Hematopoietic progenitor cell mobilization, collection, transportation, and infusion of fresh products

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ABSTRACT

Introduction: The quality of the graft is a critical determinant of transplant success. **Methods:** Literature review and expert discussion. **Results:** In this review, we highlight the essential aspects of hematopoietic progenitor cell mobilization and collection, including apheresis and bone marrow harvest. We also discuss optimal cell dose and concentration, as well as key considerations for product labeling, transportation, and infusion of fresh cell therapy products. **Conclusion:** This document contains recommendations based on the best scientific evidence available at the time of its publication. We hope it proves useful in the daily operations of a bone marrow transplant unit.

Keywords: Mobilization; Stem cell collection; Stem cell infusion; Transportation; Bone marrow harvest; Leukapheresis.

INTRODUCTION

This consensus reviews the recommendations for the mobilization, collection, labeling, transport, and infusion of hematopoietic progenitor cells, with the key points summarized in Table 1.

Table 1. Key recommendations for hematopoietic progenitor cells.

Key points	
Process	**Consensus recommendation**
Mobilization of peripheral hematopoietic progenitor cell (HPC-A)	Standard mobilization: filgrastim (G-CSF) 10 µg/Kg/day administered once or twice daily for 5 days, with the first collection on day 4 or 5[1–3]
	Chemotherapy plus G-CSF: vinorelbine 35 mg/m²; cyclophosphamide (Cy) 1,5 a 2 g/m²[1,4]
	Rescue mobilization: plerixafor (0.24 mg/Kg, 6 to 11 hours before collection) combined with G-CSF or chemotherapy plus G-CSF[1]

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Collection of peripheral hematopoietic progenitor cell (HPC-A)	$CD34^+$ target dose: 2 x 10^6/Kg per transplant (target dose of 4 to 5 x 10^6/Kg)[1]
	Large or very-large volume apheresis may improve $CD34^+$ yield in poor mobilizers ($CD34^+$ count between 10 and 20 cells/μL)[5,6]
	Products intended for infusion within 48 h or cryopreservation after 24 h should be diluted to a maximum nucleated cell concentration of 300,000/μL[7] or 200,000/μL[1], respectively. This can be done during the collection or in the cell processing lab using closed system methodology
Bone marrow harvest	Collection target: 10 to 15 mL/kg of recipient weight, not exceeding 20 mL/Kg of donor[8]
	Recommended cell dose: ≥ 3 x10^8/kg of nucleated cell (NC) (minimum of 2 x10^8 NC/kg)[8]
Labeling	ISBT 128 is the recommended standard for all cell therapy products[8]
Transport of fresh products	Maintain temperature between 2 and 24ºC (ideally near 4ºC)[6]
Infusion	Do not use leukocyte-reduction filters or irradiate the graft[1]
	Infusion rate: 6mL/Kg/hour for fresh products (maximum 4h)[6]

Source: Elaborated by the authors.

METHODS

Literature review and expert discussion.

RESULTS

Mobilization and collection of peripheral hematopoietic progenitor cell

Mobilization of CD34+ cells into peripheral blood followed by collection via apheresis is a critical step. Its goals include obtaining an adequate number of hematopoietic progenitor cells, minimizing collection-related complications, preventing mobilization failure, and optimizing resource utilization[2,9].

ALLOGENEIC TRANSPLANT

Growth factor-based mobilization

Filgrastim (G-CSF) 5–10 μg/kg/day administered once or twice daily subcutaneously for four or five days, with first collection on day 4 or 5[2,3]. The optimal CD34+ target dose is 4–5 × 10^6/kg (Table 2)[1–3]. Excessively high cell doses correlate with increased risk of chronic graft-*versus*-host disease (GVHD) and mortality[1–3].

Table 2. Recommended $CD34^+$ dosage.

Dose of mobilized CD34+ (for one transplant)	Autologous transplantation	Allogenic transplantation
Minimum	2×10^6/kg	4×10^6/kg
Optimal	2.5–5×10^6/kg	5–8×10^6/kg

Source: adapted from Worel et al.[1].

Peripheral venous access should be used whenever feasible[1,2].

Sickle cell trait (SCT) has historically raised theoretical concerns regarding vaso-occlusive complications during granulocyte colony-stimulating factor (G-CSF) mobilization and apheresis procedures. Although retrospective and prospective studies have demonstrated that peripheral blood stem cell collection from SCT donors is safe and effective, the small sample sizes in both cohorts have been insufficient to modify current clinical guidelines, which continue to advise against mobilization and apheresis in allogeneic SCT donors[10–12].

AUTOLOGOUS TRANSPLANT

Growth factor-based mobilization

Filgrastim (G-CSF) 10–20 μg/kg/day once or twice daily subcutaneously, with first collection on day 5[1–3,9]. The final dose should be administered two to three hours before peripheral blood CD34+ measurement[2,3].

If the initial yield is insufficient, G-CSF may be continued for an additional one or two days[1]. In cases of insufficient autologous hematopoietic progenitor cell collection, strategies to optimize mobilization include increasing the G-CSF dose or changing the dose schedule (such as administering G-CSF twice daily)[13,14]. However, it is important to note that increasing G-CSF dose alone has shown limited success historically[15].

G-CSF alone should not be used for remobilization[5].

Chemotherapy-based mobilization

Patients receiving chemotherapy as part of disease treatment may undergo chemomobilization[1]. G-CSF should be initiated one to five days after completion of chemotherapy, depending on the regimen[1–3]. Patients without chemotherapy indication but with risk factors for mobilization failure may also benefit[1,5]. In this mobilization, cyclophosphamide (Cy) is commonly used at a dose of 2 g/m^2[1–3,16]. Other options include vinorelbine, intermediate-dose cytarabine or Cy combined with etoposide[4,17,18].

Use of Plerixafor

Plerixafor may be administered with G-CSF or chemomobilization in patients at high risk of failure or with previous mobilization failure, or as preemptive strategy of 0.24 mg/kg of body weight per day (up to a maximum of 40 mg/day), subcutaneous, in the night before the collection, six to 11 hours before the quantification of CD34+ cells and apheresis collection[1–3,9].

In cases of renal impairment, specifically for patients with a creatinine clearance ≤ 50 mL/min, the dose must be reduced by one-third to 0.16 mg/kg, up to 27 mg/day. Regarding treatment duration, plerixafor can be administered for up to four consecutive days[19].

Plerixafor is not currently approved for allogeneic donor mobilization[20]. Therefore, its administration must be restricted to clinical trials or conducted under specific authorization[21].

Target dose of CD34+ cells to be collected

The minimum and the optimal dose of CD34+ cells to be infused are described in Table 2. Mobilization with G-CSF only typically reach CD34+ cell peak between the fourth and sixth day of use[2]. For patients mobilized with chemotherapy plus G-CSF, CD34+ quantification usually begins eight to ten days after the end of chemotherapy administration, during the hematological recovery, when the leukocyte count is over 1,000 cells/μL[1,3,9]. Many centers start the leukapheresis when a CD34+ cell count of ≥ 10/μL is reached[22,23]. The American Society for Blood and Marrow Transplantation (ASBMT) and the European Society for Blood and Marrow Transplantation (EBMT) recommends a CD34+ cell count of ≥ 20/μL[1,2].

Hematopoietic progenitor cell collection: autologous and allogeneic

Large volume (LV) and very-large volume (VLV) apheresis (processing three or four, or five or more times the patient's blood volume, respectively, significantly increase CD34+ collection yield, improves final stem cell collection and must be considered for poor mobilizers (CD34+ count in peripheral blood < 10 to 20 μL)[3,5]. Heparin combined with ACD-A is frequently used in LV and VLV procedures. However, during these procedures, continuous electrolyte monitoring and appropriate replacement are essential to prevent citrate-related toxicity, including hypocalcemia, hypomagnesemia, and hypokalemia. Post-procedure laboratory evaluation is important to assess electrolyte levels, blood counts, and coagulation status, and to correct any electrolyte disturbances or fluid imbalance[16]. Supportive transfusion with red blood cells or platelet concentrates should be performed as clinically indicated before and after the apheresis procedure[5]. For allogeneic donations, a maximum of two consecutive sessions is recommended, and each session should not exceed five hours in duration[1].

For products intended for off-site transport or infusion within 48 hours, appropriate nucleated cells (NC) concentration must be ensured. Modern apheresis equipment typically yields highly concentrated products. Therefore, the device should be programmed to automatically add plasma (*e.g.*, a 1:1 dilution) to the hematopoietic progenitor cell collection bag, and to collect additional plasma (*e.g.*, 100 mL) for the cell processing laboratory to perform further dilution if needed. The optimal NC cell concentration is ≤ 300,000/µL for fresh infusion[7] and ≤ 200,000/µL for cryopreservation after 24 hours of the end of the collection[24].

Lymphocyte collection

Lymphocyte collection by apheresis for donor lymphocyte infusion (DLI) does not require mobilization with granulocyte colony-stimulating factor, as peripheral blood mononuclear cells are obtained through unstimulated apheresis procedures. While donor evaluation must follow standardized medical suitability criteria, serological and molecular screening for blood-borne communicable diseases, as legally mandated, must be performed within seven days prior to collection[12]. Specific guidelines addressing optimal practices for lymphocyte collection and DLI strategies in Brazilian centers have been currently developed by the Laboratory Practice Committee of the Brazilian Society of Bone Marrow Transplantation and Cellular Therapy (SBTMO).

BONE MARROW HARVEST

Apheresis is the preferred source for autologous and unrelated allogenic transplants. The bone marrow harvest is an alternative for donors who decline mobilization with G-CSF or lack adequate venous access. Allogeneic bone marrow transplantation results in lower incidence of GVHD and should be the first option in patients with severe aplastic anemia[8]. Bone marrow should not be the preferred source of hematopoietic progenitor cell when cryopreservation is required.

To minimize peripheral blood contamination, multiple punctures withdrawing 2–5 mL per aspiration are recommended[8]. The syringes should be washed with a heparin solution at each aspiration. The volume to be collected should respect the target of 10–15 mL/kg of recipient, not exceeding the volume of 20 mL/kg of donor[8]. The recommended cell dose for bone marrow collection is total nucleated cell (TNC) ≥ 3×10^8/kg of nucleated cell, and it is associated with a lower rate of graft failure[8]. Minimum cell dose recommended is TNC 2×10^8/kg[8]. In the setting of ABO major incompatibility between donor and recipient, bone marrow grafts require *ex-vivo* processing—red blood cell depletion—to mitigate the risk of hemolytic complications. These manipulation procedures, however, are associated with significant loss of nucleated and progenitor cells[8]. To offset these anticipated losses and ensure that the target cell dose is achieved after processing, it is advisable to collect an additional bone marrow volume—approximately 10% above the provisional goal volume—provided that the donor's weight-based maximum harvest limit of 20 mL/kg is not exceeded[25,26]. It is recommended to add 10% ACD-A to the products that will be transported or submitted to further processing[8].

HEMATOPOIETIC PROGENITOR CELL TRANSPORTATION AND INFUSION

The transport must take place in rigid, impact-resistant outer packaging sized appropriately for the volume or number of bags to be transported[12]. The temperature should ideally be maintained at refrigerated conditions (2–8°C), but an acceptable range of 2–24°C is recognized, provided that the temperature is cooling continuously toward the refrigerated range[12,27]. The transport process must be validated by each service. The interval between the end of the collection and start of infusion should not exceed 48 hours[12].

A standard transfusion set without leukocyte-reduction filter should be used for infusion, and the recommended rate is 6 mL/kg of receiver weight/hour for fresh products. One of the possible adverse reactions, especially in haploidentical transplantation, is cytokine release syndrome. It should be considered in the differential diagnosis with possible product bacterial contamination[6].

CONCLUSION

This document updates the last SBTMO consensus about this topic, published in 2021[6]. It contains recommendations based on the best scientific evidence available at the time of its publication. We hope it proves useful in the daily operations of a bone marrow transplant unit.

CONFLICTS OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable.

AUTHORS' CONTRIBUTIONS

Manuscript preparation: Prata KL, Kondo AT, Barroso KSN, Souza AM and Ribeiro AAF; **Manuscript writing:** Prata KL, Kondo AT, Barroso KSN, Souza AM and Ribeiro AAF; **Critical revision:** Prata KL, Kondo AT, Barroso KSN, Souza AM and Ribeiro AAF; **Final approval:** Prata KL.

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