short shelf-life** due to a "fresh" final product, meaning the product is not designed for long-term storage. In the absence of stringent final product testing and commercial-scale shipping logistics, delivering fresh products, not cryopreserved, is the simplest way to manufacture cell therapies when the primary concern is to establish proof of concept for the therapy.

Using fresh products alleviates concerns about adding extra manufacturing process steps and whether or not freezing the therapy late in its manufacturing process is going to damage the quality of the product. And while that's perfectly acceptable for proof of concept of the cell therapy, as development into late-stage and then commercial production becomes the goal, different constraints arise. This is where cryopreservation of final product becomes an option and, in some cases, even a necessity.

In hospital settings and small academic settings where early phase cell therapy development often takes place, it's quite feasible to manufacture a product, carry it down the hall, and infuse it into a patient, and it makes both economic and logistical sense to do so. However, once a cell therapy developer considers the possibility of a late-stage clinical or commercial product with potentially thousands of doses a year that need to be shipped to hospitals all over a continent or the world, it becomes much more logical to have a cryopreserved final product.

For example, with a patient-specific cell therapy product that has a shelf-life of 18, 24 or even 48 hours, the process is prone to significant timing risks. Shipping and final delivery to the patient can be delayed by traffic, weather conditions, insufficient resources to support rapid turnaround lot release, and the availability of the patient, any of which could cause product expiry. Once a developer has gone through the intensive time and expense of manufacturing a cell therapy, failing that product at the very end of the process because it has expired before it can be administered could be catastrophic. Such a scenario is devastating to the patient, of course, but additionally, the cost of every failed product ends up being built into the cost of goods for successful products. The cost of a failed product in the administration phase is the most expensive point in the manufacturing process for a failure to occur. That's a very clear rationale for any cell therapy manufacturer to considering cryopreservation of final product.

Part 1

Benefits of Cryopreservation of Final Product

The logistics can be greatly **simplified and standardized when cryopreservation is part of the process**. That's a key point because standardization of administration is, on the commercial scale, going to be necessary to minimize cost of goods.

Simplified Logistics

As part of staying within a short window for shelf-life, a fresh cell therapy product will need to be extracted from and then infused into patients on certain days of the week or even certain hours—which could mean extra expenses in labor and facilities to accommodate that schedule. For example, if a cell therapy developer completes the manufacturing of a patient-specific therapy at 5:00 pm or even 10:00 pm, it now needs to ship right away in order to reach the patient within the window. That requires pick-up and delivery at off-peak hours, which adds the extra cost of a specialized courier service. In comparison, manufacture of a cryopreserved product can be completed at any point during a seven-day work week, and can be stored until it can be shipped at a time that is most convenient for the patient, receiving hospital, and so on, at much lower cost.

More Uniform Distribution of Manufacturing

Jumping off from the benefit of flexibility in terms of logistics, cryopreservation of final product also may directly impact the potential of idle capacity. Idle manufacturing capacity can easily result from the need to manufacture product tightly timed to patient treatment schedules as opposed to available capacity. By spreading out manufacturing over a given week or month, the requirements for manufacturing capacity can be more accurately forecast and investments in that capacity made more carefully.

In a hypothetical example for a fresh final product therapy with a three-day manufacturing process, let's assume all collections of input materials are made on Mondays, the only possible days to manufacture are Tuesday through Thursday, and Friday is the only possible day for treatment. If the final product is cryopreserved, collections can now be scheduled any day of the week, with manufacturing to follow and final product to be shipped out for treatment on any day of the week. In this case, cryopreservation has effectively added an additional three-day manufacturing slot to each week, decreasing idle capacity by half. Cryopreservation of the input material would also have an impact on the scheduling flexibility in this scenario.

Dendreon's Provenge®, a notable fresh formulation final product with an 18-hour shelf-life, required three apheresis collections for the production of three doses. It is easy to hypothesize that a **cryopreserved final product might have allowed for significant cost reductions** in regards to these multiple administrations.

Multiple Administrations Over Time

Should the manufacturing process be capable of producing multiple doses from a single manufacturing lot, cryopreservation of the final product allows for those doses to be administered over time, on whatever schedule is determined to be most clinically beneficial. This could also provide the developer with the flexibility to add a retreatment without requiring an additional collection if the process is designed to allow for it. By removing the requirement for an additional collection process to produce the starting material for each dose, the cost of goods is substantially reduced by leveraging the manufacturing lot over multiple doses.

Robust Release Testing

When it comes to release testing, a fresh product with a 24-hour shelf-life only allows for 24 hours within which to perform all the necessary sample testing and lot release by quality assurance, as well as shipping and administering the product. This is often not realistic or feasible, so fresh cell therapies end up having a conditional release—in which data from sample testing is not available until after the product has been administered. And though there is of course some indication that a product is safe and effective based on not only previous products but also in-process and limited-release testing, with fresh product the cell therapy developer does not have proof-positive that this is a safe and effective product in terms of sterility and functionality. Because tests used in conditional release, such as gram stain, have low sensitivity to detect contamination, conditional release will almost assuredly lead to unnecessary complications. Furthermore, regulators may choose not to approve conditional release for any given product without sufficient rationale as to why an alternate approach, such as cryopreservation, is not possible. Clearly, scenarios requiring conditional release present risks that will only escalate as products move toward commercialization.

With cryopreservation of final product, once manufacturing is complete the cell therapy can be stored and the developer has the luxury of time (providing there is flexibility in the timing of patient administration): sterility testing typically takes seven to 14 days, and mycoplasma testing can take up to 28 days. With a viral vector modified product, in some cases, regulators may require a replication competent virus assay, for which data results take between five and eight weeks. Potency assays, particularly for a cell-based product, may take several days to execute. With a cryopreserved product, all these tests can be completed and results known, allowing for the confidence that the product is both safe and functional before it is administered. This is particularly important for therapies where patient pre-conditioning is expensive or risky.

Transition from fresh to frozen final product will reduce patient morbidity and mortality over the lifetime of the product due to the increased sensitivity and robustness in safety testing afforded by frozen product.

Part 2

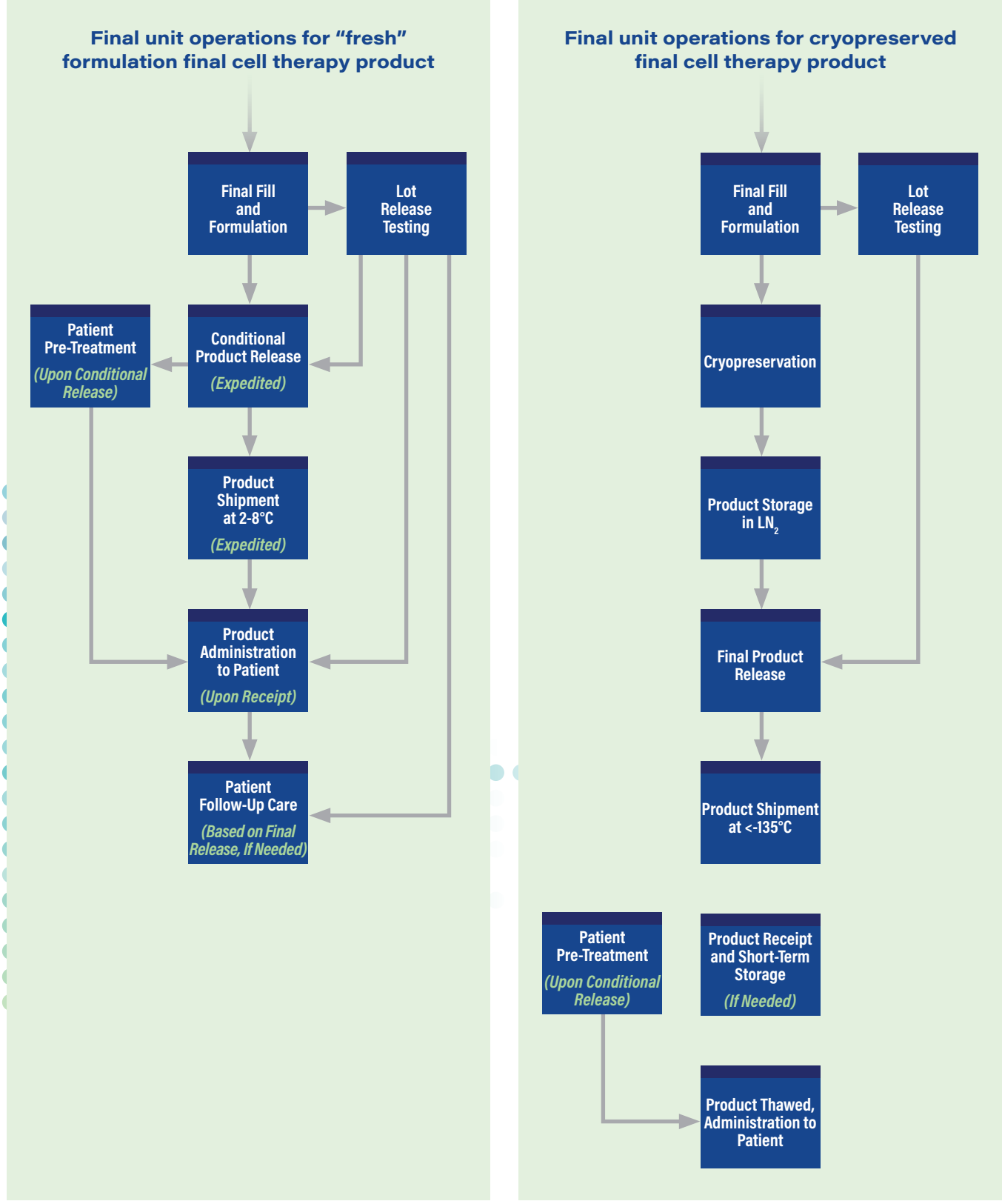
Knowing the Risks

Of course, having mentioned some of the primary benefits of cryopreservation of final product, it's worth noting that integrating this step into a manufacturing process is not without risk.

Due to the complexity and often poorly understood mechanism of action for cell therapies, it is difficult to even identify and measure the critical quality attributes of the product, such as potency. This complicates the understanding of the associated effect of cryopreservation and thawing on any cell therapy product. It's a well-documented phenomenon that there is damage that occurs to cells upon freezing and thawing due to the osmotic shock and the cellular ice formation, as well as the reperfusion injury of being in a hypothermic state. Because of these cellular insults, the function of the cells is likely to be affected, and there will be a fundamental difference in the potency, at least immediately upon thawing. There will be some impact on cell function, some reduction of viability, some cell death that occurs through cryopreservation. Nothing comes without cost.

Other risks of introducing cryopreservation of final product into a cell therapy process include:
The cost of the development work to integrate cryopreservation into the process may be significant
The addition of multiple additional unit operations to the cell therapy manufacturing process, each adding their own direct cost and accompanying risks *(see Figure 1 on next page)*
Additional unit operations (i.e. thawing) are put in the hands of the clinical sites, requiring investment in training and possibly equipment at those sites

Figure 1: Integration of cryopreservation of final product increases the number of unit operations



Part 3

Timing is Critical

To help ensure that the benefits outweigh the costs, **it is crucial to consider the introduction of cryopreservation of final product as early in clinical development as possible**, mitigating the comparability risk. If a developer integrates cryopreservation prior to Phase I, there is no comparability risk.

The same change between Phase I and II carries some comparability risk—the developer will still need to prove to regulators that the cryopreserved product is equivalent, but the burden of proof is lower in these early stages. If a developer waits to make the change until after Phase II, once efficacy has already been proven and the time has come for a pivotal trial, the burden of proof is much higher. After Phase III, proving comparability generally becomes significantly more difficult due to increased comparability risk and cost to run the necessary comparability studies.

Ultimately, there are two ways to prove comparability between cryopreserved and fresh final cell therapy products.

1. A cell therapy developer can have an extremely well-characterized and relevant *in vitro* model that shows that the product is the same, within a reasonable doubt.
2. Alternately, the developer can generate some clinical data with fresh products in a Phase II trial, for example, and then do a Phase IIb with frozen products and demonstrate similar efficacy at that time. This can be done as part of a clinical trial if the developer has appropriate trial design, but a fair amount of process development and characterization will still be necessary before moving to implementation of a frozen product.

Ideally, the path forward is to build an acceptable *in vitro* model that allows the developer to optimize the cryopreservation process and have faith that, following that optimization, what results is an appropriate product that the developer is comfortable implementing in a clinical study.

There may be some resistance to making the change to cryopreservation early in the development process because a cell therapy developer might not want to add cost and complexity *(see previous Figure 1)* to the manufacturing. Another hurdle for developers is the need to understand the characterization of the product well enough to prove comparability. Developing a characterization platform that is sufficient to show comparability is crucial and is a step that must be taken prior to successfully integrating cryopreservation. Despite these challenges, the earlier a developer can make that decision, the better off the process will be in the long run.

Part 4

Expert Guidance

Cell therapy developers should consider the integration of cryopreservation a crucial process change as they move from academic-stage into commercial-ready therapies. Like any process change in cell therapy manufacturing, cryopreservation involves taking a number of elements into careful consideration and **requires a particular set of experience and expertise to execute successfully**.

For cryopreservation specifically, one area where such expertise is necessary is the technical know-how—understanding exactly what is needed from a technical standpoint to develop and execute such a process change. This includes familiarity with cryopreservation instrumentation, reagents, and disposable technology, deep understanding of the biophysics of cryopreservation, efficient design of experiments, and experience with appropriate analytical method development, integration into a high throughput process flow, process validation, and stability validation. Such technical hurdles can be daunting and can dissuade cell therapy developers from undergoing the change to cryopreservation themselves.

Because of these hurdles and the intense time and cost that can be associated with not executing the cryopreservation process change seamlessly, it may be valuable for cell therapy developers to partner with a manufacturing development specialist that has a long history and deep expertise in cell therapy. The "cold truth" is that cryopreservation is not a process change that can be taken lightly—the stakes are simply too high.

PCT has helped multiple clients develop robust cryopreservation processes. Contact PCT to learn more about cryopreservation and other manufacturing process improvements for your cell therapy.

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