Haematopoietic Stem Cell Mobilisation and Apheresis:

A Practical Guide for Nurses and Other Allied Health Care Professionals
Widely accepted cancer treatment strategies include chemotherapy and radiotherapy. The rationale for administration of high-dose chemotherapy and/or radiation to patients with therapy-sensitive tumours is to reduce tumour burden. Delivery of these therapies with respect to higher drug doses and intensified schedule are often limited by organ toxicities (eg, bone marrow, heart, and lung) and pancytopenia. To overcome these dose limitations, autologous haematopoietic stem cell transplantation (AHSCT), high-dose therapy supported by the infusion of haematopoietic stem cells, has evolved as a medical procedure to allow for administration of intense drug doses with tolerable organ and haematopoietic toxicity. Infusion of autologous stem cells following dose-intensive treatment "rescues" the bone marrow by re-establishing normal haematopoiesis. Upon regeneration of bone marrow function, patients may be cured from their disease or receive additional cancer treatment.1,2

Autologous haematopoietic stem cell transplantation is a complex medical procedure that has been used to treat and cure patients with various malignant and non-malignant disorders. Although the first documentation of AHSCT use to treat cancer was reported in the 1890s,3 achievement of a cure in patients with a malignancy was only documented in 1978 following a clinical trial conducted at the National Cancer Institute (United States).4 Subsequent to this report, numerous advances have been made in the art of AHSCT and thousands of patients around the world have had their diseases successfully managed through the use of AHSCT.

The term "autologous haematopoietic stem cell transplantation" is frequently used interchangeably with the terms autologous bone marrow transplantation (aBMT), autologous peripheral blood stem cell transplantation (aPBSCT), and autologous haematopoietic cell transplantation (AHCT).5 "Autologous" means that the donor cells used for the procedure are from the patient himself, as opposed to "allogeneic," which refers to a cell donor other than the patient. In certain allogeneic circumstances, the term "syngeneic" is used when the cell donor is a patient's identical twin. The source of stem cells for collection is identified by the terms "bone marrow" and "peripheral blood." Cells for the patient may be collected either from the donor's bone marrow reserves, such as those stored in the iliac crest of the pelvic bones, or from the donor's peripheral blood. Additionally, umbilical cord blood (UCB), found in the umbilical cord and placenta following childbirth, is another source of progenitor stem cells used in clinical practice in the setting of allogeneic transplants.6 When 2 autologous stem cell transplantations occur in a scheduled, sequential fashion, this process is referred to as "tandem autologous stem cell transplantation."6,7

In the more than 3 decades following the first successful use of AHSCT, the utility of this treatment for malignant and non-malignant conditions has been well established (Table 1).8 In the setting of relapsed malignant conditions, standard chemotherapy regimens may produce unacceptable rates of bone marrow suppression (myelosuppression), resulting in a low white cell count, low platelet count, and anaemia. This increases the risk of potentially fatal infections and bleeds. Following chemotherapy, the patient is therefore given a transplant of stem cells to regenerate damaged bone marrow. Thus, the reinfusion of autologous stem cells has become a therapeutic modality for reducing prolonged myelosuppression.9-11 Data demonstrate that high-dose therapy with stem cell rescue has a positive impact on disease response rates; however, for some patients it fails to improve overall survival when compared to conventional chemotherapy treatments. Thus, the definitive role of AHSCT in certain situations, such as the treatment of refractory or relapsed Hodgkin's lymphoma or chronic lymphocytic leukaemia, remains inconclusive12-15 and indications for AHSCT continue to evolve.
Pluripotent stem cells express the cell surface marker antigen CD34. This marker is the indicator most frequently used in clinical practice to determine the extent and efficiency of peripheral blood stem cell collections. Although not a complete measure of the quantity and quality of collected cells, blood samples from collections are assayed to determine the number of CD34+ cells present. Once specific cell targets are achieved, cell collections are completed and stored for future use. Standard target levels can vary among treatment centres and a patient's specific goal is related to the underlying disease, the source of stem cells, and the type of transplantation to be performed. In general, a target level of $2 \times 10^6$ CD34+ cells/kg body weight is considered the minimum for autologous transplantation, with optimal levels being $\geq 5 \times 10^6$ CD34+ cells/kg for a single transplant and $\geq 6 \times 10^6$ CD34+ cells/kg for a tandem transplant.

Historically, autologous stem cell collections involved the removal of bone marrow cells from a patient's bilateral posterior iliac crest region (Figure 4) under general anaesthesia in a hospital operating room. However, due to advances in medical technology, most collections today are performed by apheresis (Figure 5). Peripheral blood stem cell collection is considered the preferred method for mobilisation prior to AH SCT due to patient convenience, decreased morbidity, and faster engraftment of WBCs and platelets. Additional comparisons between harvest of bone marrow and peripheral blood for autologous transplantation are summarised in Table 2.

### Table 2. Advantages and Disadvantages of Haematopoietic Stem Cell Collection Methods

<table>
<thead>
<tr>
<th>Collection Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Bone marrow       | - Single collection  
                   - No need for special catheter placement  
                   - Use of cytokines not necessary | - Performed in an acute care setting since it requires general anaesthesia  
                   - Slower neutrophil and platelet engraftment  
                   - Higher rates of morbidity and mortality  
                   - Potentially more tumour cell contamination of product |
| Peripheral blood  | - Does not require general anaesthesia and can be performed in an outpatient setting  
                   - Faster neutrophil and platelet engraftment  
                   - Associated with lower rates of morbidity and mortality  
                   - Potentially less tumour cell contamination of product | - Collection may take several days  
                   - Sometimes requires placement of large-bore, double-lumen catheter for collection  
                   - Haemorrhage, embolism, and infection are possible complications related to insertion of central venous catheter |

Figure 4. Bone Marrow Harvest
The concentrations of HSCs are 10-100 times greater in the bone marrow compared to the peripheral circulation. Therefore, methods to increase the circulating concentrations of HSCs are necessary to ensure adequate and successful collections. Agents used to mobilise HSCs include the administration of cytokines with or without chemotherapy prior to scheduled collection periods.

Filgrastim and lenograstim used as single-agent mobilisers have been well established, with both agents having reliably demonstrated increased concentrations of circulating HSCs. G-CSF is thought to stimulate HSC mobilisation by decreasing SDF-1α gene expression and protein levels while increasing proteases that can cleave interactions between HSCs and the bone marrow environment. The mechanism of action for G-CSF is illustrated in Figure 6. The recommended dose of filgrastim and lenograstim is 10 mcg/kg/day as a subcutaneous injection for multiple days. However, these growth factors are typically given at a total daily dosage of 3-24 mcg/kg/day. Data indicate that divided doses of G-CSF (eg, lenograstim 5 mcg/kg twice daily) are more efficacious than single-dose administration (eg, lenograstim 10 mcg/kg once daily) by producing a higher yield of CD34+ cells and the need for fewer apheresis procedures. However, current clinical practice does not favour one schedule over the other.

Figure 5. Apheresis Collection

Figure 6. Mechanism of Action of G-CSF

CXCR4, chemokine receptor 4; G-CSF, granulocyte colony-stimulating factor; SDF-1α, stromal cell derived factor-1 alpha.
Prior to initiation of the stem cell collection process, patients must be thoroughly evaluated and determined to be acceptable transplant candidates and able to tolerate all of the procedures involved. Some of the medical, nursing, and psychosocial evaluations can occur prior to a patient's first visit or referral to a transplant service or clinic. The patient's primary medical haematologist/oncologist frequently serves as a patient's first contact during the transplantation process. All of the testing, evaluation, and education involved throughout a patient's transplant involves a myriad of health care professionals working together to orchestrate this complex medical procedure.

The medical pre-evaluation is the first step a patient must complete when undergoing an AHSCT. This involves the patient's primary medical oncologist making a referral to a transplant centre or service. The physician provides the transplant team with information that often includes specifics relating to the care of the patient up to this time point, such as past medical history, cancer status, summary of cancer treatments and responses, and complications experienced during therapy. Accompanying this information are any available radiograph and laboratory testing results.

After review of the patient's medical information, the transplant team will initiate their own battery of tests and evaluations to assess a patient's eligibility to proceed with stem cell collection and transplantation. This involves restaging the patient to verify or establish current disease status, ascertaining the function of various organs (eg, kidneys, liver, and lungs), documenting the absence of certain comorbid conditions and infectious diseases (eg, congestive heart failure and the presence of human immunodeficiency virus), and evaluating the overall performance status and psychosocial condition of the patient. At this time, extensive education will be initiated for the patient and their family and/or caregivers. Often a member of the nursing profession (clinic nurse, nurse educator, or nurse coordinator) will coordinate the education process for the patient and those involved in their care (see Chapter 4).

Upon determination that a patient is eligible to proceed to transplantation, preparing them for the collection process occurs next. The preferred method for venous access is the placement of a peripheral catheter at the time of an apheresis session (eg, insertion into the antecubital vein). For those patients in whom peripheral line placement is not feasible, appropriate catheter selection and central venous placement (eg, internal jugular vein) is scheduled prior to the first stem cell collection. Catheters used for apheresis procedures must be able to tolerate large fluctuations in circulating blood volume. Therefore, these catheters are often large-bore, double lumen devices that can be used temporarily during cell collections, or placed permanently and used throughout the transplantation process. As with most catheters placed in the area of the upper extremities, patients should be monitored for signs and symptoms of hypotension, shortness of breath, and decreased breath sounds as these can be indicative of venous wall perforation, haemothorax, and/or pneumothorax, all of which are rare but serious complications that can occur. In some cases, catheters for apheresis may be placed centrally in a femoral vein if patients are at an increased risk of developing complications from a catheter placed in the upper extremity or chest wall. For centrally placed catheters, radiographic evaluation is used to verify catheter placement prior to clearance for its use. Additionally, instructions on caring for the catheter to prevent infection and maintain its integrity should be extensively reviewed with the patient and/or caregivers.1,2,16,82,83

**Preparation for stem cell collections in an apheresis centre or unit follows the pre-transplantation evaluation and catheter placement.** Patients will be advised and counselled on therapies that they will receive during mobilisation with respect to administration schedule and expected adverse effects. As previously detailed in Chapter 2, agents used during this stage of AHSCT usually include single-agent cytokines (such as filgrastim).
that are given with or without certain chemotherapy agents, a designated cycle of disease-specific chemotherapy regimen, or more recently, filgrastim or lenograstim in combination with plerixafor. Upon initiation of the mobilisation regimen, patients can expect to undergo their first apheresis session in as little as 4 to 5 days or, in some cases, 2 to 3 weeks later.1,16,83 The extent of mobilisation is ascertained through evaluation of a patient’s WBC count. Serial measurements of the patient’s WBC count will assist the clinician in determining the appropriate time to commence collection procedures. Additionally, centres may use peripheral blood CD34+ cell levels as a surrogate for mobilisation status. Established thresholds for apheresis initiation may vary across centres, but typically range from 5 to 20 CD34+ cells/microlitre. Although useful in estimating mobilisation efficacy, peripheral blood CD34+ counts can be variable within and across centres.15,84,85

Once mobilisation has reached an optimal level, a patient can be scheduled for sessions in the apheresis centre. An apheresis technician highly trained in stem cell collections is responsible for the equipment utilised in the collection process (Figure 9). Clinical nurses working in the apheresis unit are responsible for educating the patient about the stem cell collection process and monitoring patients for any adverse reactions. Patients are connected to the apheresis machine by their catheter. One lumen is used to draw blood out of the patient and into the machine. Here the blood is spun at high speeds in a centrifugation chamber housed within the cell separator machine. The desired stem cells are collected during the entire procedure, either in cycles or continuously, and the remaining blood components are returned to the patient through the second lumen of their catheter. This second lumen additionally can be used to administer intravenous fluids, electrolyte supplements, and medications to the patient. Each apheresis session lasts approximately 2-5 hours during which upwards to 30 litres of blood, or 6 times the average total human blood volume, is processed. Collections can occur on a daily basis until the target CD34+ levels are achieved. The apheresis process can last for up to 4 days depending on patient characteristics and the mobilisation regimen utilised.2,16,17,82,86-88

Apheresis procedures are relatively safe. Although the mortality rate is quite low at an estimated 3 deaths per 10,000 procedures,89 apheresis is associated with some morbidity. Citrate is an anticoagulant used during the apheresis process to prevent blood clotting. Thus, one of the most common adverse effects seen during this procedure is citrate toxicity manifested as hypocalcaemia. This occurs due to binding of ionised serum calcium, which leads to hypocalcaemia. Signs and symptoms of citrate toxicity as well as its management are further described in Table 7. Monitoring serum calcium level prior to and throughout apheresis may decrease the likelihood of hypocalcaemia.16,82,90 Additional adverse effects of citrate toxicity include hypomagnesaemia, hypokalaemia, and metabolic alkalosis. Magnesium, like calcium, is a divalent ion that is bound by citrate. Declines in serum magnesium levels often are more pronounced and take longer to normalise compared to aberrations in calcium levels. Signs and symptoms of hypomagnesaemia, hypokalaemia, and metabolic alkalosis as well as their management are further described in Table 7.16,82,90
Table 7. Common Apheresis Complications

<table>
<thead>
<tr>
<th>Adverse Effect</th>
<th>Cause</th>
<th>Signs and Symptoms</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate toxicity</td>
<td>Anticoagulant (citrate) given during apheresis</td>
<td><strong>Hypocalcaemia</strong>: Common: dizziness, tingling in area around the mouth, hands, and feet&lt;br&gt;Uncommon: chills, tremors, muscle twitching and cramps, abdominal cramps, tetany, seizure, cardiac arrhythmia</td>
<td>Slow the rate of apheresis; increase the blood: citrate ratio; calcium replacement therapy</td>
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<tr>
<td></td>
<td></td>
<td><strong>Hypomagnesaemia</strong>: Common: muscle spasm or weakness&lt;br&gt;Uncommon: decrease in vascular tone; cardiac arrhythmia</td>
<td>Slow the rate of apheresis; increase the blood: citrate ratio; magnesium replacement therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Hypokalaemia</strong>: Common: weakness&lt;br&gt;Uncommon: hypotonia and cardiac arrhythmia</td>
<td>Slow the rate of apheresis; increase the blood: citrate ratio; potassium replacement therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Metabolic alkalosis</strong>: Common: worsening of hypocalcaemia&lt;br&gt;Uncommon: decrease in respiration rate</td>
<td>Slow the rate of apheresis; increase the blood: citrate ratio</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Platelets adhere to internal surface of the apheresis machine</td>
<td>Low platelet count, bruising, bleeding</td>
<td>Prime apheresis machine with blood products in place of normal saline; platelet transfusion</td>
</tr>
<tr>
<td>Hypovolaemia</td>
<td>Patient intolerant of large shift in extracorporeal blood and plasma volumes</td>
<td>Dizziness, fatigue, light-headedness, tachycardia, hypotension, diaphoresis, cardiac arrhythmia</td>
<td>Slow the rate of apheresis session or temporarily stop it; intravenous fluid boluses</td>
</tr>
<tr>
<td>Catheter malfunction</td>
<td>Blood clot forms or catheter is not well positioned to allow for adequate blood flow</td>
<td>Inability to flush catheter; fluid collection under skin around catheter site; pain and erythema at catheter site; arm swelling, decrease in blood flow</td>
<td>Reposition the catheter; gently flush catheter; treat blood clot</td>
</tr>
<tr>
<td>Infection</td>
<td>Microbial pathogens enter bloodstream through catheter or catheter site</td>
<td>Fever, chills, fatigue, red and erythematous skin around catheter; hypotension, positive blood cultures</td>
<td>Administer antibiotics; possibly remove catheter</td>
</tr>
</tbody>
</table>

Due to large fluctuations in blood volume during apheresis, patients may experience hypovolaemia. Signs and symptoms of hypovolaemia as well as its management are further described in Table 7.16,82,90 Prior to starting apheresis, baseline pulse and blood pressure are measured and continuously assessed at regular intervals. Additionally, it is recommended that haemoglobin and haematocrit also be monitored. Patients at risk of developing hypovolaemia include those with anaemia, those with a previous history of cardiovascular compromise, and children or adults with a small frame. Preventative measures are aimed at minimising the extracorporeal volume shift by priming the apheresis machine with red blood cells and fresh frozen plasma in place of normal saline. Hypovolaemia may also be managed by providing intravenous fluid boluses and slowing the rate of flow on the apheresis machine. Another potential problem stemming from hypovolaemia is the development of a life-threatening cardiac dysrhythmia. If this occurs, apheresis should be interrupted and symptoms should subside before proceeding with collections.16,82,90

Thrombocytopenia, infection, and catheter malfunction are other complications that may be encountered during stem cell collections. When the patient’s blood is in the cell separator machine, platelets can adhere to the centrifuge device. Decreases in platelet concentrations can be precipitous and obtaining platelet counts prior to each collection is essential. If thrombocytopenia is present pre-apheresis, patients may receive platelet...
transfusions. Additional management of thrombocytopenia during apheresis is the return of platelet-rich plasma collected during apheresis to the patient at the conclusion of the apheresis session. As with any catheter, frequent manipulation in the absence of proper catheter care and maintenance may predispose the patient to infections and/or cause the catheter to malfunction. Proper sterile technique should be utilised at all times to decrease the risk of contamination with microbial pathogens that can lead to bloodstream infections. Furthermore, routine catheter care should include administration of flushes to prevent blood clot formation. Commonly observed complications during apheresis are summarised in Table 7.

At the conclusion of apheresis, stem cells are isolated from red blood cells and WBCs and then placed in infusion bags in preparation for cryopreservation and storage. Many centres have cryopreservation laboratories that maintain collected stem cell products in liquid nitrogen until the time of the patient’s transplantation. A common cryopreservative used is dimethylsulfoxide (DMSO). DMSO maintains cell viability by preventing ice crystal formation within the cells during storage. Additionally, the collected product may be manipulated by a pharmacological, immunological, or physical method to reduce contamination with tumour cells. Quality testing is performed on collections to ascertain contamination with microbes as well as to determine the number of viable cells available for transplantation. Once a patient reaches their CD34+ collection goal, apheresis sessions are complete. Reaching minimum thresholds for CD34+ cell amounts is important as cell dose appears to positively correlate with engraftment and outcome.

The next stage of the AHSCT process is preparing the patient for the actual transplant. Nurses play an important role in educating patients and caregivers about the transplant processes and procedures as well as the critical time leading up to engraftment and recovery. Whereas some patients can expect to proceed to transplant within days following mobilisation, others may undergo the transplantation procedure within a few weeks following stem cell collection. During the interim, additional chemotherapy may be given to the patient to help maintain their disease status. Once the transplant date is scheduled, approximately 1 week prior to the transplant date, patients begin their preparative regimen in the ambulatory or inpatient unit of the hospital. The preparative regimens may consist of chemotherapy alone or chemotherapy in combination with radiation therapy. Chemotherapy agents selected for use during this time can be different than those used during previous cancer treatments and during mobilisation. Often, if similar agents are chosen, the doses during this phase are higher than previously administered. The patient often benefits from cytoreduction in their tumour following this phase of treatment. As a consequence of the intensity of treatment, patients experience ablation of their marrow stores, hence the need for infusion of their previously collected cells as “rescue” therapy. Other expected sequelae from high-dose chemotherapy used in conjunction with AHSCT are summarised in Table 8.

Table 8. Effects Following High-Dose Chemotherapy Used in AHSCT

<table>
<thead>
<tr>
<th>Organ</th>
<th>Effects</th>
<th>Interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal tract</td>
<td>Nausea, vomiting, diarrhoea, anorexia, mucositis</td>
<td>Antiemetics, mouth care regimens, pain medications, nutritional supplementation</td>
</tr>
<tr>
<td>Blood components</td>
<td>Pancytopenia</td>
<td>Antibiotics, blood transfusions</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Haemorrhagic cystitis</td>
<td>Mesna, intravenous fluids, pain medications, bladder irrigation</td>
</tr>
<tr>
<td>Liver</td>
<td>Sinusoidal obstructive syndrome (veno-occlusive disease)</td>
<td>Diuretics, fluid restriction, intensive supportive care</td>
</tr>
<tr>
<td>Brain and nervous system</td>
<td>Headache, tremors, seizures</td>
<td>Pain medications, intensive supportive care</td>
</tr>
<tr>
<td>Heart</td>
<td>Oedema, hypertension</td>
<td>Fluid restriction, diuretics, antihypertensive medications</td>
</tr>
<tr>
<td>Lungs</td>
<td>Atelectasis</td>
<td>Pulmonary toilet</td>
</tr>
<tr>
<td>Skin</td>
<td>Rash, discoloration</td>
<td>Topical emollients, skin care and bathing regimen</td>
</tr>
</tbody>
</table>

AHSCT, autologous haematopoietic stem cell transplantation.
Before high-dose chemotherapy is administered to the patient, the integrity of stored stem cells is verified. On the day of stem cell infusion, the previously collected product is removed from liquid nitrogen storage (Figure 10), thawed, and prepared for patient administration. The infusion itself can occur in an ambulatory or hospital setting. Prior to infusion, the product is rigorously inspected and tested for quality control measures such as CD34+ cell counts and the presence of microbes. Several members of the health care team will also ensure that the product is the patient’s collected cells. The patient is prepared for the infusion by receiving premedications (eg, an antihistamine, an antipyretic), having their intravenous line equipped to receive the cells, and being placed on medical equipment to monitor their vital signs throughout the procedure. The actual time for infusion can vary based on the patient and the number of bags collected during apheresis, but typically ranges from 30 to 120 minutes. Patients should be frequently monitored for adverse events during the infusion that may require adjustment of the infusion rate for the product. Resuscitative equipment should be readily available should a medical emergency occur. Frequently encountered reactions during autologous stem cell infusions are listed in Table 9.

Table 9. Complications Associated With Autologous Stem Cell Infusion

<table>
<thead>
<tr>
<th>Adverse Effect</th>
<th>Signs and Symptoms</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactions to DMSO</td>
<td>Common: nausea, vomiting, abdominal cramping, headache, garlic aftertaste</td>
<td>Treatment of symptoms</td>
</tr>
<tr>
<td></td>
<td>Rare: Hypotension, rapid heart rate, shortness of breath, fever, neurologic complications</td>
<td></td>
</tr>
<tr>
<td>Oedema</td>
<td>Fluid retention, puffiness, weight gain, hypertension</td>
<td>Diuretics, fluid restriction</td>
</tr>
<tr>
<td>Contamination of stem cell product</td>
<td>Hypotension, rapid heart rate, shortness of breath, fever, chills, rigors, positive blood culture for microbial pathogen</td>
<td>Antibiotics, intensive supportive care</td>
</tr>
</tbody>
</table>

DMSO, dimethylsulfoxide.

The last component of AHSCIT is engraftment and recovery. During this critical time, the infused stem cells find their way back to the bone marrow microenvironment and repopulate depleted marrow stores. Post-transplant, cytokines are administered to enhance stem cell maturation and to re-establish blood cell components. The first sign of engraftment is the return of circulating WBCs to a sufficient level defined as an absolute neutrophil count (ANC) of > 500/mm^3 for 3 consecutive days, which typically occurs 7-14 days after the stem cells have been infused into the patient. Increased platelet levels (absent of transfusion support) is another indicator of recovery, and occurs at a later time point, averaging 2-3 weeks following the transplant. Until engraftment occurs, patients are at an increased risk of infections, so precautions must be taken to avoid exposure to microbial pathogens. Patients often require supportive care strategies and therapies, including administration of antiemetics, pain medications, antibiotics, and nutrition support to ameliorate consequences following the high-dose chemotherapy preparative regimen and the subsequent prolonged period of pancytopenia.
CHAPTER 5

Sources and procurement of stem cells

J. Larghero, J. García, E. Gluckman
1. Introduction
The source and procurement of haematopoietic stem cells (HSC) has varied and diversified over time. Nowadays, there are three possibilities: bone marrow (BM) HSC, granulocyte-colony stimulating factors (G-CSF) mobilised peripheral blood HSC and cord blood (CB). Each of them seems to give similar clinical results although the use varies according to age of the donor and the recipient, the indication and the preference of the centres and the donors. Usually BM collection is preferred in children, for adults it can be either BM or PBSC, CB depends on the availability of a suitable unit in banks and the absence of an HLA identical donor.

2. Use of different cell sources for HSCT in Europe
An EBMT survey on HSC transplantation (HSCT) activity in 2005 reported that 24,168 first HSCT were performed in Europe by 597 centres (1). Among these transplants, 15,278 were autologous and 8,890 allogeneic. Of the 8,890 allo-HSCT, 4,702 were provided by an HLA identical sibling donor, 514 by an HLA-mismatched family donor, and 57 by a homozygous (syngeneic) twin. An UD was used in 3617 patients (41%). The main indications were leukaemia (6,107 patients), lymphoproliferative diseases (1,520 patients), solid tumours (130 patients) and non-malignant diseases (1,048 patients). The number of allo-HSCT increased by 20% from 2004 to 2005, whereas numbers of auto-HSCT remained stable (1).

3. Choice of stem cell source
Traditionally, HSC were harvested from the iliac crests under general anaesthesia. Thereafter, mobilised PBSC have been increasingly used in both auto- and allo-HSCT. Mobilisation of HSC in sufficient number in peripheral blood can be achieved by the classical administration of growth factor such as G-CSF (allo-HSCT) and/or myelosuppressive chemotherapy (auto-HSCT). In the 1990s, unmanipulated CB cells collected and cryopreserved at birth have been used both in related and unrelated HLA matched and mismatched allogeneic transplants in children, and more recently in adults.
In 2005, the stem cell source for auto-HSCT was from peripheral blood in 98% of the 15,278 transplants and from BM in 2%. In the allogeneic setting, BM was used in 2,331 of 8,890 transplants (21%) and PBSC in 74%, confirming the increasing use of this stem cell source. CB was used in 395 allogeneic HSCT compared to 281 in 2004. It has become evident that there are many differences, both quantitative and qualitative, between these cell sources (1).

The main differences between cell sources are:
## Bone marrow
- Collection under general anaesthesia
- Limited number of haematopoietic stem cells
  - Median number of nucleated cells: $2 \times 10^8$/kg
  - Median number of CD34+ cells: $2.8 \times 10^6$/kg
  - Median number of T-cells: $2.2 \times 10^7$/kg

## G-CSF mobilised PBSC
- Collection easy
- No requirement for general anaesthesia
- Side effects of G-CSF
- High number of cells
  - Median number of nucleated cells: $9 \times 10^8$/kg
  - Median number of CD34+ cells: $7 \times 10^6$/kg
  - Median number of T-cells: $27 \times 10^7$/kg

## Cord blood
- Collection easy and harmless
- Immediate availability of cryopreserved units and lower risk of transmissible diseases
- Acceptable partial HLA mismatches
  - Median number of nucleated cells: $0.3 \times 10^8$/kg
  - Median number of CD34+ cells: $0.2 \times 10^6$/kg
  - Median number of T-cells: $0.4 \times 10^7$/kg

### 4. Donor work-up (2–5) (Tables 1–3)

#### 4.1. Donor risk from the procedure
The risks for the healthy donor must be minimised. A careful inquiry before donation must be performed. It is recommended that two separate physicians examine the donor prior to the procedure. Detailed information must be given and signed consent must be obtained from the donor and also from both parents in the case of donors under the age of legal consent. In some European countries, when the donor is <16 years old, the consent must be signed in front of a judge and both the family and the child must be seen by a committee of 3 independent experts. The presence of any risk from general anaesthesia for BM aspiration or from cardiac disease for PBSC collection is an absolute contraindication to the donation. The situation is of course completely different for CB, which represents a harmless and easily accessible source of haematopoietic stem cells without any risk to donors.
4.2. Recipient risk from a particular donor (2)
Potentially transmittable diseases from donors include infections, congenital disorders, and acquired diseases such as malignancies or autoimmune diseases.

5. Comparison of BMT and PBSCT (5–12)
In 1995, 3 pivotal studies demonstrated the safety and feasibility of using G-CSF mobilised PB allografts (Table 4). Patients experienced prompt engraftment with an incidence of GVHD similar to that of BM recipients (Table 5). In addition, no serious short-term complications of G-CSF mobilised PB harvesting were observed in the donors.

Direct comparison of PB and BM in allogeneic sibling donor transplantation has been reported in at least 8 randomised trials. Most of them did not show a benefit in overall

**Family Donors**

<table>
<thead>
<tr>
<th>Abnormal finding</th>
<th>Absolute contraindications</th>
<th>Relative contraindications</th>
<th>Specific consideration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td></td>
<td>BM donation and G-CSF stimulated or unstimulated apheresis</td>
<td></td>
</tr>
</tbody>
</table>

**Unrelated Donors (a)**

<table>
<thead>
<tr>
<th>Infectious</th>
<th>As for blood donation</th>
<th>Parvovirus B19, if known after collection: Gram-positive or Gram-negative bacterial graft infection</th>
<th>EBV, CMV, toxoplasmosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital</td>
<td>As for blood donation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignancies</td>
<td>Any malignancy except in situ cancer</td>
<td></td>
<td>Skin cancer removed in toto</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Any donation</td>
<td></td>
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</tbody>
</table>

**Unrelated Cord Blood (b)**

<table>
<thead>
<tr>
<th>Infectious</th>
<th>As for blood donation, Gram-positive or Gram-negative contamination</th>
<th>EBV, CMV, toxoplasmosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital</td>
<td>As for blood donation, Exclude, if congenital diseases known in family</td>
<td></td>
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<tr>
<td>Malignancies</td>
<td>Any, in child</td>
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(a) Not enough information is available for West Nile virus. Contamination of stem cell graft with epidermal bacteria might be a relative contraindication.
Safety and efficacy of hematopoietic stem cell collection from mobilized peripheral blood in unrelated volunteers: 12 years of single-center experience in 3928 donors

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We present results of peripheral blood stem cell (PBSC) mobilization, collection, and follow-up from 3928 consecutive unrelated stem cell donors. Assessments were performed prospectively at baseline, leukapheresis, 1 month, 6 months, and annually after PBSC donation. During follow-up, side effects were recorded by return post questionnaires. The median CD34+ cell counts on day 5 were 67.5 × 10^6 in male and 51 × 10^6 in female donors. Bone pain and headache were the most common side effects of recombinant human granulocyte-colony stimulating factor. Central venous access was required for 23 donations (0.6%). Throughout the follow-up, the absolute neutrophil counts were slightly below the initial baseline values but remained within the normal range. The majority of the donors reported good or very good health. Malignancies occurred in 12 donors (0.3%), among whom were 1 case of acute myeloid leukemia, 1 case of chronic lymphatic leukemia, and 2 cases of Hodgkin disease. Only the incidence of Hodgkin lymphoma differed significantly from an age-adjusted population. In conclusion, 7.5 μg/kg per day lenograstim proved to be safe and effective for mobilizing hematopoietic stem cells for allogeneic transplantation. Long-term monitoring of healthy PBSC donors remains important to guarantee the safety standards of PBSC mobilization and collection. (Blood. 2009;114:3757-3763)

Introduction

The collection of hematopoietic stem cells mobilized from the bone marrow into the bloodstream of healthy donors has now become a routine procedure throughout the world. In Germany, peripheral blood stem cells (PBSCs) mobilized by recombinant human granulocyte-colony stimulating factor (rhG-CSF) were used for 1739 (86.7% of all) allogeneic hematopoietic stem cell transplantations in 2006. The immediate side effects of rhG-CSF administration and PBSC collection have long been established; however, information as to possible long-term consequences has been limited up to now. To reduce the risk of complications in healthy donors, the dose of rhG-CSF should be as low as possible. At the same time, it is in the interests of both the donor and recipient to obtain enough stem cells to avoid graft failure. The optimal dose for achieving both these aims has yet to be precisely determined. Our prospective study at the University Hospital of Dresden has evaluated the efficiency and consequences, both short- and long-term, of PBSC mobilization and harvesting in a large population of healthy unrelated donors.

Methods

Subjects

The PBSC mobilization protocol was approved by the Ethics Committee of the University Hospital of Dresden, and donor informed consent was obtained in accordance with the Declaration of Helsinki. We studied 3928 healthy unrelated donors who consecutively donated PBSCs between January 1996 and January 2008. Within this group, 675 donors donated for recipients from the United States, but mobilization results and follow-up data have not been reported to the National Marrow Donor Program. Overall, 2813 men (71.6%) and 1115 women (28.4%), with a median age of 33.9 years (range, 18-61 years), were included in 4050 cycles of rhG-CSF administration and apheresis. In 122 donors (3.1%), a second cycle of rhG-CSF mobilization and PBSC collection was performed in response to a second donation request from the transplantation center or for a different recipient, after central review by the supervising physicians of the donor center. The median interval between the first and second PBSC donations was 265 days (range, 22-510 days).

PBSC mobilization and collection

The standard mobilization regimen, which was used in 97.3% of the donors, was 7.5 μg/kg rhG-CSF (lenograstim) administered for 5 to 6 consecutive days. In some protocols, donors received filgrastim at 10 μg/kg (2%) or PEG-filgrastim as a 12-mg single dose (1%). Experience from mobilization protocols with filgrastim and PEG-filgrastim has been reported elsewhere.2,3 The first leukapheresis was performed on day 5. If the required number of CD34+ cells per recipient body weight was not collected, rhG-CSF administration was repeated (depending on the donor’s leukocyte count), and a second PBSC collection was performed on day 6.

The rhG-CSF was injected subcutaneously either daily by the donor’s family doctor (15.3%) or every 12 hours by the donor or a family member (81.9%). PBSCs were collected by a continuous-flow blood cell separator (Cobe Spectra; Caridian BCT) via bilateral (anterior cubital and forearm) peripheral venous access, whenever possible, or otherwise via a central line in the femoral vein. In donors who needed a central line, only one
Special report

A European reference protocol for quality assessment and clinical validation of autologous haematopoietic blood progenitor and stem cell grafts

S Serke and HE Johnsen

1Department of Haematology-Oncology, Humbolt-Universität, Berlin, Germany; and 2Department of Haematology, Herlev Hospital, University of Copenhagen, Denmark

Summary:

Recently, the regulatory authorities have begun to show interest in haematopoietic stem cell products. On a professional rather than a regulatory basis, the International Society for Hematotherapy and Graft Engineering (ISHAGE) has established the Foundation for the Accreditation of Haematopoietic Cell Therapy (FACHT), which has drawn up guidelines for standards and accreditation of such activity. In Europe, the regulatory environment with regard to haematopoietic stem cell grafts, processing and storage are currently less stringent. However, in 1998 the European Joint Accreditation Committee Euro-ISHAGE/EBMT (JACIE) prepared a regulatory document ‘Standards for Blood and Marrow Progenitor Cell Collection, Processing and Transplantation’ which was approved by the EBMT General Assembly. The major objectives were to promote quality of medical and laboratory practice in haematopoietic progenitor cell transplantation. The standards extend and detail the pre-existing activity of EBMT centres including all phases of collection, processing and administration of these cells. This is the platform for the proposed reference protocol for CD34^+ cell enumeration and clinical validation of quality assessment to ensure that appropriate standards of work and product quality are established and will be maintained. Bone Marrow Transplantation (2001) 27, 463–470.

Keywords: CD34; flow cytometry; standardization; reference protocol; haematopoietic stem cell transplantation; prediction of engraftment

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Current indications for high-dose therapy as adjuvant treatment are now being applied to newly diagnosed patients. This development has required evidence-based quality and safety assessment of haematopoietic stem cell grafts and introduced a new institution in medicine, the stem cell laboratory. In most cases this speciality has evolved from or within hematological research laboratories. However, the increased routine technologies applied in quality evaluation, ex vivo manipulation and safety assessment in stem cell handling, naturally places this activity in transfusion medicine in close collaboration with the clinic.

In 1998, the European Joint Accreditation Committee Euro-ISHAGE/EBMT (JACIE) prepared a regulatory document ‘Standards for Blood and Marrow Progenitor Cell Collection, Processing and Transplantation’, which was approved by the EBMT General Assembly. In the same year the EBMT Board established a sub-committee on ‘Quality Assessment of Haematopoietic Stem Cell Grafts’ with the aim within the EBMT centres to: (1) Analyse the existing situation regarding quality assessment of stem cell grafts; (2) identify major problems in quality assessments and establish scientific protocols; (3) describe guidelines for the handling of auto- and allografts; (4) publish results of sub-committee studies; and (5) contribute a document to the EBMT operational manual.

The current situation regarding CD34^+ cell enumeration has been analysed and published as an European Survey on flow cytometric determination of CD34-expressing cells. Problems in quality assessment were disclosed during the first sub-committee meeting held at the annual 1999 EBMT meeting. One major concern was paragraph D4.130 on quality management in the standard document stating: ‘D4.130 A nucleated cell count shall be performed for any component after collection and after any subsequent processing (if applicable). D4.131 CD34^+ cell count shall be performed. D4.132 The target should be to transfuse a minimum of 2 × 10^6 CD34^+ cells per kg body weight, but lower numbers may be acceptable in specific cases. (This does not apply to bone marrow or cord blood)’.1

The 1999 subcommittee meeting concluded that the numbers given in paragraph D4.132 were inappropriate as no convincing data exist from single- or multicentre studies to document a common protocol for CD34^+ cell enumeration and strategy for clinical validation of numbers. This reflects a problem in preparing guidelines for quality assessments and is the background for this proposal of a European Reference Protocol on CD34^+ cell enumeration.
by flow cytometry and a strategy for its validation by clinical end-points.

**Laboratory analysis of progenitor and stem cells**

*First step in quality assessment was CD34 standardisation*

Culture assays of colony-forming cells were originally performed, but due to the inconvenience of this method several laboratories focused on flow cytometric analysis of CD34+ cells. In quality assessment and the first steps were taken in 1991 by Serke of Berlin, Siena of Milan and Fritsch of Vienna. This development was promoted by the farseeing Mulhouse Group when in early 1992, they hosted the First European Workshop on Stem Cell Determination and Standardization. The resulting Milan/Mulhouse manual is still a valuable procedure reference in laboratory practice.

The subsequent years have resulted in several publications about the standardisation of flow cytometry analysis as well as reports on the clinical implication of the enumeration of CD34+ cells. The CD34 antigen is stage-specific and identifies cells in the early stages of haemopoietic differentiation. This population, therefore, contains progenitors committed to the myeloid, erythroid, megakaryocytic and lymphoid lineages, as well as primitive progenitors and stem cells capable of long-term reconstitution. Enumeration of CD34+ cells has been shown to be useful in the procedure of stem cell mobilisation and harvest from blood for transplantation and it seems informative for the prediction of fast or delayed three-lineage engraftment and blood cell recovery following high-dose therapy.

We do not yet know the minimal safe number of CD34+ cells needed for clinical engraftment of all lineages, this may vary depending on the stem and progenitor cell subset composition in a given patient or autograft. However, we do know that a graft content of more than 5–10 million CD34+ cells per kg body weight is safe, resulting in fast recovery of ANC and platelets before days 14 and 21, respectively, in a major fraction of patients and, most important, only have a minor risk for engraftment failure.

In a survey of 1600 patients from nine published papers, including a minimum of 50 patients each, it is concluded that the overall median time to ANC and platelet recovery is 11 days (2–93) and 11 days (0–1000+), respectively. From 15 studies with information of engrafted low numbers (Table 1a and b), it is concluded that no definite lower level exists to document groups of patients at high risk for prolonged recovery, based on CD34 numbers below 1 million/kg, 2 million/kg, 2.5 million/kg, and 3 million/kg or 5 million/kg. From such data, it is obvious that we will never obtain an exact number of CD34+ cells delineating an insufficient or safe graft. We have to reconsider these terms and change exact numbers into probabilities of obtaining clinical efficacy and avoiding toxicity evaluated by proper end-points (vide infra).

The subsequent phase two, documented a likely clinical influence by single centres analysing retrospective material/data. The third phase prepared convincing single centre prospective evaluation evolving into the most important phase four, a multicentre prospective evaluation based upon important clinical end-points (Table 2). Ideally, phases II–III document the usefulness, convincing one or more centres to participate in a phase IV validation trial, which however in this phase is still a valuable procedure reference in laboratory practice. The third phase prepared convincing single centre prospective evaluation evolving into the most important phase four, a multicentre prospective evaluation based upon important clinical end-points (Table 2). Ideally, phases II–III document the usefulness, convincing one or more centres to participate in a phase IV validation trial, which however in this phase is still a valuable procedure reference in laboratory practice.
Apheresis System

Platelet Collection Guide
For use with COBE® Spectra system versions 4.7, 5.1–5.9, 6.0–6.9, 7.0–7.9

Part No. 777093-637
Reorder No. 701223-002
06/2008
Platelet Collection Process Overview

Install the filler according to the COBE Spectra system software version and disposable tubing set used to perform the procedure:

- Install the dual-stage filler (Figure 1-1) to use the Dual-Needle or Single-Needle ELP disposable tubing set.

![Figure 1-1: Dual-stage filler](image1)

- Install the dual-stage filler with LRS bracket (Figure 1-2) to use software version 5.1 and the Dual-Needle or Single-Needle ELP disposable tubing set with LRS chamber.

![Figure 1-2: Dual-stage filler with LRS bracket](image2)

- Install the dual-stage LRS Turbo filler with LRS bracket (Figure 1-3) to use software version 7.0 and the Dual-Needle or Single-Needle ELP disposable tubing set with LRS chamber.

![Figure 1-3: Dual-stage LRS Turbo filler](image3)

Extended Life Platelet (ELP) Collection Procedures

To perform an ELP procedure, install a dual-stage filler and use a Dual-Needle or a Single-Needle ELP disposable tubing set. You may concurrently collect plasma during an ELP procedure.

In the ELP channel (Figure 1-4), anticoagulated whole blood enters the first stage of the channel through the inlet tube. During the first stage, the red blood cells (RBCs) and most of the white blood cells (WBCs) are separated from the platelet-rich plasma.

The RBCs and WBCs exit the channel through the RBC tube. Platelet-rich plasma and a few remaining WBCs flow over the dam into the second stage, where the platelets and remaining WBCs concentrate in the plasma and exit through the collect tube. The remaining plasma flows around the channel to the plasma tube. When concurrently collecting a specific plasma volume, that volume is diverted to the plasma collection bag.
In vivo analyses of early events in acute graft-versus-host disease reveal sequential infiltration of T-cell subsets


Graft-versus-host disease (GVHD) is a major obstacle in allogeneic hematopoietic cell transplantation. Given the dynamic changes in immune cell subsets and tissue organization, which occur in GVHD, localization and timing of critical immunological events in vivo may reveal basic pathogenic mechanisms. To this end, we transplanted luciferase-labeled allogeneic splenocytes and monitored tissue distribution by in vivo bioluminescence imaging. High-resolution analyses showed initial proliferation of donor CD4+ T cells followed by CD8+ T cells in secondary lymphoid organs with subsequent homing to the intestines, liver, and skin. Transplantation of purified naive T cells caused GVHD that was initiated in secondary lymphoid organs followed by target organ manifestation in gut, liver, and skin. In contrast, transplanted CD4+ effector memory T (TEM) cells did not proliferate in secondary lymphoid organs in vivo and despite their in vitro alloreactivity in mixed leukocyte reaction (MLR) assays did not cause acute GVHD. These findings underline the potential of T-cell subsets with defined trafficking patterns for immune reconstitution without the risk of GVHD.

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Introduction

Allogeneic hematopoietic cell transplantation (HCT) has proven to be an effective therapy for a variety of life-threatening malignancies.1 The beneficial effects of HCT are due to the graft-versus-tumor reaction, which is capable of destroying residual tumor cells that persist after chemotherapy or radiation therapy.2 However, allogeneic HCT is limited by the immunologic recognition and destruction of host tissues termed graft-versus-host disease (GVHD). Acute GVHD continues to be a major source of morbidity and mortality following HCT, which limits treatment of a broader spectrum of diseases, such as autoimmune diseases or organ transplant rejection.3,4 Tissue-specific destruction of GVHD target organs, as gastrointestinal tract, liver, and skin, underlines the importance of migration capacities of alloreactive T lymphocytes.5,6 In the current study we aimed to determine the time points of organ infiltration and focused on the role of different lymphoid organs in initiating acute GVHD. We used in vivo bioluminescence imaging (BLI) to analyze the migration pattern of whole splenocytes after transplantation into allogeneic recipients. BLI has already proven to be a sensitive and accurate means of characterizing engraftment patterns of hematopoietic stem cells, of monitoring tumor cell growth, and of assessing response to conventional and biological therapies.7-9 We also aimed to clarify the role of different T-cell subsets during GVHD development. It is reported in the literature10-12 that CD4+ effector memory T (TEM) cells do not cause GVHD. This prompted us to characterize their trafficking and proliferation pattern in vivo, while comparing it to purified naive CD4+ T lymphocytes.

Materials and methods

Mice

FVB/N (H-2b, Thy1.1) mice and Balb/c mice (H-2d, Thy1.2) were purchased from Jackson Laboratory (Bar Harbor, ME). The luciferase-expressing (luc⁺) transgenic FVB/N line was generated as previously described.9 Female heterozygous luc⁺ offspring of the transgenic founder line FVB-L2G85 were used for all transplantation experiments. All animal studies were performed under institutional approval.

Flow cytometric cell purification and analysis

The following antibodies were purchased from BD Pharmingen (San Diego, CA) and eBiosciences (San Diego, CA) and used for fluorescence-activated cell-sorting (FACS) on an LSR flow cytometer (Becton Dickinson, Mountain View, CA): CD3e (145-2c11), CD4 (RM4-5), CD8a (145-2c12), CD8β (H18528), CD25 (H7) and IgG2a isotype control (MOPC-21, H11001). CD3e and CD4 were used as cell-surface markers to identify T lymphocytes, whereas CD8a was used to distinguish CD8αα from CD8αβ T cells.

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From the Departments of Medicine and Pediatrics, Stanford University, Stanford, CA.


A.B. and S.S. designed and performed research, contributed new reagents, analyzed data, and wrote the paper. J.B. and G.F.B. designed and performed research; C.B.W., E.I.H., and E.M.B. performed research and analyzed data; Y.A.C. contributed vital new reagents; C.H.C. designed research, contributed new reagents, and analytical tools; and R.S.N. designed research and wrote the paper. A.B. and S.S. contributed equally to this study.

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Reprints: Robert S. Negrin, Center for Clinical Sciences Research Building, Room 2205, 269 W Campus Dr, Stanford, CA 94305; e-mail: negrs@stanford.edu.

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1113
Mini review

Acute lung injury after allogeneic stem cell transplantation: is the lung a target of acute graft-versus-host disease?

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Summary:

Allogeneic hematopoietic stem cell transplantation (SCT) is an important therapeutic option for a number of malignant and nonmalignant conditions but the broader application of this treatment strategy is limited by several side effects. In particular, diffuse lung injury is a major complication of SCT that responds poorly to standard therapeutic approaches and significantly contributes to transplant-related morbidity and mortality. Historically, approximately 50% of all pneumonias seen after SCT have been secondary to infection, but the judicious use of broad-spectrum antimicrobial prophylaxis in recent years has tipped the balance of pulmonary complications from infectious to noninfectious causes. Importantly, noninfectious lung injury is associated with significant morbidity and mortality and responds poorly to standard therapeutic approaches. This mini review will discuss the definition, risk factors and pathogeneses of noninfectious lung injury that occurs early after allogeneic SCT.

Acute lung injury: idiopathic pneumonia syndrome (IPS)

Definition and clinical course

In 1993, a panel convened by the NIH defined widespread alveolar injury following SCT that occurs in the absence of an active lower respiratory tract infection and cardiogenic causes as idiopathic pneumonia syndrome (IPS). The panel was careful to stress that they considered this definition to be a clinical syndrome with variable histopathologic correlates and several potential etiologies. As shown in Table 1, diagnostic criteria of IPS include signs and symptoms of pneumonia, nonlobar radiographic infiltrates, abnormal pulmonary function and the absence of infectious organisms as determined by broncho-alveolar lavage (BAL) or lung biopsy. Histopathologic findings associated with IPS include diffuse alveolar damage with hyaline membranes and lymphocytic bronchitis and bronchiolitis obliterans organizing pneumonia (BOOP). However, the most frequently reported pattern is interstitial pneumonitis, a term historically used interchangeably with IPS. Interstitial pneumonitis is seen in association with diffuse alveolar damage and hemorrhage early after SCT and is accompanied by bronchiolar inflammation and epithelial damage at later time points.

Over the last several decades, allogeneic hematopoietic stem cell transplantation (SCT) has emerged as an important therapeutic option for a number of malignant and nonmalignant conditions. Unfortunately, this treatment strategy is limited by several side effects including pulmonary toxicity. Diffuse lung injury is a major complication of SCT that occurs in 25–55% of SCT recipients and can account for approximately 50% of transplant-related mortality. Noninfectious lung injury can be either acute or chronic depending on the time of onset after SCT and the tempo of disease progression. Historically, approximately 50% of all pneumonias seen after SCT have been secondary to infection, but the judicious use of broad-spectrum antimicrobial prophylaxis in recent years has tipped the balance of pulmonary complications from infectious to noninfectious causes. Importantly, noninfectious lung injury is associated with significant morbidity and mortality and responds poorly to standard therapeutic approaches. This mini review will discuss the definition, risk factors and pathogeneses of noninfectious lung injury that occurs early after allogeneic SCT.

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2Dr Cooke is a National Marrow Donor Program Amy Strelzer-Manasevit Scholar and a fellow of the Robert Wood Johnson Minority Faculty Development Program.

3Drs Cooke and Yanik are recipients of a Translational Research Award from the Leukemia and Lymphoma Society.

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Autologous hematopoietic stem cell transplantation is an increasingly accepted treatment for refractory autoimmune diseases. Refractory celiac disease with aberrant T cells (RCD type II) is unresponsive to available therapies and carries a high risk of transition into enteropathy-associated T-cell lymphoma (EATL). This study reports on the feasibility, safety, and efficacy of ASCT in patients with RCD type II. Thirty patients with RCD type II were evaluated. Seven patients (4 men, 3 women, mean age 61.5 years [range, 51-69 years]) underwent transplantation. After conditioning with fludarabine and melphalan, ASCT was performed. Patients were monitored for response, adverse effects, and hematopoietic reconstitution. All 7 patients completed the mobilization and leukapheresis procedures successfully and subsequently underwent conditioning and transplantation. Engraftment occurred in all patients. No major nonhematologic toxicity or transplantation-related mortality was observed. There was a significant reduction in the aberrant T cells in duodenal biopsies associated with improvement in clinical well-being and normalization of hematologic and biochemical markers (mean follow-up, 15.5 months; range, 7-30 months). One patient died 8 months after transplantation from progressive neuroendocrine disease. These preliminary results showed that high-dose chemotherapy followed by ASCT seems feasible and safe and might result in long-term improvement of patients with RCD type II whose condition did not respond promptly to available drugs. (Blood. 2007;109:2243-2249)
Patients, materials, and methods

Patients

Between March 2004 and March 2006, 13 patients were evaluated for ASCT. The 4 men and 3 women (mean age, 61.5 years; range, 51-69 years) with RCD II underwent ASCT. Six other patients were excluded because of the presence of coexistent coronary artery disease and heart failure (New York Heart Association classification III in 2 patients), EATL found on evaluation before transplantation (3 patients), and low performance status (1 patient). One patient could not be treated due to unsuccessful leukapheresis; she developed EATL and died subsequently despite chemotherapy and immunotherapy with anti-CD52 (alemtuzumab).22 The 2 patients with congestive heart failure died from progressive disease and cachexia (first patient) and bronchiectasis (second patient). The 3 patients with EATL all died within few months, whereas the patient with low performance status died from cachexia.

The baseline characteristics of the patients are shown in Table 1. All patients received therapy with prednisone and cladribine (2-CDA) several months before undergoing ASCT (not within 6 months of transplantation). The first 3 patients (patients A, B, and C) were diagnosed with CD at relatively advanced age, had persistent diarrhea and weight loss and failed to respond to GFD, steroids, and immunosuppressives. Because of the presence of active disease and high percentage of aberrant T cells in the small bowel mucosa, they were included in this study protocol. At the age of 48 years, patient D was diagnosed with CD in association with dermatitis herpetiformis. Furthermore, he had a clinical picture of neuroceliac disease with ataxia. After exclusion of structural brain and infectious disorders, he underwent ASCT at the age of 63.5 years. Patient E has, in addition to CD with ulcerative jejunitis, Hashimoto thyroiditis, and patient F has CD with ulcerative jejunitis. One patient (patient G) was included because of the presence of very extensive ulcerative jejunitis with multiple small bowel strictures necessitating repeated resections although initially biopsies showed a low percentage of aberrant T cells. He had clinically short bowel syndrome (remaining small bowel approximately 100-150 cm) requiring total parenteral nutrition (TPN).

Criteria for diagnosis of RCD

Patients with CD were considered to be refractory when symptoms of malabsorption due to villous atrophy persisted or recurred after a former good response despite strict adherence to a GFD for at least 1 year. Furthermore, possible underlying diseases such as autoimmune enteritis, bacterial overgrowth, giardiasis, amyloidosis, intestinal lymphangiectasia, Whipple disease, hypogammaglobulinemia, eosinophilic enteritis, EATL, and inflammatory bowel disease were excluded.11 The diagnosis of RCD was established as type II when 20% or more aberrant T cells were present.10,11,13

Inclusion criteria

Patients were included only when the diagnosis of true RCD with aberrant T cells was confirmed (except for patient G who was included based on the extensive ulcerative jejunitis with short bowel syndrome despite having only 10% aberrant T cells), after verifying their strict adherence to a GFD. Performance status according to the World Health Organization (WHO) criteria had to be 0 to 2, and no severe concomitant cardiac, pulmonary, renal or hepatic disease could be present. EATL was excluded by endoscopic examination with multiple biopsies, computed tomography (CT) scan, positron emission tomography (PET), and a trephine bone marrow biopsy. Furthermore, neither active uncontrolled infection nor HIV positivity was permitted.

Evaluation

Before proceeding to ASCT, the patients were extensively evaluated as to their performance status, the presence of concomitant diseases, and extraintestinal disease or EATL. This evaluation included clinical assessment noting particularly signs and symptoms of malabsorption, body mass index (BMI), and performance according to the WHO score23; evaluation of adherence to a GFD including frequent consultation with dietitian (advice and follow-up) in addition to checking serology (antiendomysial [EMA] and anti-tissue transglutaminase antibody [anti-tTG], both of which usually revert to negative after strict adherence to the GFD); and evaluation by upper gastrointestinal endoscopy (UGIE), video capsule endoscopy (VCE), and double balloon enteroscopy (DBE). Duodenal biopsies (4 biopsies) were classified according to the modified Marsh criteria.24-25 T-cell receptor (TCR) gene rearrangement study,13,14 T-cell flow cytometry, and IEL phenotyping were performed.15,26,27 Laboratory evaluation included whole blood cell counts and serum levels of creatinine, bilirubin, liver enzymes, lactate dehydrogenase, albumin, electrolytes, iron, ferritin, folic acid, and vitamin B12 were determined. EMA and anti-tTG assays, HLA-DQ typing, thyroid function tests, stool examination for Giardia and other parasites, and HIV serology were also performed.28 For radiologic evaluation, the patients underwent whole-body CT scanning and whole-body PET to exclude intestinal and extraintestinal localization of EATL.29,30

Immunophenotyping of IELs

IELs were isolated from 3 duodenal biopsies by passing them through nylon filters (1 × 100 μm, 1 × 40 μm, BD Biosciences, Discovery Labware, Bedford, MA). Cells were stained with fluorescent-labeled monoclonal antibodies to CD3, CD7, CD8, CD45, CD103, and TCRγδ, as well as with relevant isotype controls.

All monoclonal antibodies were from BD (BD Biosciences, San Jose, CA), except for CD103, which was from IQ Products (Groningen, The Netherlands) and analyzed by 4-color flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA). Leukocyte common antigen (CD45) was always included to identify the lymphocyte population. In some tubes cell surface CD3 staining (anti–CD3-APC) was followed by permeabilization (Cytofix/Cytoperm, BD Biosciences PharMingen, San Diego, CA) and subsequent cytoplasmic staining with anti–CD3-PTIC or isotype control.

Aberant T cells were defined either as CD7+ surface CD3− cells (expressed as percent of CD103+ lymphocytes) or as cytoplasmic CD3+ surface CD3− cells (expressed as percent of CD103+ lymphocytes).12,26

All flow cytometry analyses were performed by an analyst and interpreted by the same medical immunologist; histopathology was performed by the same pathologist to ensure uniformity, reproducibility, and consistency of results.

Assessment of TCR gene rearrangement by PCR

TCRγ gene rearrangements studies were performed in separate 3 to 4 duodenal specimens that were preserved on Histocon (Polysciences Europe, Eppelheim, Germany) and frozen at −20°C. DNA was extracted from cryosections of duodenal specimens by a standard procedure using proteinase-K digestion and ethanol precipitation of the gDNA. TCRγ gene rearrangements were analyzed by multiplex polymerase chain reaction (PCR) amplification under standardized conditions. A monoclonal and polyclonal control was included in each experiment. Clonality assessment for TCRγ gene rearrangements was done according to the Biomed-2 concerted action BM H4-CT98-3936 on PCR-based clonality studies for early diagnosis of lymphoproliferative disorders.12,14

Peripheral blood stem cells mobilization and collection

Mobilization of hematopoietic progenitor cells from the bone marrow into the peripheral blood was achieved using granulocyte colony-stimulating factor (G-CSF) 2 × 5 μg/kg by subcutaneous injection for at least 4 days. Hematopoietic stem cells were harvested from the peripheral blood by leukapheresis and kept frozen until ASCT. The target CD34+ count was more than 2 × 10⁶/kg.

Conditioning and ASCT

The conditioning regimen consisted of fludarabine given orally for 5 days (40 mg/m²/d) and melphalan (given intravenously, 2 days, 70 mg/m²/d) as shown in Figure 1. At day 0, the frozen stem cell suspension was thawed and reinfused. The rationale for this conditioning regimen was based on T-cell depletion by a purine analog combined with a modified dose of melphalan (total dose 140 mg/m²) for myeloablation.
Effect Of High Dose Chemotherapies Biology Essay

There are 2 possibilities of thawing: with flushing out or without flushing out. The flushing out procedure must be done immediately after thawing, without any delay, for the purpose of limiting the toxicity of DMSO. This procedure consists of: spin over (2000 rpm), removal of the supernatant liquid and admixture to the remaining cells of a solution composed of 10% ACD + 2% Albumine. The thawing procedure without flushing out: we out each frozen bag into a bath of water at 37˚C and we shake it with gentle movements, 2 minutes until it is completely defrosen. A number of 86 procedures of hematopoietic stem cells (HSC) harvest and cryopreservation from 64 volunteer donors, 54 adults (28 women and 26 men) and 10 children (5 girls and 5 boys) with ages between 6–66 years (on average 30.5) were carried out in the Bone Marrow Transplant Center from Clinical Institute Fundeni, Bucharest. We counted the WBC and the number of CD34+ cells from the peripheral blood, beginning the day +4 from the mobilization regimen. HSC from peripheral blood were harvested by leukapheresis procedure with the help of discontinuous flow separators (Haemonetics MCS plus) and continuous flow separators (Cobe Spectra), autoPBSC procedure and MNC procedure, by treating several total blood volumes (SVL method – Standard Volume Leukapheresis and LVL method – Large Volume Leukapheresis). A number of 24 procedures were carried out with Haemodics separator (16 donors) and 62 procedures with Cobe Spectra separator (48 donors). ACD-A was the anticoagulant agent that we used with 1:9 ratio (Haemonetics separator) and 1:12 ratio respectively (Cobe Spectra separator). HSC harvested were than combined with a cryopreservation solution (DMSO) at a final concentration of 10% (DMSO 10%). The freezing procedure was realized with the help of nitrogen liquid programmable freezer MiniDigitCool. The graft quality control was done as well before freezing procedure (native product) as after the freezing procedure (cyotubes and cryocites bags). There have been done the following tests: total blood count, blood smear, total number of CD34+ cells, cells viability (with tripan blue), number of CFU-GM. The HSC graft was thawed in a water bath at +37˚C by smooth movements, immediately followed by the infusion to the patient. HSC mobilization was achieved for all the 64 volunteer donors by administration of Filgastrim, on an average 8.4 mcg/donor weight (limits 5–16.64 mcg/donor weight), leukapheresis procedure being realized in day +5 of Filgastrim administration. We started leukapheresis procedure at a WBC level in peripheral blood of 45.9 × 10⁹/L (limits 5.1–72.1 × 10⁹/L) and a CD34+ cells level in peripheral blood of 101.5 × 10⁶/L (limits 16.2–112.8 × 10⁶/L). The optimal dose of stem cells CD34+ was achieved at 45 donors by a single leukapheresis procedure and at 18 donors by two leukapheresis procedures. There have been achieved 59 SVL procedures and 27 LVL procedures; we treated on average 2.98 total blood volumes (limits 1.8–4.2) in 331.4 minutes (limits 159–865 minutes). The medium number of CD34+ cells harvested/procedure was 7.5 × 10⁶/L (limits 0.29–52.1 × 10⁶/L) and the medium number of CD34+ cells harvested/patient was 9.5 × 10⁶/L (limits 1.3–52.1 × 10⁶/L). Cells viability obtained with the direct test was 97.6% (limits 87–100%) and cells viability after the mixing with DMSO solution was 71.4% (limits 30–100%). In vitro testing of clonogenic capacity of progenitor cells showed on average 529.9 × 10⁴ CFU-GM/body weight/sample (limits 34–3435 × 10⁴). The tests performed after the graft thawing showed the following results: cells viability 55.8% (limits 10–84%) in 71 collections; number of CD34+ cells 4.4 × 10⁶/patient body weight (limits 0.66–21.98) in 12 collections; number of CFU-GM: 98.5 × 10⁴/patient body weight (limits 0–635) in 54 collections. We performed allogeneic transplant with the source of stem cells from the peripheral blood in 50 patients; the graft that was administrated was formed on average by 5.6x10⁶ CD34+ cells/patient body weight (limits 2.11–15.09 x10⁶/patient body weight). In conclusion, a healthy volunteer donor, will undergo in most cases 4 or 5 days of Filgastrim administration. The WBC and the number of CD34+ cells from the peripheral blood will be counted beginning with the 4th day. When the number of CD34+ cells from peripheral blood will reach a certain level (usually on the 4th or 5th day), the volunteer donor will be sent to the apheresis unit for harvesting stem cells, 1 or 2 sessions of apheresis will be enough for collecting the total amount of stem cells for a graft. The apheresis session will take on average 4 hours (if it will be used MNC procedure) or 5 hours (if it will be used auto PBSC procedure). HSC will be combined with a cryopreservation solution; then the graft will be stored in liquid nitrogen at –196˚C degree until the patient will be transplanted. When it will be needed, the graft will be thawed in a water at +37˚C by smooth movements, immediately followed by its infusion to the patient.
Using G-CSF Mobilized PB Allografts Biology Essay

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G-CSF is used for increasing concentrations of circulating HSC. In 1995, 3 pivotal studies demonstrated the safety and feasibility of using G-CSF mobilized PB allografts. Patients experienced prompt engraftment with an incidence of GvHD similar to that of BM recipients. In addition, no serious short-term complication of G-CSF mobilized PB harvesting were observed in the donors.

During MNC procedure, anticoagulated whole blood enters the inlet chamber through the inlet tube. As the blood flows through the channel, the system separates it into three layers: RBC on the outside, buffy coat containing WBC in the center, and platelet-rich plasma on the inside. The system establishes the RBC plasma interface during Quick Start. After Quick Start, the operator adjusts the plasma pump flow rate to hold the interface in a constant position. The system draws the MNC from the channel through the WBC collect tube, while the platelet-rich plasma exists through the plasma tube. The RBC exit through the RBC tube. During Spectra AutoPBSC procedure, anticoagulated whole blood enters the first stage of the channel through the inlet tube. In the first stage, the system separates the RBC and WBC from the platelet-rich plasma. The RBC and granulocytes exit the channel through the RBC tube. Platelet-rich plasma flows over the dam into the second stage where the system concentrates the platelets in the plasma. The plasma exit through the collect tube to return to the donor, and the remaining plasma flows through the channel to the plasma tube. MNC accumulate above the layer of RBC. During the Harvest phase, the MNC flow over the dam into the second stage. Once the collect concentration monitor (CCM) detects cells in the collect line, the collect valve opens and the MNC flow to the collection bag. The Chase phase follows the Harvest phase during which plasma “chases” the MNC in the collect line up to the collection bag. Concurrent collection of a specific plasma volume is optional. The system determines the plasma and collect pump flow rate based on the donor/patient hematocrit, and maintains the interface position. A small volume of plasma and RBC flow into the control tube to help maintain the interface. Common apheresis complications: citrate toxicity (hypocalcemia, hypomagnesaemia, hypokalaemia, metabolic alkalosis), thrombocytopenia, hypovolemia, catheter malfunction, infection. The collected stem cell products are maintaining in liquid nitrogen at –196˚C until the time of patients transplantation.

Cryopreservation solutions:

— DMSO 10% + Albumine 4% or HES 6% for programmable freezing;

— DMSO 5% + HES 6% for freezing at –80˚C; — DMSO 10% + NaCl + autoFFP/ACD.

Reactions to DMSO:

— Common: nausea, vomiting, abdominal cramping, headache, garlic aftertaste;

— Rare: hypotension, rapid heart rate, shortness of breath, fever, neurologic complications.

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The Last Several Decades Allogeneic Hematopoietic Cell Transplantation Biology Essay

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