

**RECOMMENDED GUIDELINES FOR COLLECTION OF PBSC FOR PURGING AND
SUBSEQUENT INFUSION
IN PATIENTS WITH NEUROBLASTOMA**

Questions/problems regarding PBSC collection can be directed to Dr. Villablanca at (323) 669-4565 or Dr. Reynolds at (323) 669-5646.

PLEASE NOTE: there are NO specimens required on blood or marrow prior to PBSC collection. The only criteria to begin are ANC and/or CD34+ count.

PBSC MOBILIZATION/CRITERIA TO BEGIN PHERESIS:

Patients will receive G-CSF 5 µg/kg/day beginning 24 hours after last chemotherapy dose given prior to planned pheresis. When ANC >1000/µL for 1 day following nadir (or peripheral CD34 count is >20/µL) **regardless of platelet count**, G-CSF is increased to 16 µg/kg/day for 3 days and then PBSC harvest is performed.

If PBSC harvest cannot be performed within 4 days of meeting the above criteria (due to chemotherapy toxicity, infection, etc.) contact Dr. Villablanca or Dr. Reynolds. If WBC rises to greater than 80,000/µL prior to PBSC harvest, discontinue G-CSF. If G-CSF has been stopped, call Dr. Villablanca or Dr. Reynolds prior to beginning mobilization to confirm dose.

PBSC Pheresis Instructions:

During PBSC harvest adjust daily G-CSF dose based on post-harvest WBC as follows:

- if post-WBC is <60,000/µL, administer 16 µg/kg
- if post-WBC is >60,000/µL, administer 5 µg/kg

Discontinue G-CSF once peripheral blood stem cell harvest is complete.

The Cobe Spectra or the Fenwal CS 3000+ machines are recommended for pheresis because the continuous flow centrifugation devices are better tolerated than discontinuous flow machines. Equipment should be operated in compliance with the manufacturer's operating guidelines. "Large Volume" leukapheresis procedures should be used for PBSC collections. During each leukapheresis procedure, the volume of whole blood processed should be approximately 480 ml/kg. This is approximately 6 blood volumes per day (one blood volume = 80 ml/kg) of pheresis.

Blood Priming

Priming of the machine prior to collection should be with ACD and saline according to manufacturer's directions. For patients less than 25 kg, a secondary prime with cross-matched, IRRADIATED, leukocyte-poor red blood cells should be done. This is described in the standard operating procedures for each machine. For Cobe 6.0 software system, priming may only be needed for patients less than 15 kg. These cells should be diluted with normal saline prior to priming as appropriate. Use of a Cobe in-line blood warmer on the return line will be used for the Cobe machine. A standard blood warmer device can be used with the Fenwal machine.

IF PLATELETS ARE LESS THAN 30,000, TRANSFUSE PRIOR TO PHERESIS.

Anticoagulant

Anticoagulant to be used is Acid Citrate Dextrose Formula - A (ACD-A) in a ratio sufficient to prevent extracorporeal clotting. Heparin anticoagulation is not recommended for use in PBSC collections except for patients with an allergy to citrate. One liter of ACD-A contains 21.33 g citrate. Hypocalcemia is a well recognized side effect of citrate anticoagulation and patients must be monitored for this side effect. Hypocalcemia may be treated with oral calcium (2 Tums or 8 oz calcium rich orange juice or milk), or with intravenous calcium gluconate.

Whole Blood Flow Rate

The following rates are designed to avoid citrate reactions and thus boluses and continuous infusions of calcium can be avoided.

<2 years (<15 kg)	15 - 20 ml/min (initial)*
2 - 5 years (15 - 20 kg)	25 - 40 ml/min
>5 years	35 - 50 ml/min

*may be increased to 25 – 30 ml/min by ratio ramping.

Vascular Access

For continuous flow apheresis, two sites of venous access are required.

Laboratory Studies

A type and cross for PRBC should be performed prior to the apheresis procedure. Pre-apheresis and immediately post-apheresis a CBC with differential and platelet count must be obtained. Additional studies may be required based on the clinical condition of the patient. Studies that frequently are required include BUN, creatinine, ionized calcium, magnesium.

PBSC COLLECTION GOALS:

A **MINIMUM total of 10×10^6 CD 34+ cells/kg should be collected and shipped**, with a goal of providing $> 5 \times 10^6$ viable CD34+ cells/kg after purging and cryopreservation (**minimum of 1.0×10^6 viable CD34+ cells/kg are REQUIRED for re-infusion**).

The following schedule is suggested for PBSC pheresis:

DAY ONE PHERESIS: Collect full day (usually six hours) and store at room temperature until after day two collection is completed. Add 100U/ml of preservative-free heparin to stem cell collection at end of pheresis.

DAY TWO PHERESIS: Collection should end in time to allow shipping of both day one and two collections **together at room temperature** to arrive at Purging Laboratory by 11 AM of **DAY THREE**. Add 100 IU/ml of preservative-free heparin to day two stem cell collection at end of pheresis.

DAY THREE PHERESIS (Optional): Collect **1.5×10^6 CD34+ cells/kg as a backup (preferably 5×10^6 CD34+ cells/kg)**. This will NOT be purged, and should be stored at the institution where the phereses are performed (i.e. not shipped to CHLA).

If sufficient PBSC can be collected for purging and re-infusion into patient, AND an unpurged backup in first two days; then a third day of collection is not needed. If sufficient PBSC cannot be collected in three days, then contact Dr. Villablanca or Reynolds to discuss. The number of CD34+ cells present in the PBSC will be determined by the Purging Laboratory to allow consistent evaluation of numbers infused into the patient.

To insure optimal cell yield and viability, the purging MUST be done within 24 hours of the last day of PBSC collection, as mandated by the FDA IDE BB-2259. If you find that you have collected between day one and two $> 18 \times 10^6$ CD34+ cells/kg then you should cryopreserve some of those cells in excess of 18×10^6 CD34+ cells/kg from DAY ONE OF COLLECTION at your institution, and ship the remainder for purging. At least one half of the cells shipped for purging should be collected the same day as you ship them to the Purging Laboratory. DO NOT SHIP PBSC ON DAY ONE UNLESS DR. REYNOLDS CONFIRMS TO YOU THAT WE CAN CHANGE THE PURGING DATE.

At least 5×10^6 CD34+ cells/kg are required from Day 1 plus Day 2 to ship for purging. Therefore if 3.5×10^6 CD34+ cells/kg is obtained on day 1, at least 1.5×10^6 CD34+ cells/kg will be needed on day 2. Experience has shown that in patients with poor yields on Day 1, yields from subsequent days of collection are at best 50% of the first collection day. Please call Dr. Villablanca at 323-669-5654 or Dr. Reynolds at 323-669-5646 if any questions regarding collections or criteria to ship.

Cells to be purged should be shipped to the NB Purging Lab **at room temperature**. Cells will be frozen in Los Angeles after purging is completed. See enclosed sheet with specific shipping instructions.

STEM CELL STORAGE: (NEUROBLASTOMA PATIENTS ONLY)

Purged PBSC will be stored in the Purging Laboratory at Childrens Hospital L.A. until the patient expires. At that time, the stem cells may be utilized for research purposes including, but not limited to, culture for tumor cells, possible establishment of cell lines, and piloting novel purging methods

STEM CELL INFUSION:

The stem cell product (bone marrow or PBSC) should NEVER be irradiated prior to infusion. Only a 170 micron blood filter should be used during reinfusion. No WBC filter should be used.

Fluid Management:

Hydration with D5.45NS + / - KCL should begin 2-4 hours prior to the infusion and be continued for at least 4 hours following infusion. Intravenous fluids on the day of stem cell infusion, excluding the volume of cells infused, should total 3000 ml / m² / 24 hours.

Premedication:

The DMSO cryoprotectant may cause a histamine-like reaction when infused into the patient. Therefore premedication with Benadryl. **DO NOT USE TYLENOL**. See Section 5.24 for further information regarding pre-medication for stem cell infusion.

Thawing of Stem Cell Product:

Stem cells are thawed in a 37° C waterbath which is monitored with a mercury thermometer to ensure temperature does not rise above 40° C. Only one bag of stem cell product should be thawed at a time. In the event of bag breakage, every effort should be made to maintain sterility and salvage the stem cell component using a syringe with a large bore needle. When the infusion of one bag is completed, the next bag should be thawed. When the final bag of stem cells has been infused, the IV tubing should be flushed with normal saline.

Thawed stem cells should be infused as rapidly as tolerated through a central venous catheter. **No blood component filter is recommended.** The unit may be infused by gravity, or the cells may be drawn up into a syringe and pushed by trained personnel. Microaggregate filters and leukodepletion filters **MUST NOT** be used for infusion of stem cells. If a thawed unit appears clumpy or stringy and these particles cannot be dispersed with gentle kneading, the stem cell product could be infused through a standard 170 micron blood filter. When the final bag of stem cells has been infused, the IV tubing should be flushed with normal saline.

Possible Symptoms During Infusion

Precipitating Factor

Possible Symptoms

hemolyzed red cells
cellular clumps and debris
cold 10% DMSO
microbial contamination
plasma proteins

fever, chills, hemoglobinuria
chest pain, hypoxia, hypertension
nausea, headache
fever, chills, hypotension
urticaria