# Routine Hematopoietic Progenitor Cell Processing: HPC, Apheresis and HPC, Marrow Products

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## 4.1 Introduction: Why This Chapter Is Important

Upon collection of hematopoietic progenitor cells (HPCs), there are numerous steps that must take place to guarantee the potency and purity of the product in anticipation of transplantation. Every HPC product must have cell enumeration, flow cytometric immunophenotypic studies, sterility testing, viability studies, and, if necessary, cryopreservation and storage. It is critical that cryopreserved HPC products that may be stored for up to years be done so in a way that permits the cells to remain viable and functional in order for engraftment to occur after transplantation. In this chapter, laboratory processes specifically pertaining to HPC, Apheresis and HPC, Marrow products will be discussed. In addition, we will discuss the indications and associated matters for HPC product manipulation of both autologous and allogeneic products that are collected by either apheresis technology or bone marrow harvest. More detailed information about HPC laboratory regulation and accreditation is reviewed in Chap. 2.

## 4.2 Following the Rules: Regulation and Accreditation

HPC products are considered biologics; thus, the laboratories that process these unique products are highly regulated and must be registered or licensed with the US Food and Drug Administration (FDA) for the use of these products in the USA. If

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these facilities are Clinical Laboratory Improvement Amendments (CLIA) certified, then FDA regulations, state laws, and/or College of American Pathologists (CAP) guidelines are followed. Additionally, laboratory accreditation by the AABB (formerly known as the American Association of Blood Banks) and the Foundation for the Accreditation of Cellular Therapy (FACT) is often obtained.

Regardless of whether the product is apheresis or marrow derived, these products often require at least minimal analysis and manipulation to guarantee safety and efficacy.

## 4.3 Autologous HPC Products: The Donor Is the Recipient

The overwhelming majority of autologous HPC products are collected by apheresis technology. Autologous HPC products do not have issues involving ABO incompatibilities, as the donor and recipient are the same. However, a collected autologous HPC product may not be infused immediately and could require preservation until the patient has been prepared and deemed ready for hematopoietic cell transplantation. Each HPC product is aliquoted into storage bags based on predetermined total nucleated cell (TNC) concentrations, after which point a cryoprotectant is added prior to the freezing process. Samples for sterility testing are usually collected immediately subsequent to the addition of cryoprotectant but antecedent to cryopreservation.

### 4.3.1 Optimizing Cell Concentrations: How Much Is Enough?

The optimal TNC concentration for use during HPC product cryopreservation is currently unknown. Poor mobilizers are typically defined as (a) not having achieved a circulating $CD34^+$ count >20/μl within 6 days after G-CSF injection at 10 μg/kg/day (or 5 μg/kg/day after chemo-mobilization within 20 days) **or** (b) yielding $<2.0 \times 10^6$ $CD34^+$ cells/kg of body weight in ≥3 apheresis collection; this condition can be challenging from an HPC laboratory standpoint (Olivieri et al. 2012). For such protracted (in days) collections, large quantities of cells have been collected, but the majority of them are not the cells of interest, i.e., most commonly large numbers of granulocytes. Thus, these HPC products require dilution with either autologous plasma or another isotonic solution to a predefined concentration prior to the addition of cryoprotectant and cryopreservation.

From the perspective of the HPC laboratory, cryopreserving and storing cellular products with large volumes involve increased resources as well as increase the risk of adverse events at the time of infusion, namely, cryoprotectant-related toxicities, granulocyte-related reactions, and volume overload (Calmels et al. 2007). Though there is unease that increased TNC concentrations during cryopreservation and

storage may result in a higher risk of toxicity to the cells and associated decreased viability, it must once again be mentioned that the ideal TNC cryopreservation concentration has not been identified, and increasing this concentration to achieve smaller product storage volumes is a possibility (Lecchi et al. 2016). Additionally, volume reduction of the HPC product via centrifugation with resulting cell-free supernatant removal could further be utilized to decrease product storage volumes, particularly for those patients that are volume sensitive (i.e., pediatric patients or those with cardiac and/or kidney impairment) or have large-volume products (i.e., from poor mobilizers). These HPC products with smaller volumes would require fewer laboratory materials and reagents, decreased processing time, decreased freezer space, and potentially result in fewer infusion reactions.

### 4.3.2 Cryopreservation: Storing HPC Products for Later Use

If HPC products cannot be immediately utilized, cryopreservation can be used to preserve the mononuclear cells and maintain their viability and functionality until recipients are ready to receive them (Rowley et al. 1994). In HPC laboratories that have protocols for cryopreservation, this manipulation has been proven to be safe, minimizes adverse events, and allows for timely engraftment of the cells upon later infusion (Koepsell et al. 2014). It is important to note, however, that multiple cryopreservation protocols exist and that each laboratory is tasked with demonstrating that their specific protocols allow for the freezing, storage, thawing, and infusion of viable and functional cells (Lecchi et al. 2016).

While cryopreservation provides many benefits, most notably providing additional time to prepare a patient for transplantation, there are significant risks associated with this process that must be accounted for by the HPC laboratory. Infusion of cryopreserved HPC products is associated with toxicities of variable severity that are related to the total cellular content, cellular composition (i.e., increased number of granulocytes), cryopreservation volume, and overall total product volume (Rich and Cushing 2013). In order to cryopreserve any product, a cryoprotectant must be used in order to prevent the formation of ice crystals within and outside of cells during the freezing process. These ice crystals can result in cell injury/death, decreased viability, and associated complications in recipients. For these reasons, the amount and timing of adding cryoprotectant to HPC products are critical parts of any cryopreservation protocol. Dimethyl sulfoxide (DMSO) is a commonly utilized cryoprotectant, and its use in conjunction with albumin, electrolyte solution, and controlled-rate freezing has been demonstrated to make cryopreservation a safe and effective maneuver in the HPC laboratory. However, it should be noted that laboratories vary in their concentration of cryoprotectant; for DMSO, a commonly used concentration is 10%, but this may be different depending on a particular laboratory's policies, practices, and experience with alternatives (Pamphilon and Mijovic 2007).

### 4.3.3 Controlled-Rate Freezing Versus Uncontrolled-Rate Freezing

If an HPC product requires cryopreservation, a precise quantity of cryoprotectant is slowly added to a specific number of cells and volume, followed by freezing and subsequent storage in liquid nitrogen (LN2). The process of the actual freezing of an HPC product can transpire in one of two ways: controlled-rate freezing **or** uncontrolled freezing (i.e., dump freezing):

- In controlled-rate freezing, an HPC product is placed within a sealed chamber and cooled at a rate determined by a computer that incorporates temperature data from the product and freezer in real time (often 1–2 °C/min). An important advantage to using controlled-rate freezing is the capacity to minimize the latent heat of fusion phase (see Chap. 6). When the HPC product begins to phase shift during cooling and transitions from a liquid to a solid, energy is released that disrupts the otherwise stable cooling process; this energy is referred to as the latent heat of fusion, and it can result in the temporary warming of cells that may impact cell viability (Meagher and Herzig 1993). With controlled-rate freezing, the temperature of the HPC product is continually measured, and the increasing temperature due to the latent heat of fusion is detected by the computer and is accommodated for by temporarily increasing the rate of cooling until a stable cooling curve is re-achieved. Ultimately, the temperature of the product is decreased to −80 °C or lower prior to transfer to an LN2 storage freezer. During this entire process, the temperature of the product is monitored and recorded.
- In contrast, uncontrolled-rate freezing involves the simple transfer of a HPC product into a −80 °C freezer and subsequently to a LN2 storage freezer (<−150 °C). This process also cools at a general rate of 1–2 °C/min but does not have a mechanism to detect and precisely accommodate for the latent heat of fusion. The actual cooling rate is difficult to document, and the freezer should be left undisturbed, which can be a potential logistical problem for facilities with multiple HPC products to process each day.

In many HPC laboratories, controlled-rate freezing is the choice for cryopreservation of cellular products.

### 4.3.4 Vapor Phase Liquid Nitrogen Versus Liquid Phase Liquid Nitrogen

At a temperature of −80 °C or lower, a HPC product can be transferred to a LN2 freezer for long-term storage. There are two freezer options for long-term storage of cryopreserved products: vapor phase or liquid phase LN2. Traditionally, liquid phase freezers were known to maintain cell therapy products with fewer temperature fluctuations compared to vapor phase models. However, newer jacketed vapor phase freezers have shown to minimize temperature gradients on par with liquid

**Table 4.1** Temperatures for HPC product storage and transport

| Product storage | HPC, Apheresis | HPC, Marrow |
|---|---|---|
| Fresh HPC product | Room temperature or 1–6 °C up to 72 h | Room temperature up to 48 h[a] |
| Frozen HPC product in vapor phase LN2 | ≤−150 °C | ≤−150 °C |
| Frozen HPC product in liquid phase LN2 | −196 °C | −196 °C |

*LN2* liquid nitrogen
[a]Specific facilities may store for longer periods of time and/or at 1–6 °C

phase freezers. Another important benefit of vapor phase LN2 freezers over liquid phase LN2 models is the abrogation of risk associated with viral and bacterial cross-contamination between HPC products housed within the same freezer. In other words, the probability of a potential infectious agent within a cryopreserved cell therapy product contaminating adjacent HPC units is practically zero if storage is conducted in freezers with a gaseous medium versus a liquid medium. There is currently no defined expiration of cryopreserved HPC products, and components frozen for over 10 years old have been successfully thawed and infused with no engraftment issues (see Chap. 6) (Attarian et al. 1996; Veeraputhiran et al. 2010; Winter et al. 2014). See Table 4.1 for temperatures associated with HPC products.

### 4.3.5 HPC Product Thawing: Fast as You Can

Once the time of transplantation is set, many facilities will thaw a recipient's cryopreserved HPC products as close as possible to the planned infusion time. This is done to minimize the amount of time cells that are retained in the liquid state after thawing prior to infusion. Since some studies have demonstrated that DMSO is cytotoxic to cells at room temperature, rapid thawing in a 37 °C water bath followed by infusion as quickly and safely as possible is recommended (Cameron et al. 2013). While local hospital and laboratory policies must be followed for recipients with multiple products to be infused at a given time, many facilities thaw products sequentially for a given infusion; in this way, confirmation that the antecedent product has been infused successfully and that the recipient is doing well is obtained by the laboratory team from the clinical team prior to thawing of the subsequent HPC product.

### 4.3.6 DMSO Adverse Effects and Prevention: Taking the Bad with the Good

While the cryoprotectant DMSO has allowed for the effective freezing, storage, and successful transplantation of HPC products, it is also associated with many clinically significant side effects that include nausea, vomiting, cardiovascular events,

respiratory distress, kidney injury, and allergic reactions (Tormey and Snyder 2009). Rare fatalities associated with DMSO have even been reported (Zenhausern et al. 2000). In consideration of the documented adverse effects attributed to DMSO, a maximum exposure of 1 g/kg/day is allowed. Depending on the number of TNC collected, the HPC product cell concentration in each bag, product volume, and the weight of the recipient, infusions of HPC products may have to be performed over more than 1 day in order to prevent DMSO-associated toxicities. Due to the medical and logistical challenges associated with DMSO, alternative approaches utilizing decreased concentrations of DMSO (typically 5%) in addition to extracellular protectants like hydroxyethyl starch (HES) have been successful in cryopreserving HPC products. For example, initial data demonstrate improved viability with HES (3%) and DMSO (5%) versus DMSO (10%) alone (Berz et al. 2007). However, there is concern about the use of such solution combinations as there is a paucity of long-term data regarding products cryopreserved in this fashion. Although HES can be a valuable supplement to DMSO that can possibly decrease cryoprotectant-associated adverse events while maintaining or improving cryopreserved HPC product characteristics, the optimal ratio of these agents has not been defined, and the use of such alternatives requires further investigation.

### 4.3.7 HPC Product Washing: To Wash or Not to Wash?

Due to the adverse events known to be associated with cryoprotectants such as DMSO, it is logical to consider the option of washing HPC products after thawing in order to remove the offending chemical. However, washing of the cells to remove DMSO and other additives risks loss of critical HPCs due to cytotoxicity associated with increased exposure time of cells to DMSO as well as HPC losses that would occur during washing process; both of these would result in a lowering of the cell dose. In selected circumstances, such as in patients with a documented severe DMSO allergy, washing of HPC products may be the safer choice. But as a matter of routine practice, it is more common to infuse the HPC product directly into the patient after the thawing process is completed.

## 4.4 Allogeneic Products: Unique Concerns and Unique Processes

In contrast to autologous HPC products, allogeneic HPC products harbor attendant risks just as any routine blood component from the blood bank. These risks include, but are not limited to, infectious disease transmission, allergic reactions, immunologic reactions, hemolytic reactions, and graft-versus-host disease. Additionally, unlike in solid organ allografts, transplantation across ABO barriers with HPC components is routinely performed. However, the many donor and product factors which might make a routine blood donor and corresponding product ineligible and

**Table 4.2** ABO mismatches in HPC allogeneic transplantation

| | O donor | A donor | B donor | AB donor |
|---|---|---|---|---|
| O recipient | Compatible | Major | Major | Major |
| A recipient | Minor | Compatible | Major and minor | Major |
| B recipient | Minor | Major and minor | Compatible | Major |
| AB recipient | Minor | Minor | Minor | Compatible |

unsuitable for donation or transfusion may not automatically exclude the same individual for donation of HPC products. In contrast to donors of routine blood components, the potentially detrimental factors associated with an HPC donor and corresponding product are weighed against the benefit of transplantation of the impacted HPC product for a given recipient on a case-by-case basis.

A donor-recipient pair is considered to have a major ABO mismatch if the recipient's plasma has naturally occurring isohemagglutinins that are incompatible with the donor's red cells (e.g., A donor and O recipient). Conversely, the donor-recipient pair is considered to have a minor ABO mismatch if the donor's plasma contains naturally occurring isohemagglutinins against the recipient's red cells (e.g., O donor and B recipient). Certain donor-recipient pairs can have both major and minor (also termed bidirection) ABO mismatches (e.g., A donor and B recipient). See Table 4.2 for a complete presentation of ABO mismatches between donors and recipients.

### 4.4.1 HPC, Apheresis Allogeneic Products and ABO Incompatibility

HPC, Apheresis products collected from the peripheral blood usually have hematocrits <5%; thus, issues of major ABO incompatibility due to incompatible red cells rarely occur. The apheresis instruments are excellent at isolating the buffy coat and limiting the red cell contamination of the HPC product. On the other hand, these products can have up to several hundred milliliters of plasma, and thus possible hemolytic reaction due to incompatible isohemagglutinins can occur. This may necessitate plasma reduction as part of the HPC processing. See Tables 4.2 and 4.3 for complete information on donor-recipient ABO mismatches and associated HPC laboratory processes to mitigate the risk of acute reactions. FACT/JACIE Standards require that the transplant physician specify the modifications that should occur to the HPC product based on the ABO incompatibilities present between the donor and recipient (Foundation for the Accreditation of Cellular Therapy, Joint Accreditation Committee-ISCT and EBMT 2015).

For HPC, Apheresis products that have incompatible isohemagglutinins against recipient red cells (i.e., minor ABO incompatibility), plasma reduction to remove the isohemagglutinins can be achieved by centrifugal separation. This can be performed by either manual centrifugation of the product bag and expressing off excess plasma or by using an automated apheresis instrument to remove plasma. However,

**Table 4.3** Required HPC product modifications based on ABO mismatches

| Donor | Recipient | Manipulation to the product |
|---|---|---|
| O | O | None |
| O | A, B, AB | Plasma reduction |
| A | A | None |
| A | O | Red cell reduction |
| A | B | Red cell and plasma reduction |
| A | AB | Plasma reduction |
| B | B | None |
| B | A | Red cell and plasma reduction |
| B | O | Red cell reduction |
| B | AB | Plasma reduction |
| AB | AB | None |
| AB | O, A, B | Red cell reduction |

Red cell reductions are routinely performed on HPC, Marrow products only. Plasma reductions are routinely performed on HPC, Apheresis and HPC, Marrow products

the benefits of plasma reduction must be weighed against the risk of cell losses that may occur during the separation.

HPC, Apheresis products may be infused either fresh or after cryopreservation and subsequent thawing. If cryopreservation is applied, the identical methods previously discussed for autologous HPC products can be utilized.

### 4.4.2 HPC, Marrow Allogeneic Products and ABO Incompatibility

HPC, Marrow products collected from anesthetized donors in the operative suite can routinely have volumes of up to 2000 ml with hematocrits of up to 35%; thus, hemolytic reactions due to incompatible donor red cells can occur. Most facilities determine their own maximum limit for the allowable quantity of incompatible red cells, with 20–30 ml of incompatible red cells being regarded as acceptable (Daniel-Johnson and Schwartz 2011). However, if this threshold is exceeded, red cell reduction must be performed.

All methodologies for red cell reduction are based upon densitometric separation of red cells with a specific gravity of approximately 1.08–1.09 from MNCs with a similar specific gravity of approximately 1.06–1.07 (Areman and Loper 2016). These methods include procedures previously discussed such as centrifugation and automated apheresis separation, as well as two additional methods: hydroxyethyl starch-mediated densitometric separation and densitometric gradient separation. When red cells come into contact with the hydroxyethyl starch, red cell rouleaux occur, and the specific gravity of the red cell fraction increases. This results in a better densitometric separation between the sedimenting red cells and the mononuclear cells that remain afloat. The red cells can then be removed, leaving behind a MNC-enriched product.

Densitometric gradient separations utilize agents, such as Hypaque-Ficoll, to create a density barrier. Red cells and granulocytes have a higher specific gravity and, after a centrifugation step, end up below the density gradient barrier. Cellular elements with a lower specific gravity, such as the mononuclear cells containing the cells of interest, remain above the gradient and can be subsequently isolated. Similar to plasma reduction, the benefits of any red cell reduction strategy versus the risks of HPC losses must be considered.

Additionally, HPC, Marrow products also contain large quantities of plasma. If a minor ABO incompatibility exists between the donor and recipient, the product would require a plasma reduction, as described previously.

HPC, Marrow products may be infused either fresh or after cryopreservation and subsequent thawing. If cryopreservation is applied, the identical methods previously discussed for autologous HPC products can be utilized. HPC, Marrow products undergo red cell reduction prior to cryopreservation to minimize hemolysis and the deleterious effects of free hemoglobin (Rother et al. 2005).

### 4.4.3 Donor Lymphocyte Infusion: Small Infusions for Big Issues

In cases of allogeneic HPC transplantation where recipients have disease relapse or there is evidence of failing engraftment (e.g., worsening chimerism studies), few treatment options are available short of a second allogeneic transplant. In these situations, donor lymphocyte infusion (DLI) may be considered to re-induce remission by eliciting a graft-versus-tumor effect and/or to provide support to a failing graft in the hopes of improvement. The exact concentrations and frequency of DLI can vary from patient to patient and from disease to disease. Often DLI dosing is utilized to achieve a specific improvement endpoint or until adverse events manifest (graft-versus-host disease or marrow toxicity) (Castagna et al. 2016).

For the HPC laboratory, DLI is processed from one of two sources: either from the original allogeneic HPC product prior to transplantation or from a subsequent leukocyte collection at a later time after transplantation from the original allogeneic donor. When processing DLI for potential future use from the original allogeneic HPC product, the $CD3^+$ cells need to be quantified and cryopreserved for use at a later time. $CD3^+$ cells are most commonly enumerated and dosed per kg of recipient body weight. The volumes of DLI are much smaller than typical HPC transplant volumes. However, it should be noted that $CD3^+$ cell populations are not necessarily directly proportional to $CD34^+$ cell populations; thus, depending on the dose(s) of DLI requested by the transplant physician and the $CD34^+$ dose requested, clear communication should be provided to the clinical team taking care of the recipient so that the updated $CD34^+$ cell dose, reduced as a result of any requested DLI processing and storage, is known and verified as this may alter the original request for DLI doses. After preparation of DLI at requested doses, these aliquots of cells are cryopreserved per standard protocols as

described above and are thawed and infused similar to traditional HPC products. DLI processed from a subsequent collection from an allogeneic donor is similarly enumerated, processed, cryopreserved, stored, thawed, and infused. However, the majority of DLIs are provided fresh. Donor collection volumes are proportional to T-cell collection and thus can be tailored to the requested dose. The donor must be reevaluated prior to each new collection to ensure safety of the product and the donation.

## 4.5 Infectious Disease Testing: Impact on the HPC Laboratory

While an in-depth discussion of infectious disease testing for HPC donors is covered elsewhere in this volume, it is important to highlight the impact that results of these tests have on the HPC laboratory. Testing for infectious disease agents must be performed per manufacturer's instructions using FDA-licensed and FDA-approved donor screening tests. While testing is not required for autologous donors, any untested products must be labeled as "Not Evaluated For Infectious Substances" and stored in quarantine vapor phase LN2 freezers. Additionally, for any donor (either autologous or allogeneic) that has an "incomplete" or "ineligible" status based on the results of the donor screening questions and/or testing, the corresponding product must have the appropriate labeling and be stored in quarantine vapor phase LN2 freezers.

As stated previously, some allogeneic donors may not meet all donation requirements but may still be approved for donation. In these situations, a summary of records that contains information regarding why those requirements have not been met must be provided to the transplant center prior to product procurement. The recipient's physician has the ability to authorize the use of the product if the recipient has been advised and the product is labeled appropriately and released under urgent medical need. Clear and timely communication between the transplant physicians (for both donor and recipient) and the HPC laboratory is critical to ensure the appropriate labeling, processing, storage, and handling of such HPC products.

## 4.6 Potency of the HPC Product

Regulatory and accrediting standards of HPC laboratories require processes and protocols to confirm product identity, trace the product from donor to recipient, and characterize product integrity for quality and quantity. For each institution, release criteria are established for donor eligibility, total cell count, HPC cell dose, viability, and sterility, and acceptable values and ranges must be defined. There is a need for some variability in what is "acceptable" as these products, which are derived from and for individuals, are potentially irreplaceable and needed urgently. With regard to viability, post-processing (pre-cryopreservation) TNC viability release criteria is typically >90%, with post-thaw viabilities having a lower threshold of >70%. The

equipment, reagents, and supplies used in all HPC laboratory processes must be qualified, written definitions of the type and volume of samples to be obtained must be stated, and time points during production for sampling must be determined. It should be clearly defined whether quality control is an in-process control or whether it is a control of the final product. Even minor manipulations, such as wash steps, volume reduction steps, and cryopreservation, require quality testing for cell numbers and bacterial and fungal contamination. Autologous and allogeneic HPC products have a well-established and proven clinical benefit for patients, and these unique products can be released despite quality control parameters being out of specification. The final decision to release an HPC product that does not meet specifications should be guided by the consideration that the benefits outweigh potential risks for the recipient. Lastly, any adverse events that occur during or after HPC infusion that might be related to the product should be documented and reported to the HPC laboratory. Only with this information can processes be improved, errors identified and corrected, and products ultimately made safer and better for patients.

### 4.6.1 Product Release Testing

Testing requirements for the release of cellular therapy products must be defined. Product testing and characterization ensure product safety, purity, and potency, but currently no standardization exists for what to test, when to test, and how to test HPC and other cellular therapy products. Additionally, the combination of manual and automated methods commonly employed further contributes to the variability observed between different facilities. Commonly performed tests include TNC count, hematocrit, viability, sterility, $CD34^+$ cell content for HPC products, and T-cell content for allogeneic products. These tests may also play a role in determining processing procedures, such as RBC removal, MNC and/or subset enrichment, or depletion of other target cells. Protocols for test utilization are established with consideration of timing for each particular manipulation in the overall processing of a product. For example, determination of $CD34^+$ cell content both before and after density gradient separation is performed, as this process is known to decrease $CD34^+$ cell content.

TNC and $CD34^+$ counts are general measures of product quantity but do not provide information about viability or potency. Commonly used viability assays include flow cytometry-based assays and dye exclusion assays. The use of 7-aminoactinomycin D (7-AAD), a fluorescent chemical compound with DNA affinity, in flow cytometric analyses offers advantages over traditional trypan blue staining that include decreased subjectivity, increased accuracy (particularly with thawed HPC products), and the ability to be done in conjunction with $CD34^+$ assessment. However, depending on the processing laboratory setup, turnaround times for flow-based assays may hinder the lab's ability to release a product for fresh infusion; in this circumstance, the use of trypan blue can be advantageous.

Potency assays are performed to assess the ability of a specific cellular therapy product to affect a specific result, the most common example being the use of a

hematopoietic progenitor cell transplant to result in marrow reconstitution. These assays have been found to be associated with time to engraftment (Stroncek et al. 2007) Examples of these include TNC count, $CD34^+$ assessment, colony-forming unit assays, and measurement of $CD133^+$/aldehyde dehydrogenase (ALDH) bright cells. Emerging methods include gene and microRNA expression profiling.

Finally, assessments of sterility are performed to query the product for aerobic and anaerobic bacteria and fungus. Culture-based methods are the most common in US labs and require validation by each processing center for the products and reagents used. Other rapid methods are needed for more than minimally manipulated products and may include gram staining, endotoxin measurement, and mycoplasma testing.

Additional considerations for release testing include labeling and assessment of product appearance (e.g., color, turbidity, and container integrity). Cell composition, storage conditions, product expiration, patient identification, product identification, processing center name and address, warnings, and precautions are common release requirements. The implementation of ISBT labeling has helped to move standardization forward in this matter (Slaper-Cortenbach 2010).

## 4.7 Expert Point of View

HPC transplantation, whether autologous or allogeneic, is a routine treatment in many institutions. The HPC laboratory plays a critical role in guaranteeing the safety and efficacy of these products regardless of whether they are immediately infused or cryopreserved and stored for years prior to use (Koepsell et al. 2014). There are many things about HPC product processing that are defined and required. However, some issues that can impact patient safety or efficacy are not well defined and/or vary from facility to facility.

For autologous HPC product processing, the optimal concentration of TNC in the HPC products prior to cryopreservation is unknown. Autologous HPC products are almost always cryopreserved, and DMSO is the most commonly used cryopreservation agent at this time (we currently use a 10% concentration). Cell concentration in HPC product bags and the percent DMSO used in cryopreservation are two laboratory variables that have direct impacts on how much DMSO a patient will receive at the time of transplant (Windrum et al. 2005). Autologous donors with high peripheral white blood cell counts and low circulating $CD34^+$ counts (i.e., poor mobilizers) can have large volumes of product collected, processed, and stored, which can lead to large-volume infusions at the time of transplantation. These transplants often require infusion over multiple days to ensure patient safety and limit adverse side effects related to the cryoprotectant as well as cellular content. Some institutions have even limited the daily DMSO dose of cryopreserved products to decrease infusion-related adverse events (Khera et al. 2012). Regardless of the freezing method, HPC products are carefully cryopreserved to maintain cellular viability and functionality. Cell viability is critical to engraftment of transplanted HPCs; therefore, the time from addition of cryopreservation media to start of

controlled-rate freezing (our preferred option) must be minimized, as DMSO is cytotoxic to cells when in the liquid state (Rowley and Anderson 1993). Regulatory and accrediting agencies require the validation and monitoring of a cryopreservation method and storage that preserves cellular viability both post-processing (pre-cryopreservation) and at infusion (post-thaw). Post-processing (pre-cryopreservation) TNC viability release criteria is typically >90%, with post-thaw viabilities typically at lower levels of >70%.

Unlike autologous HPCs, allogeneic HPCs may be ABO incompatible. If a donor-recipient ABO incompatibility exists, the ordering stem cell transplantation team member must indicate the type of ABO incompatibility, and if RBC reduction is needed for the removal of incompatible RBCs (our recommended threshold is <20 ml), plasma reduction is needed to remove incompatible plasma or both in the donor HPC product. Similarly, DLI doses to be prepared and cryopreserved must be requested, with the understanding that if these cells are to be taken from the original HPC product, a smaller $CD34^+$ transplantable dose will be an obligatory effect.

## 4.8 Future Directions

There are many unanswered questions in the area of HPC processing. We still do not know the optimal TNC concentration prior to cryopreservation. This, of course, may be impacted by the concentration and/or type of cryoprotectant utilized. While 10% DMSO is currently the most common cryopreservative in use, lower concentrations of DMSO in conjunction with other agents (such as hydroxyethyl starch) could decrease recipient exposure to this chemical associated with a variety of adverse events (Windrum et al. 2005). However, long-term stability data of cryopreserved HPCs in predefined and optimized alternative solutions are needed. Lastly, adverse event monitoring, which might be considered a clinical problem, has direct connections with the HPC laboratory. Through the reporting, tracking, and reviewing of adverse events via a robust quality program that monitors adverse events and associates them with HPC laboratory variables, a safer and more effective HPC laboratory and overall program can be created.

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