CRYOPRESERVATION

Hematopoietic stem cells (HSC) isolated from the peripheral blood (PBSC) are frozen and stored using the same techniques previously developed for bone marrow hematopoietic cells. The general parameters include cryopreservation in dimethylsulfoxide (DMSO) and a source of plasma protein with or without hydroxyethylstarch (HES), cooling at 1 to 3 °C/minute, and storage at -80 °C or colder.

The major difference for the cryobiologist is the large number of cells collected during apheresis, averaging 4.3x10^10 in a series of patients at this center, that complicates cryopreservation of PBSC. Cryopreservation of cells at the concentrations frequently used for bone marrow could result in volumes in excess of 400 ml for each collection. Infusion-related toxicities appear to be related to the amount of DMSO infused. The maximal tolerated dose of dimethylsulfoxide is not known for humans. The LD50 of DMSO for dogs has been reported to be 2.5 gm per kilogram body weight, or about 22 ml/kg of a 10% DMSO solution. We limit the amount of cryopreserved cells to be infused to 10ml/kg/day for this reason.

Patients with larger volumes will receive the cells over more than 1 day. To minimize the volume infused, we concentrate the cells before cryopreservation. In one series of patients, the average cell concentration of cryopreserved PBSC was 5.59x10^8 nucleated cells per ml. High concentrations of red cells and platelets were also frozen. We found no detrimental effect of cryopreservation at these high cell concentrations on the recovery of nucleated cells, mononuclear cells, CD34+ cells, or CFU-GM, although others reported a decreased CFU-GM recovery at high cell concentrations.

No clinical studies have addressed the effect of cell concentration on engraftment speed or toxicity of infusion. Of concern is that about twelve patients out of several hundred infused at this center have developed alterations in mental status after infusion of PBSC. It is very unlikely these events are related to the small volume of DMSO infused. Also, we routinely infuse cryopreserved cells through a 170 micron blood administration filter so it is unlikely these events are embolic in nature. Alternatively, the cells can be washed after thawing, with removal of most of the DMSO. This technique is used at some centers but again, clinical studies demonstrating differences in engraftment speed or infusion-related toxicities have not been published.

A popular cryopreservation technique uses 5% DMSO in combination with 6% HES. This also reduces the quantity of DMSO infused. Only one clinical study comparing cryopreservation with 10% DMSO to cryopreservation with the DMSO/HES mixture has been reported. This study of 24 patients did not find a difference in engraftment kinetics, although the detection of small differences in engraftment could require a study population 10-15 times as large. Such a study is ongoing at this institution.

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PBSC cryopreserved with DMSO-containing solutions are cooled at 1-3°C/min and this can be achieved by use of computer-controlled devices or by immersion of bags of about 50 ml into a -80°C freezer. Cooling by immersion into a -80°C freezer is not limited to HES-containing solutions, but may be used for cells frozen with DMSO alone.

STORAGE
The storage temperature depends upon the cryoprotectant used and the anticipated time before transplantation. Under proper storage conditions, long-term storage is feasible. Appelbaum reported the loss of canine HSC stored for 18 months in the vapor phase of LN2. This loss may have resulted from a large temperature gradient that typically occur in vapor phase refrigerators, or from repetitive temperature shifts occurring when the refrigerator was opened. In contrast, Stiff reported successful engraftment of bone marrow stored for up to 22 months at -80°C. Possible explanations for these differing results could be the use of an extracellular cryoprotectant by Stiff or the storage of an amount of HSC in excess of that needed for engraftment which would obscure progressive loss over time of storage at -80°C. We believe it is prudent to store cells at as low a temperature possible if the cells are intended for long-term storage.

Tedder reported the transmission of hepatitis between patients by immersion into LN2. A sealed overwrap bag would reduce the risk of microbial cross-contamination, although this author is not aware of any such bags made of material that will withstand nitrogen immersion. Alternatively, HSC may be stored in the vapor phase. As noted, vapor phase refrigerators may have a large temperature gradient so that bags stored at the top of the refrigerator are stored at a much warmer temperature. We constructed our racking system from aluminum which is a much better conductor of heat than steel; the gradient in our refrigerators is only about 6°C from bottom to top.

SHIPMENT
Shipping of cells must be viewed simply as mobile storage - maintenance of adequate storage conditions despite the hazards of handling. Transportation of frozen HSC can be within the same building, the same metropolitan area, or more distant. It is probably the practice of most laboratories to transport cells within the same building or locality in LN2 carriers. These insulated devices will hold adequate nitrogen for several hours and are large enough for several storage bags. That nitrogen will spill if they are tipped and the limited quantity of nitrogen limits the practical use of these devices to local transportation. We routinely clean the devices between each patient’s use to reduce the risk of microbial cross-contamination that may occur with LN2 immersion. Transportation of LN2 on public streets must be in compliance with public law regarding the transportation of hazardous material. Cells may also be transported using dry ice.
although it is unknown if damage will occur if the cells stored in LN$_2$ are allowed to warm to –79 C and then placed back into a LN$_2$ storage device.

Shipping of HSC frozen in LN$_2$ to distant transplant centers must be in “dry shippers.” These are devices that contain absorbent material in the wall of the canister that absorbs LN$_2$ and reduces the risk of tipping. Canisters with a neck of adequate size to allow insertion of storage bags also hold many liters of nitrogen and are capable of maintaining cryogenic temperatures for several days, frequently 10-14 days. The quantity of nitrogen cannot be determined by use of a dipstick or visual inspection. The shipper must be weighed to determine the quantity of LN$_2$ contained. The relationship between time since the device was charged with LN$_2$ and the weight of the container is very strong and can be used to predict the amount of time remaining before warming will occur. (These devices will not warm appreciably until virtually all the LN$_2$ is expended.) However, these devices are exposed to considerable handling and may lose vacuum. Therefore, these devices must be inspected before each use and the laboratory must have a quality control program in which the loss of LN$_2$ over time is routinely measured and compared to the initial performance of the device when first placed into service.

Cells may be shipped in the passenger compartment of some air carriers with prior arrangement. The size of a dry shipper requires that a seat be purchased just for the shipper. Frequently, cells are shipped using the service of an overnight carrier. These carriers do not have a 100% on-time delivery record, so the dry shipper must contain adequate LN$_2$ to allow for delays. The dry shippers are large and awkward devices. We have experienced the destruction of one dry shipper during shipping with one of these services. A third option is to employ the services of a same-day courier service. These companies send packages as “unaccompanied baggage” in the cargo hold of the aircraft. Transport of a dry shipper is more complex, but these services will typically deliver the shipper to the air carrier and have a representative waiting for the shipper at the terminus of the flight for delivery to the transplant center.

SUMMARY
PBSC are routinely and successfully cryopreserved for transplantation using the same techniques used for bone marrow. However, important questions regarding the cryobiology of hematopoietic stem cells, whether bone marrow or peripheral blood derived, remain to be answered. One very important question concerns the long-term storage of PBSC at various storage temperatures, and whether some cryoprotectant solutions will allow storage at the relatively warm temperatures of mechanical freezers without progressive loss of stem cell viability. There is also ongoing investigation of cryoprotectant solutions without DMSO. Such solutions would avoid the infusion-related toxicities associated with DMSO.
REFERENCES


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