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How do I perform hematopoietic progenitor cell selection?

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Abstract

Graft-versus-host disease remains the most important source of morbidity and mortality associated with allogeneic stem cell transplantation. The implementation of hematopoietic progenitor cell (HPC) selection is employed by some stem cell processing facilities to mitigate this complication. Current cell selection methods include reducing the number of unwanted T-cell (negative selection) and/or enriching CD34+ hematopoietic stem/progenitors (positive selection) using immunomagnetic beads subjected to magnetic fields within columns to separate out targeted cells. Unwanted side effects of cell selection as a result of T cell reduction are primary graft failure, increased infection rates, delayed immune reconstitution, possible disease relapse and post-transplant lymphoproliferative disease. The Miltenyi CliniMACS Cell Isolation System is the only device currently approved for clinical use by the FDA. It uses magnetic microbeads conjugated with a high affinity anti-CD34 monoclonal antibody capable of binding to HPCs in bone marrow, peripheral blood or umbilical cord blood products. The system results in significantly improved CD34+ cell recoveries (50–100%) and consistent three log CD3+ T cell reductions compared to previous generations of CD34+ cell selection procedures. In this article, the CliniMACS procedure is described in greater detail and the authors provide useful insight into modifications of the system. Successful implementation of cell selection procedures can have a significant positive clinical effect by greatly increasing the pool of donors for recipients requiring transplants. However, before a program implements cell selection techniques, it is important to consider the time and financial resources required to properly and safely perform these procedures.

Introduction

Although numerous improvements in allogeneic stem cell transplantation have occurred over the past several decades including high resolution human leukocyte antigen (HLA) typing technology[1,2], better patient selection methods and conditioning regimens[3,4], and enhanced supportive care measures[5,6], graft-versus-host disease (GVHD) remains the single most important source of morbidity and mortality associated with this procedure[7–9]. To address this difficult problem, laboratory methods have been established to eliminate or reduce the incidence and/or severity of GVHD while attempting to retain the beneficial graft-versus-tumor (GVT) effects that have been documented in allo-transplantation[7–9].

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Principles of cell selection

Hematopoietic progenitor cell (HPC) graft engineering currently consists of *ex-vivo* laboratory methods designed to reduce the number of unwanted T-cell (negative selection) and/or to enrich CD34+ hematopoietic stem/progenitors (positive selection) through the use of different immunological methods, the most successful of which includes immunomagnetic beads or super-paramagnetic microbeads conjugated with anti-CD34 antibody or anti-T-cell antibodies (e.g., CD2, CD3, CD4/CD8, T-cell receptor α/β) and separation of target cells in strong magnetic fields applied to separation columns[10–15]. T-cell reduction methods for allogeneic transplant grafts also subject recipients to higher risks of primary graft failure, increased infection rates, delayed immune reconstitution, possible disease relapse and post-transplant lymphoproliferative disease (PTLD)[16–19]. In order to reduce the incidence of graft rejection for selected products, some investigators have supplemented the HPC graft with a fixed dose of CD3+ T-cells[11]. Although the optimal dose of donor T-cells to administer is unknown, clinical evidence suggests that T-cell doses of 1–2×10E5 CD3+ cells/kg for unrelated, HLA matched products may be protective against primary graft failure[11].

Comparison of previously used cell selection methods

Early ex-vivo attempts to T-cell deplete bone marrow prior to administration used soybean agglutinin and sheep red blood cell rosetting (SBA/SRBC) performed on bone marrow grafts in pediatric patients with immunodeficiency syndromes[20,21]. Although the methods were basic they nevertheless resulted in approximately 2.0 logs of T-cell depletion of the cell graft. In adults, the use of partially T-cell depleted haploidentical bone marrow using anti-CD3 antibodies combined with intensive total body irradiation (TBI) achieved similar results[22]. With the establishment and availability of high quality monoclonal antibodies developed for characterization of T-cell subsets and hematopoietic (CD34+) progenitors, more effective positive and negative cell selection methods were established over the past two decades[23]. Different cell selection systems were developed such as the Ceprate SC immunoaffinity column (CellPro, Bothell, WA), the Isolex 300i magnetic cell selection system (Nexell/Baxter, Irvine, CA) and the CliniMACS CD34 reagent system (Miltenyi, Cambridge, MA), Dynabeads (Dynal/Invitrogen, Carlsbad, CA) for immuno-panning of target cells, and flow cytometric cell sorting techniques[24–27]. Although each method has benefits and drawbacks only the magnetic isolation methods became widely accepted for clinical use due to the more efficient T-cell reduction (depletion) achieved with these systems. The Cell Pro Ceprate SC and the Baxter Healthcare Isolex 300i were the first instruments to receive FDA approval for enrichment of CD34+ progenitors intended for transplantation but patent disputes forced Cell Pro out of business[24,27]. The Isolex 300i Magnetic Cell Selection System was FDA approved for processing autologous peripheral blood progenitor cell (PBPC) products to obtain a CD34+ cell-enriched population intended for hematopoietic reconstitution after myeloablative therapy in patients with CD34-negative tumors but it was quickly employed to perform CD34+ cell enrichment (passive T-cell reduction/depletion) of allogeneic HPC, apheresis grafts. The semi-automated system consisted of an anti-CD34 monoclonal antibody reagent (mouse anti-human CD34) and paramagnetic beads (Dynal/Invitrogen) with sheep anti-mouse IgG conjugates that were

incubated with HPC(A) and passed through a column surrounded by a strong magnetic field. Column-bound target CD34+ cells were washed free of unwanted (contaminating) cells and then released from the beads by chymopapain which non-enzymatically competed for the CD34+ antibody binding site on the targeted CD34+ cells[27]. The released cells were collected by flushing them out of the column with the magnetic beads remaining attached to the column. The system was functionally closed using sterile pathway tubing sets. The starting cell product and reagents were prepared and attached to the device; the process was then automated through CD34+ cell enrichment and harvest. The Isolex 300i cell selection system was used worldwide until Baxter withdrew support for the hematopoietic stem cell market.

Currently employed cell selection methods

The lone clinically approved device is the CliniMACS Cell Isolation System which employs a colloidal suspension of super-paramagnetic microbeads directly conjugated to a CD34 antibody (mouse anti-human monoclonal) capable of binding to HPC contained in bone marrow, peripheral blood and umbilical cord blood products[26]. The CliniMACS system is similar to the Isolex 300i system in that it uses an anti-CD34 monoclonal antibody chemically conjugated to dextran beads having an iron oxide/hydroxide core. Labeled cells are passed through a single-use sterile, disposable tubing set with proprietary cell separation columns. A sterile, isotonic phosphate-buffered saline solution (PBS/EDTA buffer) is used as external wash and transport fluid for the *ex-vivo* cell processing. When the paramagnetically tagged CD34+ cells pass through the column and strong magnetic field, they are retained in the column which results in a high level of CD34+ cell enrichment[26]. The anti-CD34 monoclonal antibody used for the target cell labeling step in the CliniMACS system is a more specific and higher affinity antibody which results in improved CD34+ cell recoveries as compared to Isolex 300i CD34+ cell recoveries. Also, the dextran microbeads conjugated to the anti-CD34 antibody do not require cleavage to remove them which also results in higher cell recovery for the CliniMACS instrument. It has been shown that the microbeads are ingested by the cells and stripped of their iron content which is then recycled intracellularly. An important step of HPC processing prior to labeling the CD34+ cells with antibody is a product wash step to remove platelets. The presence of platelets in the cell suspension during the antibody incubation procedure can result in excessive clumping or cell aggregate generation which can adversely affect the fluidics system (filter clogging etc.) and result in a poor target cell recovery and reduced purity. The CliniMACS device recently received Food and Drug Administration (FDA) Humanitarian Use Device (HUD) authorization[28]. The CliniMACS CD34 Reagent System indication is for processing hematopoietic progenitor cells collected by apheresis (HPC, Apheresis) from an allogeneic, HLA-identical, sibling donor to obtain a CD34+ cell-enriched population for hematopoietic reconstitution following a myeloablative preparative regiment without the need for additional graft-versus-host disease (GVHD) prophylaxis in patients with acute myeloid leukemia (AML) in first morphologic remission.

In addition to acceptable CD34+ cell recovery following selection, the CliniMACS system also achieves a final post-selection product with a consistent three (3) log reduction of CD3+ cells which is a more efficient and consistent T-cell reduction than that achieved with the

Isolex 300i system. Therefore, this technology was chosen as the standard for TCD of peripheral blood progenitors collected by apheresis, HPC(A) as well as for TCD of allogeneic bone marrow grafts. The following procedure (table 1) will describe the system in greater detail, discuss the benefits and shortcomings of the system and provide useful insight into modifications of the system that permit our Processing Facility to provide a safe, effective and cost-efficient product for allogeneic hematopoietic stem cell transplant.

Clinical Implications

Graft modification by means of immunomagnetic selection can greatly influence the clinical outcomes for the transplant recipient. HLA-mismatched HPC grafts are associated with an increased incidence and severity of GVHD[7–9] which can be mitigated by the removal of the effector immune cells (T-cells). Both positive selection (CD34 enrichment) and negative selection (CD3 or TCR alpha/beta T-cell reduction) methods[29,30] have been employed clinically to provide T-cell reduced HPC products as a form of GVHD prophylaxis. Early studies demonstrated that the reduction of T-cells in the HPC products resulted in markedly reduced GVHD but that the incidence of graft rejection, disease relapse, and prolonged impaired immune reconstitution were increased. More recently, in pre-clinical experiments, immunomagnetic depletion of CD45RA+ (naïve) and late effector T-cells demonstrated sustained interferon-γ secretion in response to cytomegalovirus, Epstein-Barr virus, Aspergillus, and Candida antigens, while effectively reducing CD8-mediated alloreactivity, suggesting a lower risk of inducing GVHD[31].

Another aspect to consider when pursuing this type of graft modification is in the acquisition and assessment of the starting product. For example, when performing CD34+ cell enrichment, CD34+ cell recoveries of 50–100% are observed which means that up to twice as many CD34+ cells may be required to achieve the desired cell dose since as little as half may be recovered. In order to surmount this challenge some collection centers have begun to employ plerixafor in conjunction with GCSF as a mobilization augmentation strategy[32,33]. The use of peripheral blood CD34+ cell counts the day before anticipated collection has been employed for autologous patient collections[34] and it is also being investigated for its utility for allogeneic donor collections intended for subsequent cell selection.

Conclusion

The successful implementation of cell selection procedures allows for a marked increase in possible donors for any given patient population by allowing safer haploidentical and other HLA-mismatched transplants. While cell selection procedures allow for great strides in expanding the pool of patients who have suitable donors, large investments in laboratory infrastructure must also be made. These procedures are highly resource intensive which include expensive reagents/disposables, multiple flow cytometry assays and the need for highly specialized staff training. Programs that wish to implement these advancements must carefully plan with both clinical and laboratory faculty and staff to determine the number of procedures a year to offer, contingency plans should the selected donor not sufficiently mobilize, and logistical considerations, such as access to rapid flow cytometry testing to name a few critical issues.

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Table 1

CD34+ Cell selection workflow and troubleshooting

Issue	Solution
Pre-processing HPC(A) Product Storage	Products are diluted with 2.5% human serum albumen buffer to a cell concentration of <200 × 10E6 mL whenever possible.
Platelet Washes	Perform two (2) platelet washes using the COBE 2991 cell washing device (Terumo BCT, Lakewood, CO) to remove the majority of platelets, prior to the antibody incubation phase. Excess platelet contamination causes product clumping at subsequent stages of processing which can interfere with successful cell selection and cell recovery.
Human IVIG	Add to the washed product at a concentration of 1.5mg/mL and incubate product five (5) minutes at room temperature on an orbital rotator. This step is intended to reduce non-specific binding of the target cell antibody during antibody incubation step. Follow manufacturer's instructions for antibody incubation.
Post-incubation Wash Step	Wash product once to remove any excess unbound antibody, by centrifugation using a refrigerated floor model centrifuge. (e.g. Sorvall RC3BP, 655 × g, 10 minutes, Thermo Fisher, Waltham, MA)
Product Resuspension & Examination	Resuspend product to the appropriate volume for loading on the machine. Examine product for signs of clumping. If clumping is observed, pass product through a platelet filter (Blood Component Recipient Set with Standard Blood Filter and Luer Adapter, 170 to 260 micron filter). We use as many filters as necessary. Load filtered product on the device following standard procedures.
Product at upper limit of total nucleated cells (TNC) for device tubing set (120×10E8 cells) and/or product is still clumpy after several rounds of filtering	Split the product and run over two large scale columns. Allows us to successfully avoid fluidic problems during the selection run and retain high levels of pure cell recovery.
Concentration of final CD34+ selected cell fraction	At completion of CD34+ selection procedure, transfer CD34+ cell fraction to four 50 mL conical centrifuge tubes. Spin tubes at 840 × g for 10 minutes at room temperature. Resuspend cell pellets and pool into one tube at a predetermined volume. Perform nucleated cell count manually using a hemacytometer. Samples are removed for other testing, following standard procedures.
Product Testing Performed:	
➢ Pre Selection Tests	**a.** Flow Cytometry (CD34, CD3 with subsets) **b.** 5 day sterility (Bactec bottles) **c.** Hematology (WBC, HCT, PLT, DIFF) **d.** ABO/Rh Confirmation
➢ Post Selection Tests	**a.** Flow Cytometry (CD34, CD3 with subsets) **b.** LAL endotoxin **c.** 14-day sterility **d.** Stat Gram Stain **e.** CFU