

## **Childrens Hospital Los Angeles Stem Cell Processing Laboratory**

### **Expanded Access Protocol for Purging of Peripheral Blood Stem Cells or Bone Marrow from Patients with High-Risk Neuroblastoma Prior to Autologous Transplantation Under FDA Investigational Device Exemption BB-IDE-2259**

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## STUDY ABSTRACT

Patients with high risk neuroblastoma have a poor prognosis with standard therapy. The recently completed CCG-3891 study documented that increased dose intensity of induction therapy and consolidation with purged bone marrow transplant improves outcome for these patients. Peripheral blood stem cells (PBSC) have been shown to have fewer contaminating neuroblastoma cells than bone marrow. In addition, we have adapted methods used for purging of autologous bone marrow to use for purging of PBSC. The impact of purging of PBSC on clinical outcome is currently being investigated in A3973, a Children's Oncology Group (COG) Group-wide phase III study, in which patients are randomized to non-purged PBSC, or PBSC that are purged at CHLA by magnetic immunobeads. Purging is carried out under an Investigational Device Exemption (IDE) from the FDA, BB-IDE-2259. While the benefit of purging in terms of increased event-free survival remains unknown pending results of the COG A3973 study, prior clinical studies have established that the magnetic immunobead method used at CHLA for purging is safe, does not compromise engraftment of the stem cells and restoration of hematopoiesis when compared to non-purged products, and removes multiple logs of neuroblastoma cells from bone marrow or PBSC. Purging has been conducted to date by the CHLA laboratory on marrows or PBSC from > 1000 children with neuroblastoma.

The purpose of this study is to provide access to the purging device to patients who are not enrolled on a clinical trial that enables purging under BB-IDE-2259. Patients are eligible to enroll in this study if they have high-risk neuroblastoma, are planning on having stem cells collected and used to support intensive chemotherapy. Stem cells purged under this protocol, if deemed suitable for re-infusion in support of intensive therapy by criteria specified under BB-IDE-2259 may be used to support intensive therapy given as best-available therapy, or may be used to support therapy given under any protocol that allows use of stem cells as purged in this protocol.

This study has no projected accrual and is expected to remain open for approximately 5 years, during which time it is anticipated that the A3973 study can be completed and analyzed. Future clinical use of stem cell purging for high-risk neuroblastoma patients will depend on the outcome of the A3973 study. As this protocol will be used to provide best-available therapy using an investigational device that has been intensively studied, the data collected will be limited to those data required for quality control of the investigational device by the FDA, and those data required of all stem cell products processed at CHLA by various regulatory agencies. As excess stem cells that can be used for research purposes are commonly available from stem cell collections submitted for purging, and studies with such excess stem cells integral to ongoing quality control of the purging process, and use of such already obtained cells poses minimal risks to the patient, patients will be asked to donate excess cells for research.

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## **1.0 SPECIFIC AIMS**

### **1.1 Primary Aim**

#### 1.11

To provide expanded access to stem cell purging under BB-IDE-2259 for patients with high-risk neuroblastoma who are not enrolled in a clinical study that utilizes FDA BB-IDE-2259.

#### 1.12

To monitor tumor removal, viable cell recovery, safety of shipping of stem cells, and engraftment of the cells in patients as required by FDA BB-IDE-2259.

### **1.2 Secondary Aim**

To make available for research and quality control purpose stem cells collected under this protocol that are excess beyond those needed for the patient's clinical care.

## 2.0 OVERVIEW OF PURGING OF STEM CELLS FOR NEUROBLASTOMA

Treatment of high-risk neuroblastoma with myeloablative therapy followed by 13-cis-retinoic acid has been established as standard-of-care by a large, randomized Childrens Cancer Group Study (29). Comparison of purged autologous bone marrow transplantation (ABMT) in support of myeloablative therapy provided an apparent better event-free survival (but not statistically significantly different) than use of allogeneic marrow (30). Because of these prior studies and the high incidence (~33%) of marrows that are harvested for ABMT that contain neuroblastoma cells detectable by immunocytology, purging of bone marrows for ABMT in neuroblastoma is routinely utilized in many centers in the USA. The role of purging of peripheral blood stem cells (PBSC) is less well defined, and an ongoing Children's Oncology Group phase III study seeks to define the role of PBSC purging by randomizing patients between purged and non-purged PBSC transplants. As the safety of purging is well established and the purging process poses only moderate risks of a loss of stem cell product (the greatest risk being the potential loss during shipping the product for centralized purging) this protocol will serve to provide expanded access to purging for patients with high-risk neuroblastoma until such time as the role of purging is clearly defined by clinical studies.

Harvest of PBSC or marrow is scheduled to be performed during hematopoietic recovery following induction therapy. Patients whose PBSC or marrow product is positive for tumor cells by immunocytology (sensitivity of 1/100,000)(positive is  $\geq 1/100,000$  tumor cells) will not have these cells used for myeloablative consolidation.

Harvest of a stem cell product for use during myeloablative consolidation therapy should occur ideally after two cycles of Induction Chemotherapy. The bone marrow does not need to be negative for tumor for PBSC harvest to occur. Patients generally proceed to ablative Consolidation therapy at completion of Induction if they have an immunocytologically negative stem cell product available. PBSC harvests to be purged will be shipped to the CHLA Stem Cell Processing Laboratory for purging on this study. Although all bone marrow and PBSC products will also be evaluated in the future for tumor content by RT-PCR these results will NOT be available to the treating physician and will NOT be used to determine if a product is to be infused for transplant. The RT-PCR studies are part of BB-IDE-2259 quality control testing and are not currently used to determine suitability of stem cells for clinical use.

To be eligible to proceed to myeloablative Consolidation therapy, patients must have adequate stem cells cryopreserved after purging. For PBSC this is a minimum of  $1.5 \times 10^6$ /kg viable CD34+ cells, or  $0.8 \times 10^6$ /kg viable CD34+ cells with an unpurged "backup" of  $1 \times 10^6$ /kg viable CD34+ cells. For bone marrows the minimum is  $0.5 \times 10^8$ /kg viable nucleated cells, but special permission of the study chair is required to use  $< 1 \times 10^8$ /kg viable nucleated cells. The stem cells must be demonstrated to be free from microbial contamination and free of neuroblastoma cells by immunocytology.

### 3.0 BACKGROUND AND RATIONALE

#### 3.1 Potential Risks of Tumor Contaminated Stem Cell Source

No randomized prospective trials to determine if purging of stem cells affects event free survival in neuroblastoma have been performed. Because the relapse rate for high risk neuroblastoma patients is very high due to lack of effective *in vivo* purging, it is difficult to demonstrate the impact that purging of stem cells has on survival following myeloablative therapy. A case control study from the European BMT Solid Tumor Registry (1), found no significant difference between the two year progression free survival in neuroblastoma patients transplanted with allogeneic (35%) versus autologous (41%) marrow. When the subgroup who received unpurged autologous marrow was analyzed, the only significant factor was residual skeletal disease detected pre-BMT by MIBG. Garaventa et al also reported no significant difference in progression-free survival in 18 stage IV neuroblastoma patients transplanted with purged (n=18) versus unpurged (n=21) autologous bone marrow following similar chemotherapy regimens (2). Another study from Japan found no difference in relapse rates between autologous marrow and PBSC in 15 children transplanted for neuroblastoma (3).

However, evidence for the tumorigenicity of reinfused neuroblastoma cells exists in the reports of miliary lung metastases following bone marrow infusion (4-6), the high rate of relapse on the CCG-321P3 study for patients with >0.1% tumor in bone marrow at the time of harvest, and the gene marking studies of Rill et al. showing the presence of the neomycin resistance marker gene in neuroblastoma relapsing post-ABMT (28). In the study by Rill et al, autologous bone marrow cells marked with the neomycin-resistance gene were reinfused to patients with neuroblastoma after myeloablative chemotherapy. All patients had pre-harvest bone marrow samples morphologically negative for tumor. In the three patients who relapsed, the marker gene was detected. Therefore, in performing consolidation with myeloablative therapy with stem cell rescue, it is desirable for the stem cell product infused to be negative for detectable neuroblastoma cells.

PBSC have been reported to have a lower incidence of tumor cell contamination than bone marrows in the same patients. Circulating tumor cells may also be reduced by effective induction chemotherapy. A study by Moss et al. found neuroblastoma tumor cells in peripheral blood specimens in 75% of samples at the time of diagnosis, 36% during therapy and 14% at the time of PBSC harvest (7). On CCG-3891, 25% of patients had detectable tumor in marrow by immunocytology at the time of harvest (median 10 tumor cells per 100,000 bone marrow cells) while 6.5% had positive peripheral blood immunocytology at the same time point (8). Patients with neuroblastoma have been shown to have circulating tumor cells with clonogenic properties *in vitro* (9).

Based on the data suggesting that PBSC have lower tumor cell content than bone marrow, and may engraft more rapidly than bone marrow, PBSC will be utilized as the preferred stem cell sources in this study.

### 3.2 Detection of Tumor in Marrow or PBSC

Since the number of neuroblastoma cells that will give rise to a tumor after intravenous infusion is unknown and since this number may vary for different patients when purging is performed the goal is to remove all detectable tumor. A sensitive method to detect residual tumor after purging is essential. Human neuroblastoma cells often have a cloning efficiency in tumor stem cell assays of < 10% (10), and can show “no growth” in marrows with histologically or immunologically detectable tumor (11). Such growth assays do not provide a sensitive means of detecting residual neuroblastoma cells after purging. One advantage associated with methods of purging that rely on physical removal of the tumor cells is that it is easier to detect the *presence* of small numbers of tumor cells than it is to distinguish small numbers of live tumor cells from dead cells.

Although useful in model studies, the pre-labeling method does not allow the assessment of clinical marrow samples for purging efficacy. For analysis of clinical samples, the Neuroblastoma Immunocytology Lab (Director, R Seeger, MD) developed a highly sensitive immunocytologic procedure for detecting neuroblastoma cells in bone marrow, which is capable of detecting 1 tumor cell per  $10^5$  marrow cells (8, 12). Although other methods for immunological detection of neuroblastoma in marrow have been described (11, 13, 14), their reported sensitivity is less than this immunocytology method. Even with a sensitivity of detecting 1 tumor cell/ $10^5$  marrow cells up to  $10^4$  tumor cells could be present in infused marrow of  $10^9$  cells.

### 3.3 Impact of Tumor Cells Detected by RT-PCR

Methods utilizing reverse transcriptase polymerase chain reaction methodology (RT-PCR) have reported detection of as few as one neuroblastoma tumor cell per  $10^6$ - $10^7$  mononuclear cells (15, 16). Mattano et al reported detectable neuroblastoma tumor cells in peripheral blood in two patients with negative immunocytology (15). The neuroblastoma reference and purging labs will employ an RT-PCR assay, using both tyrosine hydroxylase (TH) and the neuronal gene (PGP 9.5) which has a sensitivity of 1 tumor cell per million hematopoietic cells (Seeger et al, in preparation). As the clinical significance of RT-PCR positive stem cells is currently unknown, RT-PCR analyses will not be used to quality control the stem cells used in this protocol, but will be conducted at a later time on samples of cells from this protocol as a quality control measure for the purging process in general, should data from ongoing clinical studies validate the usefulness of RT-PCR assays in assessing clinically-relevant tumor contamination.

Immunocytology and RT-PCR studies will be used on bone marrow, peripheral blood, and PBSC collections to examine the question of tumor contamination, although only the immunocytology and light microscopy results will be used to determine whether the stem cell or marrow product is suitable for protocol use. The clinical implications of the possibly more sensitive PCR have not yet been defined, but will be evaluated on this study. The incidence of RT-PCR positivity in stem cell products infused will be studied in patients on this protocol as a retrospective quality control procedure, once ongoing clinical studies have established the clinical relevance of RT-PCR positivity, and once assays that generate established clinically relevant information have been established and validated.

### 3.4 PBSC Purging

Because of the high tumorigenicity of small numbers of neuroblastoma cells in bone marrow (28) it is necessary to consider that tumor in PBSC poses a possible danger of re-establishing the disease after myeloablative therapy if infused into the patient. Even tumor cells below the level of detection by immunocytology might contribute to relapse in patients.

One approach to decreasing tumor in PBSC is to do CD34 + selection. The antigen CD 34 is present on almost all committed and primitive progenitor cells, but not on most malignant cells (17, 18). CD 34+ cells have been positively selected from both peripheral blood progenitor cell and bone marrow harvests prior to transplantation in patients with breast cancer, melanoma, NHL, neuroblastoma, and PNET using one of several available CD 34+ cell selection techniques (19-22). CD 34 selected PBSC and unselected PBSC cells have identical time to hematopoietic engraftment, and engraftment time has been shown to be more rapid than bone marrow (19-23). Transplant with CD34 enriched products results in long term engraftment (72-73). Tumor cell depletion of 1.2 - 4.5 logs has been reported resulting from CD 34 selection on BM (20, 24), and may be even more effective on PBSC, where initial tumor contamination is lower. In a recent abstract Leung and colleagues reported a median 3.3 log tumor depletion in PBSC of neuroblastoma patients following immunomagnetic CD 34+ cell selection compared to 2.5 log tumor depletion following bone marrow selection (22).

However, it has been shown that CD34 selected PBSC preparations from breast cancer patients still contain tumor (25). Investigators have also found residual neuroblastoma cells in CD34 selected PBSC from advanced neuroblastoma patients, and an alarmingly high incidence of lymphoproliferative disorders and other events has caused many investigators to be cautious about the use of CD34+ selection when other methods with safer track records are available

The CHLA Stem Cell Processing Laboratory developed methods to more effectively purge PBSC collections using similar methods they have used for bone marrow (26). Because of the large numbers of PBSC cells relative to bone marrow it is necessary to fractionate the PBSC before purging. A method for rapid reduction in cell numbers from PBSC harvests was developed that reduces total cell numbers to 30% of the starting number while retaining 70% of the starting CD34+ cells. This method utilizes carbonyl iron, a very non-toxic iron particle preparation (26), which adheres to monocytes and neutrophils, allowing their removal with the same high energy magnetic device used for purging the magnetic beads. They have shown neither CD34+ cells nor myeloid progenitors (CFU-GM) are depleted by carbonyl iron, use of this approach will allow fractionation of PBSC such that the amount of PBSC needed to be purged with magnetic immunobeads will be less than the usual number of bone marrow cells purged with their current method. This will allow purging PBSC in a cost-effective manner. Their studies have shown that there is no significant difference in the efficacy of purging of marrow or PBSC fractionated with carbonyl iron compared to unfractionated cells.

PBSC shipping to CHLA from a variety of institutions to enable centralized purging with immunomagnetic beads was piloted on the CHLA 91LA6 study, and then implemented across the COG in the A3973 Phase III study, and has now been performed for > 200 patients. There have been occasional incidences of delayed marrow engraftment, but all data to date point toward no increase in incidence of delayed engraftment with purged products relative to unpurged products. A true comparison of this question is part of the A3973 COG randomized trial and will not be available until the study is closed and analyzed. However, the A3973 study is being monitored carefully by the COG Data Monitoring and Safety Board (DSMB), and the DSMB has not raised any safety concerns with the purging.

## **4.0 ELIGIBILITY CRITERIA AND PATIENT ENTRY**

### **4.1 Eligibility**

#### 4.11 Age

Patients must be  $\leq 30$  years of age at the time of initial diagnosis.

#### 4.12 Clinical Stage/Histology

Patients must have a diagnosis of neuroblastoma (ICD-O morphology 9500/3) verified by histology and/or demonstration of clumps of tumor cells in bone marrow with elevated urinary catecholamine metabolites.

#### 4.122

Patients must have a risk-group of neuroblastoma that requires stem cell transplant in support of intensive therapy to be eligible for this protocol. *Patients enrolled in any phase III study must have the permission of the study chair in order to be eligible for this study.*

#### 4.16 Organ Function

Patients must have adequate hematopoietic function to enable PBSC or marrow harvest, as determined by medical standards at local site of stem cell collection. General guidelines of peripheral stem cell mobilization are outlined in section 6.3.

#### 4.18 Informed Consent

The patient and/or the patient's legally authorized guardian must acknowledge in writing that consent to become a study subject has been obtained, in accordance with institutional policies approved by the U.S. Department of Health and Human Services.

#### 4.19 Protocol Approval

Approval for the use of this treatment protocol by the individual institution's Human Rights Committee must be obtained, in accordance with the institutional assurance policies of the U.S. Department of Health and Human Services.

4.2 Investigator Qualification. Patients must be entered on this study by the physician responsible for collecting their stem cells. That investigator must provide an FDA 1572 to the Study Chair in order to be eligible to enter patients, and must comply

with their local institution's IRB requirements for this study. In addition, the investigator must provide written assurance that they will promptly report any adverse events to the study chair and that they will promptly provide data on marrow engraftment after stem cell infusion.

#### 4.3 **Patient Entry**

##### 4.31 Patient Registration

Patients will be registered with the CHLA Stem Cell Collection Coordinator, Robert Torres Phone: **(323) 669-4565**. Prior to registration, the following must be confirmed:

1. A memorandum of understanding is in place between the site collecting stem cells and CHLA regarding payment for purging.
2. IRB approval of this protocol at collecting site must be documented with copy of written memo, which is also copied to CHLA CCI Office.
3. A copy of the signed informed consent must be faxed to CHLA Transplant Office at 323-664-9455.

## 5.0 Scheduling Stem Cell Harvest and Purging

### 5.1 Timing/scheduling of stem cell collection

Peripheral blood stem cell harvest is recommended to be performed after cycle 2 of induction chemotherapy for newly diagnosed patients. (Delays of harvest beyond this point should be discussed with study chair). For relapsed/recurrent patients, timing of peripheral blood stem cell collection will be determined by the treating physician, after discussion with the study chair. Do not base decision to harvest PBSC on bone marrow tumor content. PBSC harvest may proceed even if there is persistent tumor in bone marrow.

Timing of bone marrow harvest is dependent on assessment of tumor content in bone marrow (see section 6.9 for details).

**Prior to any PBSC or bone marrow collection for purging,, call Robert Torres who carries out the Stem Cell Purging Laboratory Scheduling at (323) 669-4565 to schedule purging. A tentative purging date should be scheduled as soon after diagnosis as is possible.**

### 5.2 Criteria for reinfusion of purged stem cells

#### 5.21 Dosage

Purged PBSC: A minimum of  $1.5 \times 10^6$  viable CD34 cells/kg (optimum  $2 \times 10^6$ /kg) must be available. If no other stem cell source is available, patients with PBSC collections of  $0.8-1.5 \times 10^6$  viable CD 34 cells/kg may be allowed with permission of Study Chair. If purged PBSC has  $< 1.5 \times 10^6$  CD34/kg, these patients must have unpurged back-up available).

Purged Bone Marrow: A minimum of  $10^8$  mononuclear bone marrow cells/kg (optimum  $> 2 \times 10^8$  cells/kg or  $> 8 \times 10^4$  CFU-GM/kg).

#### 5.22 Tumor cell content

Stem cell product must be free of detectable tumor by immunocytology after purging.

### 5.3 Stem Cell Infusion

#### 5.31 Infusion

Stem cells will be infused intravenously within 1½ hours of thawing. Timing of stem cell infusion will be determined by treating physician, as medically indicated by preceding therapy regimen.

#### 5.32 Toxicities

Toxicities may include an anaphylactic reaction, respiratory difficulty, hypotension, and volume overload. Other transfusions should be avoided if possible on the day of the stem cell infusion. Furosemide should be administered if clinically indicated.

### 5.33 Premedications/Monitoring

- a) Fifteen minutes prior to stem cell infusion, premedicate with acetaminophen (10 mg/kg PO) and Benadryl (1 mg/kg IV).
- b) Ambu bag, Benadryl and epinephrine at bedside.
- c) Place patient on cardiac monitor during infusion and for 1-2 hours following completion.
- d) Discontinue all other IV fluids as possible during stem cell infusion to avoid volume overload.
- e) Hydrate for 24 hours post stem cell infusion with 3000 ml/m<sup>2</sup>/day total IV fluids.

### 5.4 Scheduling of Purging

Arrangements will need to be made in advance with the Neuroblastoma Purging Laboratory for shipping and purging of the stem cell product, for both PBSC or bone marrow. For each PBSC to be shipped for purging, the investigator needs to discuss the plans for timing of harvest and length of PBSC collection procedure with the Neuroblastoma Purging Laboratory in advance (Call Robert Torres at (323) 669-4565 or FAX at 323-664-9455). A tentative purge date will be scheduled at this time. A follow-up phone call is then required to confirm purge date once patient meets criteria to escalate G-CSF in preparation for harvest. If it is clear that a patient will require a harvest either earlier or later than the tentative harvest date, contact the Robert Torres as soon as this is known to change the purging date. This will prevent delays in scheduling the purging.

## 6.0 PROCEDURES FOR STEM CELL MOBILIZATION AND HARVEST

### 6.1 **Recommended Procedure for PBSC Mobilization and Collection**

For patients < 10 kg contact study chair prior to scheduling purging to discuss apheresis plan.

### 6.2 Catheter Use

PBSC may be collected using a large bore double lumen central venous catheter specifically designed for apheresis or, if the patient is of sufficient size, two large bore peripheral IVs can be placed in the arms for the procedure. It is anticipated that most neuroblastoma patients will require placement of a temporary or tunneled apheresis catheter. Most centers have not had success in collecting peripheral blood progenitor centers using the standard Hickman or Broviac central lines and therefore a specific apheresis catheter is recommended (see Appendix V for listing of suitable catheters).

### 6.3 Recommended Protocol for Peripheral Stem Cell Mobilization

Patients should continue on G-CSF 5 mcg/kg/day while recovering from the prior cycle of chemotherapy until the ANC is > 1,000/ $\mu$ L following the nadir, then the G-CSF dose should be increased to 16 mcg/kg/day for 3 days (regardless of platelet count). Collection then begins the day after the third dose of G-CSF. G-CSF is given daily until PBSC collection is complete. (Alternatively, the G-CSF dose can be increased to 16 mcg/kg/day when the ANC reaches 1,000 following the nadir and PBSC harvesting can

begin thereafter when a circulating CD34 cell number is  $> 20$  cell/ $\mu$ L. When feasible, this method is preferred). If patients are off G-CSF prior to planned PBSC harvest, they should receive G-CSF 16 mcg/kg/day for 3 days prior to the first day of scheduled PBSC harvest and continue daily G-CSF until PBSC collections are completed. In this situation peripheral CD 34 cell determinations are recommended to help determine optimal day to begin collection.

#### 6.4 G-CSF Dose during PBSC Harvest

Adjust daily G-CSF dose based on post-harvest WBC as follows:

- If post-WBC is  $< 60,000$ , administer 16 mcg/kg.
- If post-WBC is  $> 60,000$ , administer 5 mcg/kg.

#### 6.5 Required CD34 Yield for PBSC Collections

It is recommended that large volume apheresis be performed on all patients for each collection. Large volume apheresis is defined as processing 6 blood volumes from the time of opening the collect valve. See Appendix III: Guidelines for the Collection, Cryopreservation and Infusion of PBSC. CD 34+ cell counts and CFU-GM assays should be done at local institution on all collections. CD 34 counts will also be performed on PBSC specimens sent to the CHLA Stem Cell Processing Lab.

For Purged PBSC: The goal is to obtain  $10 \times 10^6$  CD34+ cells/kg to be shipped for purging, with a goal of providing a minimum of  $1.5 \times 10^6$  CD34+ cells/kg for infusion, with optimally  $\geq 5.0 \times 10^6$  CD34+ cells/kg for infusion post-purging. Do not ship collections for purging if  $< 5.0 \times 10^6$  CD34+ cell/kg are collected in the first 2 days unless discussed with protocol chair first. DO NOT cancel shipping prior to discussing low yield with study chair.

The following schedule is suggested for PBSC pheresis:

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

**DAY TWO:** End pheresis in time to allow shipping of BOTH day one and two collections together at room temperature, to arrive at Purging Laboratory on DAY THREE, preferably by 11 AM. Add 100 IU/ml of preservative free heparin to day two stem cell collection at end of pheresis. **DO NOT POOL DAY 1 AND DAY 2 PBSC COLLECTIONS.**

**DAY THREE:** Backup collection recommended if  $\leq 5 \times 10^6$  CD 34+ cells/kg shipped for purging and concerned about low post-purge yields. Recommend a minimum collection of  **$2.0 \times 10^6$  CD34+ cells/kg (preferably  $5 \times 10^6$  CD 34/kg)** as a backup. This will NOT be purged and should be stored at collection site. Confirmation of a sufficient backup to CHLA Purging Laboratory is required prior to beginning myeloablative therapy if post-purge CD 34+/kg  $\leq 1.5 \times 10^6$ .

PBSC should be shipped after DAY TWO collection if a minimum of  **$3.5 \times 10^6$  CD34+ cells/kg** can be obtained from DAY ONE of pheresis. **If DAY ONE yield is less than  $3.5 \times 10^6$  CD34+ cells/kg, please contact Robert Torres at the Purging Laboratory**

**(323-669-4565) or Dr Patrick Reynolds (323-669-5646, pager 1-877-743-8622), or Dr. Judy Villablanca: (323-669-5654, pager: 213-209-1916) to discuss prior to shipping. Do NOT cancel shipping prior to discussion regarding recommendations. Please call the Purging Lab (323-669-5632), Dr. Reynolds,, or Dr. Villablanca for any questions regarding collection or criteria to ship.**

**If sufficient cells for purging cannot be collected in 2 days, then patients will not have a purging done. Instead, collection of PBSC should be done for 2-4 days to obtain a minimum of  $2 \times 10^6$  CD34+ cells/kg (optimal goal  $> 5 \times 10^6$  CD34 cells/kg). These should be cryopreserved without other manipulation. These unpurged PBSC will then be available for consolidation stem cell transplant. A second attempt to obtain sufficient PBSC for purging can be attempted after additional chemotherapy Immunocytology should be negative for the unpurged PBSC collection to be used for transplant.** For patients with insufficient cells collected for purging, a purged bone marrow harvest can be used instead of the stored unpurged PBSC, if this is physician or parental preference.

#### 6.6 Shipping PBSC Collection for Purging

PBSC may be collected at any center approved for BB-IDE-2259 as a Bone Marrow Transplant Center and shipped to Children's Hospital Los Angeles for purging. Scheduling of the remote site harvest MUST be done in coordination with the Neuroblastoma Purging Lab. If possible, PBSC should be shipped to arrive Monday-Friday, non-holidays. Please contact the Purging Lab to make arrangements ahead of time if your patient's collection needs to be shipped to arrive on a Saturday, Sunday, or holiday.

All PBSC must be shipped in a sealed bag suitable for blood products, without leaks or any external blood on the container. All PBSC must have preservative-free heparin added (100U/ml). PBSC bags should be labeled and sealed inside an external plastic bag and packaged in a minimum of 8 cubic feet of insulation inside a styrofoam container. PBSC should be sent with the Stem Cell Harvest Shipping Form and a signed consent for purging. When the day two pheresis is complete, please call the Purging Laboratory by telephone at 323-669-4565 and provide shipping information, including carrier, tracking number, and planned arrival time. If the PBSC do not arrive by 11 AM the day after the collections were completed, then the Purging Lab will contact the shipping institution and the courier.

#### 6.7 Analysis of Peripheral Blood Stem Cells for Tumor Content and CD 34 Number

If the PBSC product is sent to the Neuroblastoma Purging Laboratory for purging, an aliquot will be removed there and analyzed for CD34 cell number as well as tumor content by immunocytology and RT-PCR pre and post purging. This sample is required for analysis of neuroblastoma cell content using immunocytochemical staining and RT-PCR in order to quality control the PBSC prior to release for infusion. Cells are also tested for microbial contamination and viable cell recovery after cryopreservation.

#### 6.8 Immunocytology - Detected Tumor Contamination of Stem Cell Product

If tumor is detected by immunocytology in any harvested stem cell product, it may not be used for myeloablative consolidation on this protocol. Patients whose unpurged or post-purge PBSC harvest is contaminated by tumor may undergo another attempt to obtain an immunocytology negative stem cell product. **Call Study Chair to discuss the options.**

#### 6.9 **Procedure for Purged Bone Marrow Harvest**

(See Appendix II for procedural details)

Bone marrow harvest is only for patients who do not have an immunocytology negative PBSC product or are unable to collect sufficient PBSC for purging and who are still eligible to proceed to myeloablative consolidation. To be eligible for BM harvest the patient must have a diagnostic bilateral bone marrow aspirate done ten days prior to scheduled bone marrow harvest which demonstrates  $< 100$  tumor cells/100,000 marrow cells and adequate bone marrow cellularity ( $> 15 \times 10^6$  mononuclear cells/ml of marrow).

#### 6.91

Patients who have purged bone marrow harvest will have purging performed at CHLA. Bone marrow can be harvested at a participating BMT Center and shipped to the Neuroblastoma Purging Laboratory for processing. The Neuroblastoma Purging Laboratory must be contacted to schedule marrow harvests/purging at (323) 669-4565 (FAX 323-664-9455, Attention: ABMT Coordinator) once it is determined that a purged bone marrow harvest will be done.

#### 6.92

Eight to ten days before marrow harvest, patients must have a bilateral bone marrow aspirate performed. Aspirate 5 ml bone marrow from each posterior iliac crest in preservative free heparin (100 u/ml). Send the two samples kept separate to the CHLA Neuroblastoma Immunocytology Reference Laboratory for nucleated cell count and immunocytologic analysis (See Section 15). Be sure to indicate that this sample is pre-harvest evaluation. Patients must have adequate cellularity defined as  $15 \times 10^6$  nucleated cells/ml of marrow and have  $< 100$  cells/ $10^5$  marrow cells to proceed to harvest. The referring institution will be notified by the Neuroblastoma Reference Laboratory of the results, and to confirm the harvest date.

#### 6.53 Tumor Content

The marrow product must have NO detectable tumor cells by immunocytology following completion of purging process (limit of detection 1 tumor cell: 100,000 mononuclear cells). If tumor cells are detected in the purged marrow, this marrow cannot be used for transplant.

#### 6.93 Cell Dose

A minimum dose of  $1.0 \times 10^8$  cells/kg must be available for infusion. The optimum dose is  $> 2 \times 10^8$  cells/kg.

#### 6.94 Timing of Bone Marrow Harvest

For newly diagnosed patients, bone marrow harvest should be scheduled following induction cycles 3-6. Perform as early on in induction as possible, preferably after cycle 3 or 4. If bone marrow harvest is to be done, following cycle 5, when surgery is scheduled, consider performing BM harvest during the same anesthesia as used for surgery, if the surgery will be minor. Otherwise, schedule surgical resection when ANC  $> 500$  and perform bone marrow harvest one week following resection. Perform bone marrow harvest once bone marrow cellularity requirements are met. (Past experience on CCG-3891 indicated that 4-5 weeks from last chemotherapy was required to obtain an adequate harvest). **Prior to any purged PBSC or bone marrow harvest, call the Stem Cell Purging Laboratory at 323-669-4565 to schedule purging.**

## **7.0 REQUIRED OBSERVATIONS**

### **7.1 IMMUNOCYTOLOGY**

Prior to bone marrow harvests, bilateral bone marrow aspirate (2-4 cc in preservative free heparin) at room temperature for immunostaining for neuroblastoma.

Ship specimens for immunocytology before harvest to:

**NOTE: DO NOT SEND PBSC  
OR MARROW FOR PURGING  
TO THIS ADDRESS, ONLY  
PRE-HARVEST SAMPLES FOR  
IMMUNOCYTOLOGY**

Robert C. Seeger, M.D.  
Neuroblastoma Biology Resource Laboratory  
Smith Research Tower, Room 509  
Children's Hospital of Los Angeles  
4546 Sunset Blvd.  
Los Angeles, CA 90027  
Telephone: (323) 669-5630  
Fax: (323) 664-9324  
Contact person: Rich Gallego

### **7.2. ENGRAFTMENT DATA**

For all patients, marrow engraftment data must be reported in writing to the CHLA Stem Cell Processing Laboratory within 30 days of stem cell infusion, and every 2 weeks after that point if sustained engraftment of ANC  $\geq$  500 and platelets  $\geq$  20,000 without transfusion was not reported at day 30. Data required are the date of stem cell infusion, regimen given prior to stem cell infusion (with dates administered), and the date at which an ANC  $\geq$  500 was first observed that remained  $\geq$  500 for 3 days, and the date in which platelets  $\geq$  20,000 was sustained without platelet transfusion. Actual ANC and platelet counts from stem cell infusion until engraftment are preferred but not required.

## **8.0 REQUIRED SPECIMENS**

### **8.1 Quality Control Specimens**

Five vials, each containing approximately 40 million cells, will be viably cryopreserved for quality control purposes in relationship to clinical care. These cells will be available for repeating the standard quality control tests (FACHT and FDA mandated) of the Stem Cell Processing Laboratory of a) viable cell recovery and CD34 recovery; b) microbiology; and c) tumor content by immunocytology or quantitative RT-PCR should there be a clinical indication for such.

## 8.2 Excess cells for research

It is common for cells beyond the capacity to purge to be collected and submitted to the purging lab. In addition, when a patient dies, and cells have been stored for autologous transplantation, they are not useable by that patient (or anyone else for clinical purposes). Such cells are designated as "excess" and are made available in an anonymized fashion for research purposes. The research that they are used for is to improve the purging process, improve methods of analyzing marrow or blood for detecting tumor cells, or for comparison of non-tumor cells to tumor cells in various biological assays. The data collected is not linked to the specific patient, as the samples are anonymized. The investigator using the cells for research will not have knowledge of the patient identity when the cells are used for research.

There is also the need to bank material for quality control purposes, as required under BB-IDE-2259, to analyze for tumor content. For this purpose we will bank cryopreserved cells before and after purging, generally ~50 million cells are stored for this purpose. In addition, nucleic acids are isolated from the beads that are discarded, to enable PCR detection of tumor present on the beads. This material will be banked along with the stem cells for quality control purposes

## **9.0 ADVERSE EVENT REPORTING**

### 9.1 What To Report

1. Any life-threatening (Grade 4) or fatal (Grade 5) adverse event with an attribution of possible, probable or definite to purging must be reported.
2. Time to ANC  $\geq$  500 that is longer than 28 days
4. Time to platelets  $\geq$  20,000 without transfusion longer than 56 days
5. Infusion of additional stem cells after the initial stem cell dose on day 0 of transplant

### 9.2 When To Report

These events should be reported within ten (10) working days.

### 9.3 Where To Report

These adverse events with commercial agents must be reported to the FDA using the MedWatch form. A copy of the MedWatch form can be obtained from the FDA's MedWatch Web site (see below). The MedWatch report can be sent by the following mechanisms:

| <b>To the FDA:</b>  | <b>CHLA</b>   |
|---|---|
| On Line:<br><a href="http://www.fda.gov/medwatch">www.fda.gov/medwatch</a>                | Copy  |
| Mail:<br>MedWatch<br>5600 Fishers Lane<br>Rockville, MD 20852-9787<br>Fax: 1-800-332-0178 | Mail:<br>Eric Bubbers, PhD<br>Director Regulatory Affairs & Administrative Director IPCR<br><br>Childrens Hospital Los Angeles, MS 55<br>4650 Sunset Blvd<br>Los Angeles, CA 90027<br>Email: <a href="mailto:ebubbers@chla.usc.edu">ebubbers@chla.usc.edu</a><br>Phone: 323-644-8717<br>FAX: 323-644-8790 |

## 10.0 RECORDS AND REPORTING

All data for this protocol will be maintained in the CHLA Stem Cell Processing Laboratory. Written records are secured in locked file cabinets, electronic records on pass-word protected computers. The lab is locked when not in use.

Required data to be submitted:

For all patients, marrow engraftment data must be reported in writing to the CHLA Stem Cell Processing Laboratory within 30 days of stem cell infusion, and every 2 weeks after that point if sustained engraftment of ANC  $\geq$  500 and platelets  $\geq$  20,000 without transfusion was not reported at day 30. Data required are the date of stem cell infusion, regimen (with dates administered) given prior to stem cell infusion, and the date at which an ANC  $\geq$  500 was first observed that remained  $\geq$  500 for 3 days, and the date in which platelets  $\geq$  20,000 was sustained without platelet transfusion. Actual ANC and platelet counts from stem cell infusion until engraftment are preferred but not required.

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## APPENDIX I: BONE MARROW HARVEST AND SHIPPING INSTRUCTIONS

### Specific Bone Marrow Harvest Guidelines

Marrow is withdrawn in the operating room under general anesthesia and sterile conditions and then is taken to the laboratory where it is treated to remove any neuroblastoma cells. These treatments include sedimentation, filtration, and monoclonal antibodies with magnetic immunobeads. Afterwards, the normal marrow cells are viably frozen and stored. A small portion of this treated marrow is analyzed to determine if all detectable neuroblastoma cells were removed and to determine if the normal bone marrow cells (CFU-GM) are able to grow *in vitro*.

Since 400-700 ml of bone marrow are usually harvested, 1-2 units of PRBC are given during the procedure. Any blood products administered before or during the procedure must be irradiated. The bone marrow will be harvested from either the posterior and/or anterior iliac crests. The patient's bone scan should be carefully reviewed to ensure that there is no active disease in the pelvis. If disease is present, the area of the pelvis with tumor metastases must be avoided at the time of harvest. Each aspiration should contain 5 ml or less of marrow. The medium for collection of the marrow is L-15 made under FDA certified GMP conditions and containing <0.005 ng/ml endotoxin. The medium which meets these standards is L-15 medium, catalogue #9083, Irvine Sci. Co., Santa Ana, CA (phone 800-437-5706, ext 231). Prior to harvest, 10,000 units of preservative-free heparin (sterile, suitable for injection) should be added to 100 ml of L-15. **Do not add DNase or any other additives to the medium for the harvest.** Do not strain marrow; straining the marrow may break up tumor clumps and decrease the efficacy of purging. After collection, place the marrow into one or more plastic bags suitable for blood products, and double seal entrance ports, and label with patient's name and birth date. Then seal the bag in an outside plastic bag and place inside a minimum of 8 cubic feet of insulation inside a styrofoam box. Ship by a carrier that will guarantee delivery to CHLA prior to 11 AM the following day. Notify the Purging Laboratory by fax and telephone as described above and notify the Operations Center.

Marrows may be harvested at any approved C.O.G. Bone Marrow Transplant Center and shipped to Children's Hospital Los Angeles for purging. A pre-harvest marrow as outlined in Section 6.52 is still required, and scheduling of the remote site harvest **MUST** be done in coordination with the CHLA ABMT Office. The goal is to obtain  $1 \times 10^9$  nucleated cells/kg at time of harvest; a minimum of  $0.8 \times 10^9$  nucleated cells/kg is required. Marrows must be shipped in a sealed bag suitable for blood products, without leaks or any external blood on the container. All marrows must have preservative-free heparin added to the medium in which they are collected. Marrow bags should be labeled and sealed inside an external plastic bag and packaged in a minimum of 8 cubic feet of insulation inside a styrofoam container. Marrows should be sent with the Stem Cell Harvest Shipping Form. When the marrow harvest is complete, please call the Purging Laboratory by telephone at 323-669-4565 and provide shipping information, including carrier, tracking number, and planned arrival time. If the marrow does not arrive by 11 AM the day after the harvest, then the Purging Lab will contact the shipping institution and the courier.

## **APPENDIX II: GUIDELINES FOR PBSC COLLECTION, STORAGE, AND INFUSION**

### **Peripheral Blood Stem Cell (PBSC) Collection Procedure**

#### **Apheresis Machine**

The Cobe Spectra or the Fenwal CS 3000+ is recommended 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. The Standards of Care protocols should be written and available in the Apheresis Unit. The standard operating procedure will be specific for each machine.

#### **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 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 necessary for patients <15 kg. The blood prime will be performed with cross-matched, irradiated, filtered red cells.

#### **Procedural Support**

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 patients platelet count is <30,000, transfuse with platelet prior to apheresis procedure.

#### **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. If patient becomes symptomatic from hypocalcemia then give oral calcium (2 Tums, 8 oz calcium fortified orange juice or 8 oz milk) or alternatively a calcium gluconate infusion can be used.

#### **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

### **Collection Goals**

During each leukopheresis procedure, the volume of whole blood processed should be approximately 480 ml/kg (6 blood volumes).

Optimal collection goal (total for all collections) is  $10 \times 10^6$  CD 34+ cells/kg for PBSC collections that are to be purged. The targeted number of cells can usually be obtained in 2-3 collection days. May obtain CD 34+ cell/kg analysis at midpoint in daily collection to determine need for additional day of PBSC collection (midpoint CD34 counts found to be equal to half the final CD34 yield).

### **Patient Monitoring**

Patients should be observed continuously during the collection. Vital signs should be obtained q 1 hour.

### **Laboratory Studies**

For patients < 25 kg, a type and cross for PRBC should be performed one day prior to procedure.

Pre-apheresis and immediately post-apheresis the following lab values should be obtained: CBC with differential and platelet count, ionized calcium and magnesium.

### **Vascular Access**

For continuous flow apheresis, two sites of venous access are required. See Appendix V for specific types of apheresis catheters available.

### **PBSC Products**

The following studies will be performed on each PBSC collection at CHLA:

- 1) culture for bacterial and fungal contamination,
- 2) nucleated cell count and differential,
- 3) assay for in-vitro progenitor colony growth (e.g. CFU-GM),
- 4) CD 34+ cell enumeration 5) Immunocytology/(PCR analysis only if clinically indicated) for tumor cell content (sent to Neuroblastoma reference Lab).

### **Cryopreservation of PBSC Products**

Each collection should be processed and cryopreserved using 10% dimethyl sulfoxide final concentration, controlled-rate freezer, and liquid nitrogen storage. Stem cells should be frozen at a final concentration of  $0.5$  to  $1.2 \times 10^8$  nucleated cells/ml. Cells to be purged will be shipped to the NB Purging Lab at room temperature. Cells will be frozen in Los Angeles after purging is completed.

### **PBSC Infusion**

**The PBSC product 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 0.45 NS +/- 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 PBSC infusion, excluding the volume of cells infused, should total 3000 ml/m<sup>2</sup>/24 hours.

### **Premedication**

The DMSO cryoprotectant may cause a histamine-like reaction when infused into the patient. Therefore premedication with Benadryl and Tylenol is recommended.

### **Thawing of PBSC**

PBSC 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 PBSC should be thawed at a time. In the event of bag breakage, every effort should be made to maintain sterility and salvage the PBSC 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 PBSC has been infused, the IV tubing should be flushed with normal saline.

Thawed PBSC 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 PBSC. If a thawed unit appears clumpy or stringy and these particles cannot be dispersed with gentle kneading, the PBSC product could be infused through a standard 170 micron blood filter.

### **Possible Symptoms During Infusion**

#### Precipitating Factor

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

#### Possible Symptoms

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

### **APPENDIX III: GUIDELINES FOR ADMINISTRATION OF G-CSF BEFORE/DURING PBSC COLLECTION**

#### **I. G-CSF Dose following chemotherapy preceding PBSC collections**

Patients will receive G-CSF at a dose of 5 µg/kg/day beginning 24 hours after last chemotherapy dose for cycle of induction following which PBSC collection is planned. Patients will continue on G-CSF 5 µg/kg/day until the ANC >1000/µL for 1 day following the nadir (regardless of platelet count).

Once these criteria are met, the G-CSF is increased to 16 µg/kg/day for 3 days and then PBSC harvest is performed. (Alternatively, the G-CSF can be increased to 16 µg/kg/day and PBSC harvesting can begin when the peripheral CD34+ count is > 20 cells/µL).

If PBSC harvest can not be performed within 4 days of meeting the above criteria (due to chemotherapy toxicity, infection, etc.), **CONTACT STUDY CHAIR**. If WBC rises to greater than 80,000/µL prior to PBSC harvest, discontinue G-CSF and **CONTACT STUDY CHAIR**. If G-CSF has been stopped, call Study Chair prior to beginning mobilization to confirm dose.

#### **II. G-CSF Dose During PBSC Harvest**

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.

#### **III. G-CSF Adjustments for Toxicity**

Patients that develop Grade IV toxicity due to G-CSF will be changed to GM-CSF. Call Study Chair to notify of plan to give GM-CSF.

## **APPENDIX IV: GUIDELINES FOR PLACEMENT OF APHERESIS CATHETERS AND CATHETER FLUSHING PROCEDURES**

### **CATHETER PLACEMENT**

In patients less than 15 kg use the MedComp 8.0 French permanent or temporary catheter as required. For patients greater than 15 kg, the MedComp 8.0 French or 11.5 French Vas-Cath can be used.

### **TYPES OF CATHETERS AVAILABLE**

Vas-Cath Soft Cell PC  
Permanent double lumen dialysis catheter  
French Size: 11.5, Length: 23 cm

available from: Vascath Incorporated  
2380 Tedio Street  
Mississauga Ontario  
Canada L5A3V3  
Tel: 416-848-5800  
Toll Free: 1-800-387-9482

MedComp  
Permanent cuffed double lumen dialysis catheter  
French Size: 8, Length: 18 cm

available from: MedComp  
1499 Delp Drive  
Harleysville, PA 19438  
Tel: 215-256-4201

### **Pheresis Catheter Flushing Protocol**

- NEVER flush lumen before withdrawing 5 cc of blood.
- Pheresis catheter should be flushed with 1,000 units of heparin per lumen 3 times per week.
- Use 1,000 units heparin per 1 cc concentration.
- Draw up 1 cc (1,000 units) in a 3 cc syringe.
- Dilute with normal saline to equal the intraluminal volume for each lumen. If intraluminal volume is less than 1 cc - flush with correct amount of undiluted heparin.
- Repeat steps 1-3 for each lumen.

Note: the intraluminal volume usually differs between lumens. This information can be found on the package insert.

## **APPENDIX V: PROCEDURE FOR IMMUNOLOGIC PURGING OF MARROW AND PBSC PRODUCTS**

### **Standards for Bone Marrow/PBSC Harvest Center**

Only institutions approved for BB-IDE-2259 are eligible to perform remote PBSC/bone marrow harvests. Detailed methods on the collection and shipping of marrow or PBSC from the harvesting institution to the purging laboratory are found in the protocol. Should any one harvest center perform below minimum standards of sterility, cell count, or shipping for two consecutive patients, or for 2/6 patients, they will be suspended from remote harvesting until they have addressed the performance in writing to the Study Chair and purging laboratory director, and received written reinstatement.

### **Purging of Bone Marrow or PBSC**

Harvested marrow or PBSC is transported to the Purging Laboratory where it is treated to remove any detectable neuroblastoma cells. An aliquot of the purged product is analyzed to determine if all detectable neuroblastoma cells were removed and to quantitate viable cells, CFU-GM and CD34+ cells (before and after freezing). If no tumor cells are detectable by immunocytology, if there is no microbial contamination, and if adequate normal cells are present, the marrow or PBSC is suitable for reinfusion; if not, the product cannot be used for reinfusion and a second harvest or alternative therapy is necessary. Once the stem cells are cryopreserved they will be shipped back to the originating hospital where the transplant will be conducted.

All processing is done in a laminar flow room in a closed system using sterile technique. Bacterial and fungal cultures are obtained prior to purging and at the time of cryopreservation. Cultures to detect fungi are not considered negative prior to 7 days of culture. The specific procedures for purging marrow and PBSC are described below:

### **PURGING OF BONE MARROW:**

#### **1. Separation of marrow leukocytes and neuroblastoma cells by sedimentation**

Because neuroblastoma cells often grow in the marrow as clusters of 5-50 cells, they sediment with erythrocytes in hetastarch. To avoid break-up of tumor cell clumps, marrow is *not* filtered through wire mesh screens in the operating room. Upon receipt by the Purging Laboratory, it is mixed with an equal part of 3% hetastarch and allowed to sediment in a 1 L Vialflex bag (Travenol, Deerfield, IL). The sediment, which contains red cells and tumor clumps, is left behind when the supernatant of plasma and leukocytes is pumped out. This separation yields approximately 90% of marrow mononuclear leukocytes in the supernatant and removes up to 1 log of tumor cells.

#### **2. Removal of neuroblastoma by filtration**

The leukocyte/plasma supernatant in the Vialflex bag is pumped through a 40 µm multi-layer mesh filter (Pall Biomedical, Fajardo, PR) and then a 20 µm PDF-20 Pediatric Transfusion Filter (Travenol, Deerfield, IL); effluent cells are collected into

a DuPont Stericell Processor and centrifuged at 400 x g (continuous flow). The cells are then washed by the Stericell with L-15 and resuspended in L-15 + 5% human serum albumin. Filtration removes approximately 0.5 logs of tumor cells with recovery of 60% of marrow mononuclear cells and 60% of CFU-G,M.

**3. Removal of neuroblastoma with immunomagnetic beads**

See Table 1. and description under Purging of PBSC

**PURGING OF PBSC**

**1. Carbonyl Iron Fractionation**

To concentrate cells for purging, PBSC are placed in blood bags and 100-200 mg of sterile carbonyl iron (CI) per 100 million cells is added in Leibovitz's L-15 medium (pre-warmed to 37°C) supplemented with 10% human serum albumin (27). Carbonyl iron is sterilized by dry heat (180 degs C for 8 hours). Incubation in carbonyl iron is carried out by placing the bags horizontally in the 37° C incubator for 1 hour. The carbonyl iron and cells which attach to it are then attached to the sides of the bags using the magnetic field from 2 opposing samarium cobalt magnets as described in US Patent # 4,710,472 for 3-5 minutes. Cells in suspension not adhering to the sides of the bags are then transferred to a new bag. The original bag is washed twice by gentle addition of 50 ml each to the same medium to recover all CI free cells. Cells collected after separation are then used for purging with magnetic immunobeads. Since tumor cells in PBSC are likely to be in smaller clumps than those found in bone marrow, the sedimentation and filtration steps in the purging process are omitted for PBSC.

**2. Removal of neuroblastoma with monoclonal antibodies and immunomagnetic beads**

PBSC fractionated by carbonyl iron are purged as described below using 5 monoclonal antibodies and magnetic beads.

**TABLE 1.**  
**Method for Removing Neuroblastoma Cells from Marrow or PBSC**  
**with Monoclonal Antibodies Attached to Magnetic Beads**

1. Prepare 4.5  $\mu\text{m}$  polystyrene-magnetite immunobeads: Dynabeads M-450, uncoated,  $13.3 \times 10^9$  beads per mg in sterile water.
  - a) Suspend beads in Dulbecco's PBS-A, pH 7.4.
  - b) Rotate with GAM (50  $\mu\text{g}/\text{mg}$  beads/0.1 ml) for 18 hr at 4°C, wash 2x, rotate 1 hr at 4°C, wash 2x.
  - c) Attach monoclonal antibodies to GAM coated beads:

| ANTIBODY | $\mu\text{g}$ antibody/mg beads | ISOTYPE |
|----------|---------------------------------|---------|
| HSAN 1.2 | 5                               | IgG1    |
| 459      | 10                              | IgM     |
| BA-1     | 10                              | IgM     |
| HNK-1    | 10                              | IgM     |
| Mab126   | 10                              | IgM     |

Rotate antibodies with beads for 180 min at 4°C, wash 2x, rotate in L15-10% FCS at 4°C for 1 hr, wash 2x, resuspend in L15-2.5% human serum albumin (HSA) sonicate, and count. Beads are tested for sterility (one-week incubation minimum).

2. Immunobeads may be used immediately or stored in L15-FCS for up to 6 weeks without losing activity. If beads are stored, wash 1x, suspend in L15-2.5% HSA sonicate, and count before using.
3. Add magnetic immunobeads to marrow or PBSC (bead to total cell ratio, 1:1;  $10^7$  cells per ml in a 600 ml transfer pack); and rotate them together in a transfusion bag for 30 min at room temp.
4. Remove tumor cells bound to magnetic immunobeads by placing transfusion bag with marrow in magnetic depletion device followed by draining cells into a second bag (for second cycle of purging) and pass cells from the second bag between eight double magnetics at a flow rate 10 ml per min.
5. Collect cells that pass by the magnets for cryopreservation.

An antibody "cocktail" is used to overcome the potential problem of heterogeneity in antigen expression by tumor cells. Bead-tumor cell conjugates are removed by applying "debulking" magnets to the outside of the 600 ml bag. For the second cycle of purging, fresh magnetic immunobeads are added to marrow cells after they have been transferred to a second bag following debulking in the first bag. After debulking of the second bag twice, the marrow is passed through tubing compressed between 8 pairs of samarium cobalt magnets, and collected into fresh 600 ml transfer packs.

Two cycles removes 3.6 to 4 logs of tumor cells with recovery of 35-40% of mononuclear leukocytes and 75-90% of CFU-G,M.

Our approach to the specificity of monoclonal antibodies is an operational one since it is difficult, if not impossible, to define absolutely tumor specific antigenic determinants. Thus, although monoclonal antibodies 459, 390, HSAN 1.2, and Mab 126 react with some normal cells outside of the bone marrow, they are useful for this *ex vivo* treatment because they react with neuroblastoma but not normal marrow cells. BA-1 reacts with approximately 6% of normal marrow cells but does not react with pluripotent stem cells. Other antibodies used in this cocktail (HNK-1) provide increased efficacy in pre-clinical experiments because they bind to neuroblastoma cells, but do not decrease CFU-G,M. Our experience with >300 patients using our current antibodies indicates that they do not compromise marrow engraftment.

Monoclonal antibodies and goat anti-mouse immunoglobulin (GAM) are prepared in sufficient quantities to meet our estimated needs for at a time, so that standardized, high quality reagents will be employed for each treatment. All antibodies are purified and passed through 0.22  $\mu$ m pore diameter filters for sterilization. Testing of aliquots of these purified lots demonstrated that the antibodies are functional, sterile, pyrogen-free. The master cell bank for producing the antibodies has been shown to be free of 12 adventitious murine viruses (MAP test), ecotropic murine leukemia virus (XC plaque assay), and xenotropic murine leukemia virus (S+/L-assay). Testing of antibody coated beads for a variety of viral products is also carried out as mandated by the FDA. Purging with magnetic beads and monoclonal antibodies is carried out under FDA monitoring (BB-IDE 2259 "Purging of Stem cells with Monoclonal Antibodies to Neuroblastoma").

### **CRYOPRESERVATION OF STEM CELLS**

After the last passage of marrow or PBSC over the magnets, sterile, pyrogen-free DMSO (Cryoserv, Research Industries Corporation, Salt Lake City, UT) is diluted to 20% in L-15 medium with 2.5% hetastarch and 2.5% human serum albumin. Cells are resuspended in L-15 medium with 2.5% human serum albumin at a concentration of 4-10 x 10<sup>7</sup>/ml. The 20% DMSO solution (ice cold) is then added slowly to the cell suspension while the cells are on ice. The cell suspension is then aliquoted into 100 ml freezing bags (Baxter). Several test vials of 2 mls are prepared for subsequent thaw-recovery analysis, and 2 to 4 vials are saved for future analyses that may contribute to the understanding of autologous PBSCT (e.g., more sensitive assay for tumor cells, assay, or future assays that may correlate with engraftment). The vials are frozen using a Cryo-Med freezing system (Mt. Clemens, Michigan) with a cooling program provided in hardware with the freezer. The cooling rate is -1°C/min, and the program maintains the cooling rate as close to -1°/min during phase change. The cooling rate is maintained at -2°/min after phase change until -40°C to avoid the damage shown to result from more rapid cooling rates after release of fusion heat. Frozen bags are placed into racks such that the bags are horizontal with respect to floor and stored in the vapor phase of a liquid nitrogen freezer.

Marrow or PBSC are cryopreserved in Cryocyte bags manufactured by Baxter and aluminum cassettes, which we specifically designed to protect the bags from pressure on fragile areas. The CHLA purging laboratory has only experienced one of these bags breaking in > 400 bags that have been shipped for ASCT. For any given patient, a minimum of 2 bags (usually 3) are used.

### **ANALYSIS OF STEM MARROW OR PBSC FOR TUMOR CELLS**

Analysis of marrow or PBSC for tumor cells is essential for assessing the efficacy of purging and for interpreting results of clinical trials. Stem cells will be tested for neuroblastoma cells with monoclonal antibodies against cell surface antigens by the CHLA Neuroblastoma Immunocytology Laboratory (RC Seeger, MD, Director). Immunocytology is used to assess the purging; details are provided below.

Mononuclear cells are prepared from a small aliquot of stem cells by equilibrium sedimentation of cells over Ficoll-Hypaque (e.g.= 1.077). Mononuclear cells that are buoyant at the density include essentially all neuroblastoma cells, while erythrocytes and granulocytes are sedimented. This enriches for neuroblastoma cells making immunocytologic examination more sensitive.

Detection of tumor is carried out with five monoclonal antibodies that are reactive with cell surface antigens of neuroblastoma but not normal bone marrow cells (390, 459, HSAN 1.2, 14G2A and BW-575). Viable cells are incubated with a mixture of all five monoclonal antibodies ( $10^5$  cells with 10  $\mu$ g of each antibody in 100  $\mu$ l of L 15-FCS, 30 min, room temp), washed three times, resuspended at  $10^6$  cells per ml, and cytocentrifugated onto coverslips ( $5 \times 10^4$  cells in 0.05 ml). After drying, cells are fixed with 2% paraformaldehyde and then sequentially incubated with 10 mM phenylhydrazine (one hr, 37 °C), biotinylated goat anti-mouse immunoglobulin, and then avidin-biotin-peroxidase complexes (one hr each, room temperature; Vector Laboratories). Bound peroxidase is visualized with diaminobenzidine/ $H_2O_2$  (5 min, room temperature), and cells are counterstained with Mayer's hematoxylin. Cells are washed between all steps for 15 min with PBS.

To provide enhanced detection of tumor, pre and post purge samples will also be examined for tumor by RT-PCR (sensitivity 1 per million) using primers for the tyrosine hydroxylase and PGP 9.5 genes. Because the clinical significance of detecting tumor by RT-PCR is currently unknown, results from the RT-PCR testing will not be done for this protocol, and will not be used to make decisions concerning the suitability for infusion of a stem cell product (either purged or unpurged). However, RT-PCR will be done on samples from this protocol at a future time for quality control of the Investigational Device.

### **Analysis of treated and cryopreserved marrow and PBSC for hematopoietic cells**

For bone marrows the total number of viable cells and CFU-G,M that can be recovered from a cryopreserved test vial will be compared to the numbers that were frozen. Although the presence of adequate viable cells ( $>0.5 \times 10^6$  per kg) and CFU-G,M ( $>10^4 \times 10^4$  per kg) in the cryopreserved marrow does not insure that sufficient pluripotent stem

cells are present, these are the most practical tests currently available, and several studies have correlated CFU-G,M in marrow with engraftment after transplant. The total number of CD34+ cells will also be determined. For PBSC the bulk of clinical data concerning restoration of hematopoiesis utilizes CD34 cell content prior to cryopreservation. Thus, for this study, the viable cell recovery and CFU-GM after cryopreservation for marrows and the CD34+ cells content just prior to cryoreservation for PBSC will be used to determine the suitability of the stem cell product for providing recovery after myeloablative therapy.

### **Testing of cryopreserved marrow.**

The 2 ml test vial is thawed rapidly in a 37°C water bath, and the contents are quickly transferred to a 15 ml plastic centrifuge tube. The vial is rinsed with 1 ml of Iscove's DMEM medium with 2% fetal bovine serum. The cell suspension is further diluted 5-fold with Iscove's DMEM medium containing 2% fetal bovine serum. Nucleated cell is counted with Turk's solution, while an additional count with trypan blue is used to assess cell viability.

#### **1. CFU-G, M Assay**

CFU-G,M is assayed using 0.5% agar-Iscove's DMEM medium, and recombinant human GM-CSF is used to stimulate colony formation. Cells are cultured at a concentration of  $2 \times 10^6$  cells/35 mm dish. Colonies of more than 50 cells are enumerated with a dissecting microscope after 14 days.

#### **2. CD34 determination**

Triplicate samples of  $1 \times 10^6$  cells are stained with CD45-FITC/CD34-PE (Becton Dickinson, San Jose, CA) for each different analysis. A single isotype control is concurrently stained with CD45- FITC/IgG1- PE not present on human leukocytes. Cells are incubated with antibody at an approximate concentration of 0.5ug per  $1 \times 10^6$  cells at 4°C for 15 minutes. After incubation, mature erythrocytes are lysed by incubating for 10 minutes with a commercial lysing reagent (10% FACS Lysing Solution (Becton Dickinson, San Jose, CA) in deionized water). Washing out the lysing solution using Dulbecco's Phosphate Buffered Saline (PBS- 1X, pH 7.4, Irvine Scientific, Santa Ana, CA) with 5% goat serum (Sigma, St Louis, MO) prepares the cells for flow cytometric analysis.

Using the EPICS Elite ESP Flow Cytometer and Expo for Elite software package (Beckman Coulter, Miami, FL), a minimum of 20,000 CD45 cells are acquired and analyzed using the four parameter flow methodology adopted by the International Society of Hematotherapy and Graft Engineering (ISHAGE). The percent CD34 is determined by dividing the number of events in the defined region for CD34 positive cells by the total number of CD45 positive cells acquired above a noise discriminator (threshold) set on forward light scatter. Cells from the isotype control sample that fall within the gated region for CD34 are subtracted out as a non- specifically stained cells.

**Shipping cryopreserved stem cells**

If tests for tumor and normal hematopoietic progenitor cells are acceptable, cryopreserved marrow or PBSC will be shipped by overnight air freight to the transplant center in liquid nitrogen vapor cryogenic container. Two 2 ml test vials of product will be sent with the marrow: one will be thawed and tested for viable cell recovery at the recipient institution (CFU-G,M testing will be optional, and the other will be returned immediately (still frozen) in the shipping container to CHLA, where it will be thawed and tested for viability and CFU-G,M. This procedure will help us detect unusual accidents during transport, such as irradiation or temperature shifts, that cause loss of cell viability. In addition we utilize a digital temperature monitor which records the temperature of the cryogenic container at hourly intervals throughout transport. Cryogenic containers are monitored for ability to hold cryogenic temperatures after each return shipment over a 5 day period using the same temperature recording system. If a backup product was prepared, it is shipped at a different time using the same procedure.

**Payment**

A standard fee will be collected for each PBSC or marrow that is purged and cryopreserved, to cover the expenses of that procedure and administrative costs. Prior to performing the stem cell harvest, the harvesting institution must enter into a legally binding memorandum of understanding (MOU) with CHLA. This MOU will be provided by CHLA and will obligate the harvest institution to pay the full purging and cryopreservation fee for each stem cell product they ship to CHLA. Payment is due to CHLA no later than 90 days after the purging date. The harvest institution is responsible for payment in full, regardless of the ability of the patient or the third party payer to cover the fee. Fees collected are for cost recovery of the purging, as allowed under BB-IDE-2259.

## **Bibliography for Appendix V**

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*This is a sample of an Informed Consent Document. The actual document will need to be approved by the IRB of the institution doing the stem cell collection. Certain elements of this example consent (such as the CA choice of law portion) are not optional and so any deletion of portions of this example must be discussed prior to utilization.*

**Childrens Hospital Los Angeles**

**<sup>1</sup>ASSENT/CONSENT/PERMISSION FOR THE PURGING, STORAGE AND SHIPPING OF AUTOLOGOUS STEM CELLS BY CHILDRENS HOSPITAL LOS ANGELES FOR NEUROBLASTOMA PATIENTS**

**A Compassionate Use Protocol For The Purging, Storage, and Shipping Of The Stem cells of Patients With Neuroblastoma Who Are Not Enrolled On A Clinical Trial**

|                 |       |                   |
|-----------------|-------|-------------------|
| Subject's Name: | _____ |                   |
| CHLA #:         | _____ | Birth Date: _____ |

• **INTRODUCTION**

You are being offered **compassionate treatment** (meaning it is not part of a clinical trial) using an experimental process called **Autologous Stem Cell Purging** by Drs. C. Patrick Reynolds, Judith Villablanca, and Robert Seeger, from the Childrens Center for Cancer and Blood Disorders at Childrens Hospital Los Angeles. You have a diagnosis of neuroblastoma, and are not part of a clinical trial that would include the use of the purging process to prepare your stem cells. The Food and Drug Administration makes investigational (experimental) processes such as purging available to doctors to treat patients outside of current clinical research studies under certain conditions. Your decision about the preparation of your stem cells using the purging process is completely voluntary. Please read the information below, and ask questions about anything you do not understand, before deciding whether or not you want this procedure to be used on your stem cells.

**Compassionate Treatment** means that there are no other standard therapies (or treatments) available and that the treatment being offered may have some benefit, however there is not

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<sup>1</sup> This form will also serve as the permission form for the parent to read and sign. In this case "you" refers to "your child"

enough information yet to prove the process will be of benefit. Not all the possible side effects of a compassionate process may also be known. When compassionate treatment is offered, the side effects and that occur in you will be reported to the Food and Drug Administration to track the safety record of the procedure, **but this information will not be used for other purposes.**

- **PROCEDURES**

This consent document will discuss the **Purging, Storage, and Shipping** of Autologous Stem Cells – it will **not** discuss cancer treatment, the harvesting (collection) of stem cells or the re-infusion (giving back) of stem cells. These other topics will be discussed with you by the doctors treating your Neuroblastoma.

What is **Purging**, and how is this different from what is normally done? Purging is a way of removing neuroblastoma (cancer) cells in the laboratory from your stem cells. Purging can be used on stem cells that are collected from peripheral blood, or from bone marrow. Purging of stem cells is an experimental procedure. Purging stem cells is designed to remove tumor cells that are detectable by the standard detection method (immunocytology) and also those that are too few in number to be seen by immunocytology. It is possible that tumor cells too few in number to be seen by current detection methods may still be in the stem cell product after purging. It is not known if there is any benefit to using stem cells that have been purged vs. using unpurged stem cells. We are trying to answer that question in other studies. All patients receive stem cell products that have been tested and found to not have tumor cells by the current (standard) detection methods.

Purging is done in Dr. Reynolds' laboratory at CHLA. During the purging process there are a number of steps used to remove the neuroblastoma cells. First a non-toxic iron particle called **Carbonyl Iron** is mixed with the stem cells. This product sticks to some normal blood cells - which may be mixed in with the stem cells - making it possible to filter them out by passing the stem cells past a magnet, and then discard them. This is similar to what happens when you pass a magnet over a dish of sand – the tiny iron fragments are picked up by the magnet, but the sand (the stem cells) stay put.

The second part uses tiny magnetic beads, and what are called **Monoclonal Antibodies, which are** proteins that “recognize” and attach to cancer cells. The antibodies are attached to the tiny magnetic beads, so when they are mixed with the Stem Cells they attach to any cancer cells and can then be filtered out, and then discarded (again similar to the magnet over the dish of sand).

Stem cells collected from **bone marrow** have an additional step at the very beginning where they

are mixed with a starch-like solution called **Hetastarch**. This solution causes cancer cells to be clumped together with red blood cells that are in the bone marrow, making them easy to separate from the stem cells, which are then discarded.

What about the **Storage** of the purged Stem cells? Once stem cells are purged they are tested to see if there are 1.) Any remaining cancer cells, 2.) Any Bacteria or Fungus (these are types of infections), and 3.) To see how many stem cells there are. It is rare that these tests show the presence of cancer cells, bacteria or fungus – if they were present you would not be given the stem cells, and you would need to discuss other options with your Oncology doctor.

The purged stem cells are then stored in carefully controlled freezers in Dr. Reynold's laboratory until they are needed.

What about the **Shipping** of the stem cells? If you are having your stem cells re-infused here at CHLA, shipping is not an issue for you. However if for any reason you were having them re-infused at another hospital the stem cells would be shipped to you there in a special container by overnight express. For patients whose stem cells are collected at sites other than CHLA, your stem cells will be shipped to CHLA to have the purging process done.

- **REQUIRED INVESTIGATIONS**

Frozen purged stem cells are stored in liquid nitrogen storage freezers until they are needed for therapy that requires stem cell support. There is no stated expiration date for frozen stem cells. Stem cells are stored until your treating doctor requests them. Testing of the stem cells at various points in the purging process is necessary to insure the process is effective. This will involve the use of small amounts of the stem cells.

- **POTENTIAL RISKS AND DISCOMFORTS**

Childrens Hospital Los Angeles takes all possible precautions to protect your stem cells from damage during testing, purging, storage and shipping. However, if there is ever any dispute or claim related to the testing, purging, storage and/or shipping of your stem cells by Childrens Hospital Los Angeles, you agree that the dispute or claim will be decided under California law, regardless of where you may live.

**Risks of Purging:**

- **Tumor cells may still be present in the stem cells** – The best standard tests available are used to test for the presence of tumor cells, but it is possible that some could “be missed”. It is possible these tumor cells could grow in your body when your stem cells are given back, however it is not known how many tumor cells are needed to cause tumor re-growth.
- **The purging process may damage the stem cells** – every care is taken to insure that the stem cells are not damaged, but it is possible that they may be damaged or enough cells lost in the purging that a 2<sup>nd</sup> harvest would be required to have enough stem cells for you to undergo stem cell transplantation.
- **The stem cells could become contaminated with infectious agents** – every care is taken to make sure that stem cells are kept sterile (germ-free), but there is the possibility that there could be a contamination. If they were reinfused, they could cause an infection in your body, so if an infection is found on testing, the stem cells will not be used.

#### **Risks of Storage:**

- **Freezer malfunction** – every care is taken to make sure that the temperature that the stem cells are stored at is kept the same until the stem cells are needed, but there is the possibility that they may become thawed and therefore damaged at some point before they are needed.

#### **Risks of Shipping:**

- **Loss or damage during shipping** – every care is taken to make sure that stem cells are shipped by an overnight express courier, but it is possible that they could be lost, delayed, and/or damaged in the shipping process.

#### • **ANTICIPATED BENEFITS TO SUBJECTS**

As we have explained, the purging process – while experimental – is felt to be beneficial to patients with neuroblastoma because it may improve the removal of microscopic cancer cells, which may be present in stem cells. Other studies have shown promising results, but this process is not yet approved for routine use by the United States FDA. There may, or may not, be any benefit to taking part in this study.

#### • **ANTICIPATED BENEFITS TO SOCIETY**

Since this study is being done for compassionate purging of stem cells, future patients may or may not benefit from your participation.

- **EMERGENCY CARE AND COMPENSATION FOR INJURY**

Childrens Hospital Los Angeles maintains professional liability insurance to protect patients from financial losses including the costs of necessary medical treatment resulting from injury caused by the fault of the hospital, its employees or its agents. If an injury is not caused by the hospital's fault, the hospital does not provide reimbursement for treatment expenses or other compensation for the injury, and payment for care of such injury will be billed to you and/or your health benefit plan. You are not waiving any legal claims, rights or remedies because of your child's participation in this research study.

- **ALTERNATIVES TO PARTICIPATION**

Participation in this compassionate-use protocol is completely voluntary. There are other options available to you, and they are;

- The use of un-purged stem cells, which are tested and stored according to current standard methods.

- **FINANCIAL OBLIGATION**

The direct health care costs which result from your participation in this compassionate-use study are your financial responsibility. All such costs will be billed to your insurance or other third-party payor. If there are any questions regarding this financial disclosure, please ask your doctor or nurse.

Childrens Hospital Los Angeles takes all precautions to protect your stem cells from any damage during testing, purging, storage and shipping. If there is ever any dispute or claim related to the testing, purging or handling of your stem cells by Childrens Hospital Los Angeles, you agree that the dispute or claim will be decided under California law.

- **FINANCIAL INTEREST OF THE INVESTIGATOR**

Compensation of the investigators is NOT based upon the number of subjects enrolled. If your physician is an investigator for this study, s/he is interested in both your healthcare, and the conduct of this compassionate study. You are not under any obligation to participate in a compassionate study conducted by your doctor.

- **PRIVACY AND CONFIDENTIALITY**

Your stem cells will be stored with your name and other identifiers, so that we can guarantee that your own stem cells are given back to you when needed. For extra stem cells that are not needed by you, patient confidentiality will be maintained by labeling these specimens for research with an identifier number rather than your name. Authorized representatives of the Food and Drug Administration (FDA) the Department of Health and Human Services, and the CHLA Committee on Clinical Investigations may need to review records of individual subjects. As a result, they may see your name, but are bound by rules of confidentiality not to reveal your identity to others. No information about you, or provided by you during this compassionate study, will be disclosed to others without your written permission, except:

- if necessary to protect your rights or welfare (for example, if you are injured and need emergency care; or

- if required by law (i.e. child abuse, reports of certain infectious diseases)

Because this study involves the treatment of a medical condition, a copy of this consent form will be placed in your medical record. This will allow doctors that are caring for you to obtain information about what medications or procedures you are receiving in the study and treat you appropriately.

- **PARTICIPATION AND WITHDRAWAL**

Your participation in having this procedure performed on your stem cells is VOLUNTARY. Your choice about whether or not to participate will have no effect on your care, services, or benefits. If you agree to participate and then change your mind, you may do so without affecting your rights to health care, services , or other benefits.

- **WITHDRAWAL OF PARTICIPATION BY THE INVESTIGATOR**

The investigator may withdraw you from participating in this compassionate study if necessary to protect your health and safety, or if other situations arise that make it necessary to do so. The investigators, Drs. Reynolds and Villablanca, will make the decision and let you know if it is not possible for you to continue.

- **NEW FINDINGS**

If there is significant new information found regarding the purging process or the treatment plan is changed in ways that might affect your decision to participate, you will be informed, and your consent to continue participating may be obtained again.

- **RIGHTS OF RESEARCH SUBJECTS**

You may withdraw from this compassionate protocol at any time and discontinue participation without penalty. You are not waiving any legal claims, rights, or remedies because of your participation in this compassionate study. If you have questions regarding your rights as a study subject, you may contact \_\_\_\_\_.

- **OTHER RESEARCH**

We are also asking you for permission to use small amounts of marrow or PBSC sent for purging to be used for research purposes or banked for future research, including those portions of the marrow or peripheral blood stem cells would be discarded in the process of purging  
You will make your choice at the end of this consent form.

- **DIFFICULT QUESTIONS**

We understand that the following paragraphs may be difficult to think about now, but we need to ask you about what to do with your stem cells if you died before they were used for your care. We are asking now so that we do not have to come to your family at that time.

Your stem cells are frozen for your use only and cannot be used for any other person. Thus, if you were no longer living, the stem cells must either be used for research purposes or destroyed.

Research done with stem cells may include studies of new methods to improve minimal tumor cell detection, function of normal cells of the immune system against tumor cells, and new methods of removing tumor cells from normal stem cells (purging).

**If you choose not to give your permission at this time by signing the appropriate line at the end of this consent form the stem cells may then be discarded in a manner consistent with the Childrens Hospital Los Angeles policies for discarding of human blood products.**

**We are asking to use of your frozen – unused - stem cells for research purposes.**

**Now, please read the instructions below. After you understand each instruction,**

**Initial the answer that is right for you.**

**Instruction 1:**

Initial **YES** if you agree to let your stem cells (that would be otherwise be discarded) be used for research purposes.

Initial **NO** if you do not want these extra stem cells products used for research.

# 1 YES \_\_\_\_\_

NO \_\_\_\_\_ (initials)

**Instruction 2:**

Initial **YES** if you agree to let your stem cells that would be otherwise be discarded if you died (before they were used for your care) be used for research purposes.

Initial **NO** if you do not want your stem cells used for research.

# 1 YES \_\_\_\_\_

NO \_\_\_\_\_ (initials)

**SIGNATURE OF SUBJECT**

Your signature(s) below indicates

You have read this document and understand it's meaning;

- You have had a chance to ask questions and have had these questions answered to your satisfaction;
- You consent to your participation in this compassionate-use study; and
- You will be given a copy of the signed permission form and of the Experimental Subject's Bill of Rights.

\_\_\_\_\_  
Name of Subject

\_\_\_\_\_  
Signature of Subject (if applicable)

\_\_\_\_\_  
Date

**SIGNATURE OF PARENT(S)/GUARDIAN (If patient is under 18 years old)**

Your signature below indicates that you have read this document; understand its meaning; have had a chance to ask questions; have had these questions answered to your satisfaction; and agree to your child's participation in this compassionate-use study. You have been given a signed copy of this assent/permission form and of the *Experimental Subject's Bill of Rights*.

\_\_\_\_\_  
Name(s) of Parent(s)/Guardian

\_\_\_\_\_

\_\_\_\_\_  
Signature of Parent (Guardian) and Date

\_\_\_\_\_  
Signature of Parent (Guardian) and Date

**SIGNATURE OF INVESTIGATOR**

I have explained the research to the subject and/or the subject's parent(s)/guardian and answered all of their questions. I believe that they understand the information described in this document and freely give permission to participate.

\_\_\_\_\_  
Name of Investigator

\_\_\_\_\_  
Signature of Investigator

\_\_\_\_\_  
Date (must be the same date as subject's)

**SIGNATURE OF WITNESS (If A Translator Is Used)**

My signature as witness certifies that the subject and/or the subject's parent(s)/guardian signed this assent/consent/permission form in my presence as their voluntary act and deed.

\_\_\_\_\_  
Name of Witness

\_\_\_\_\_  
Signature of Witness

\_\_\_\_\_  
Date (must be the same date as subject's)

Please check appropriate box and sign below.

Investigator's Statement of Certification for Subjects less than Seven Years of Age (Assent)

The undersigned investigator, \_\_\_\_\_, hereby certifies that he/she has discussed the information contained in the study consent to the subject, including any risks that may reasonably be expected to occur. The undersigned further certifies that the subject was encouraged to ask questions, that all questions were answered, and that assent was obtained.

Assent was not obtained for a subject under 18 years of age. (Please state the reason. Examples include: child is an infant; child is comatose; child lacks cognitive abilities to understand the information.)

\_\_\_\_\_  
\_\_\_\_\_

Date: \_\_\_\_\_

Time: \_\_\_\_\_

Signature: \_\_\_\_\_

Routing of signed copies of the consent form:

- 1) Give to Parent/Adult subject
- 2) Place in the CHLA Medical Record
- 3) Place in the Principal Investigator's research file.

**CHILDRENS HOSPITAL LOS ANGELES**  
**EXPERIMENTAL SUBJECT'S BILL OF RIGHTS**

Any person who is requested to consent to participate as a subject in a research study involving a medical experiment, or who is requested to consent on behalf of another, has the right to:

1. Be informed of the nature and purpose of the experiment;
2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
3. Be given a description of any attendant discomforts and risks reasonably to be expected from your (your child's) participation in the experiment;
4. Be given an explanation of any benefits reasonably to be expected from your (your child's) participation in this experiment;
5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to you (your child), and their relative risks and benefits;
6. Be informed of the avenues of medical treatment, if any, available to you (your child) after the experimental procedure if complications should arise;
7. Be given an opportunity to ask any questions concerning the medical experiment or the procedures involved;
8. Be instructed that consent to participate in the medical procedure may be withdrawn at any time and that you may discontinue participation in the medical experiment without prejudice;
9. Be given a copy of this form and the signed and dated written consent form;
10. Be given the opportunity to decide to consent or not to consent to the medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on your decision.

You have carefully read the information contained above in the "Experimental Subject's Bill of Rights" and you understand fully the rights of a potential subject in a medical experiment involving people as subjects.

\_\_\_\_\_  
(Date)

\_\_\_\_\_  
(Parent/Legal Guardian)

\_\_\_\_\_  
(Time)

\_\_\_\_\_  
(Patient)

\_\_\_\_\_  
(Witness)

**CHILDRENS HOSPITAL LOS ANGELES**  
**DECLARACION DE DERECHOS DE SUJETOS EXPERIMENTALES**

Cualquiera persona a quien se pida consentimiento para participar como sujeto en una investigacion medica, o a quien se pida consentimiento de parte de otra persona tiene el derecho:

1. Que le informen acerca de la clase y el proposito de la clase y el proposito de la investigacion;
2. Que le expliquen los procedimientos que van a seguir en la investigacion medica, y cualquier droga o aparato que van a utilizar;
3. Que le describan las molestias y los riesgos que razonablemente puedan resultar de su participacion o de la de su nino en la investigacion;
4. Que le expliquen cualquier beneficio que razonablemente se espera de su participacion o de la de su nino en la investigacion;
5. Que le indiquen otra alternativas procedimientos, drogas, y aparatos apropiados que puedan ser beneficiosos y los riesgos y de estos;
6. Que le informen si hay tratamiento medico para las posibles compicaciones de la investigacion;
7. Que le den la oportunidad de hacer preguntas acerca de la investigacion medica o el procedimiento;
8. Que le aseguren que puede retirar su consentimiento para participar en la investigacion en cualquier momento y que usted o su nino puede discontinuar su participacion en la investigacion medica sin perjudicarse;
9. Que le den copia de esta forma y de la forma excrita de consentimiento firmada y fechada; y
10. Que le den la oportunidad de decidir a consentir o a no consentir a la investigacion medica sin intervencion de elemento alguno de coaccion, fraude, fuerza, engano, obligacion, o influencia indebida en su decision.

Usted ha leído cuidadosamente la informacion contenida en la presente "Declaracion de Derechos De Sujetos Experimentales" y entienda completamente los derechos del paciente que posiblemente participe en una investigacion medica que usa humanos como sujetos.

\_\_\_\_\_  
(Fecha/Date)

\_\_\_\_\_  
(Padres/Guardines Legales)  
(Parent/Legal Guardian)

\_\_\_\_\_  
(Hora/Time)

\_\_\_\_\_  
(Paciente/Patient)

\_\_\_\_\_  
(Testigo/Witness)