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Expansion and Homing of Umbilical Cord Blood Hematopoietic Stem and Progenitor Cells for Clinical Transplantation

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A B S T R A C T

The successful expansion of hematopoietic stem and progenitor cells (HSPCs) from umbilical cord blood (UCB) for transplantation could revolutionize clinical practice by improving transplantation-related outcomes and making available UCB units that have suboptimal cell doses for transplantation. New cytokine combinations appear able to promote HSPC growth with minimal differentiation into mature precursors and new agents, such as insulin-like growth factor–binding protein 2, are being used in clinical trials. Molecules that simulate the HSPC niche, such as Notch ligand, have also shown promise. Further improvements have been made with the use of mesenchymal stromal cells, which have made possible UCB expansion without a potentially deleterious prior CD34/CD133 cell selection step. Chemical molecules, such as copper chelators, nicotinamide, and aryl hydrocarbon antagonists, have shown excellent outcomes in clinical studies. The use of bioreactors could further add to HSPC studies in future. Drugs that could improve HSPC homing also appear to have potential in improving engraftment times in UCB transplantation. Technologies to expand HSPC from UCB and to enhance the homing of these cells appear to have attained the goal of accelerating hematopoietic recovery. Further discoveries and clinical studies are likely to make the goal of true HSPC expansion a reality for many applications in future.

INTRODUCTION

Since the first umbilical cord blood transplant (UCBT) was performed in 1988 to successfully treat a patient with Fanconi's anemia [1], over 2 decades of clinical and preclinical work have been established umbilical cord blood (UCB) as a Food and Drug Administration–approved source of hematopoietic stem and progenitor cells (HSPCs) [2]. To date, over 30,000 UCBT have been performed worldwide to treat various blood diseases and genetic disorders. Among all hematopoietic stem cell transplantations (HSCT) performed worldwide, approximately 10% of patients undergo UCBT annually, and this number is expected to plateau in the next few decades. Compared with use of bone marrow (BM) or mobilized peripheral blood stem cells (PBSC), the use of UCB for transplantation has several advantages, including greater ease of finding a donor and prompt availability (with over 700,000 units stored worldwide) [3,4]. More importantly, the greater tolerance across human leukocyte antigen (HLA) barriers and lower incidence of graft-versus-host-disease (GVHD) [5] makes UCB an ideal alternative for a significant number of HSCT patients (approximately 40% of Caucasians and up to 55% to 80% of non-Caucasians) who do not have access to a HLA-matched donor [6,7]. Furthermore, in several meta-analyses, UCBT has also been shown to lead to equivalent outcomes to fully matched BM transplantations in both adult and pediatric patients, thus serving as an effective donor source for allogeneic HSCT for patients without a matched sibling donor [8,9].

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Table 1
Clinical Strategies to Enhance Expansion and Homing of UCB Grafts

1. Ex vivo expansion
   - Hematopoietic cytokines in liquid culture
     - SCF, TPO, and Flt-3L is the most potent cocktail for HSPC maintenance, whereas cytokines such as IL-3, IL-6, IL-11, and G-CSF rapidly generate differentiated cells. Growth factors secreted by differentiated cells deter self-renewal capacity of HSPC.
     - Novel growth factors include IGFBP-2, Angptl proteins, and pleiotrophin.
   - MSC cocultures recreate the microenvironment of the BM
     - Allogeneic BM-derived MSC eliminate the need to perform stem cell selection and control vital functions of HSPC through direct cell-to-cell contact and proteomic secretion.
   - Chemical molecules and proteins that regulate signaling pathways of HSPC
     - Molecules, such as tetraethylenepentamine TEPA (copper chelator), NAM (sirtuin 1 inhibitor), SR1 (aryl hydrocarbon receptor antagonist), and immobilized delta-1 (Notch ligand).
   - Bioreactors and devices that allows continuous perfusion culture
     - Large-scale expansion that maintains an optimal concentration of the potent cytokines while minimizing inhibitory factors.

2. Homing
   - Inhibition of CD26/Dipeptidylpeptidase IV (DPP-4)
     - Interfere in the chemotaxis of $CD34^+$ human hematopoietic cells through DPP-4 enzymatic cleavage in SDF-1.
   - Complement fragment 3a (C3a) priming
     - Anaphylatoxin C3a receptor, which binds to C3a (modulates SDF-1 dependent chemotaxis of $CD34^+$ cells) to allow influx of calcium into the $CD34^+$ cells.
   - Dimethyl-prostaglandin E2
     - Regulates Wnt pathway via degradation of β-catenin and increases expression of CXCR4.
   - Fucosylation
     - UCB $CD34^+$ cells exhibit poor homing due to a defect in binding BM endothelial selectin as its ligand possess inadequate alpha1-3 fucose required to form glycan determinants, such as sialyl Lewis x (sLe^X).

3. Other clinical strategies
   - Choosing UCB unit that has higher TNC dosage and the optimal HLA match.
     - High TNC is the most critical factor in mediating a successful UCBT; however, it lowers chances of finding such units for patients who belong to racial minorities.
   - Improving methods of UCB collection along with minimizing post-thaw cell loss
     - Placental perfusion increases collected cells but requires specialized technical expertise to perform.
   - Increasing the number of infused TNC using either dUCB units or a single UCB unit along with haploidentical $CD34^+$ cells.
     - Dual UCB units or coinfusion of haploidentical $CD34^+$ cells enhances UCB engraftment but requires complex HLA matching with potentially increased incidence of GVHD.
   - Improved homing by targeted transplantation of UCB graft via intra-bone or intraosseous infusion
     - Compared with i.v. infusion, intraosseous infusion is an invasive procedure that causes site morbidity.
   - Coinfusion with accessory cells, such as allogeneic BM-MSC
     - Enhances homing and engraftment of the transplanted HSPC by creating a suitable microenvironment and alleviating symptoms of GVHD.
   - Infusion of mature immune cells derived from part or whole UCB unit
     - Overcomes the current limitation of DLI in UCBT, which could treat post-transplantation complications, such as relapse, infections, and GVHD.

TEPA indicates tetraethylenepentamine; DLI, donor lymphocyte infusion.

UCBT has a characteristically slower rate of hematopoietic recovery, relative to transplants of BM or PBSC, as a consequence of a lower absolute HSPC content [4]. Median time to neutrophil recovery is typically more than 25 days for unmanipulated UCB grafts versus a median of less than 20 and 24 days, respectively, for PBSC or BM grafts [10,11]. The profound delay in hematopoietic reconstitution increases risk of opportunistic microbial and viral infection in the pancytopenic recipients, thus contributing to the high transplantation-related mortality of >30% after UCBT [12]. However, the mortality risks appear to be lower with a higher infused cell dose for transplantation [13].

Current strategies for improving the clinical outcome of UCBT for adult patients, as outlined in Table 1, focus on increasing the effective cell dose of the graft in an effort to enhance hematopoietic cell engraftment. Although the simultaneous administration of 2 unmanipulated UCB grafts has demonstrated some possible improvement over single UCBT, the rate of hematopoietic recovery still remains suboptimal, with the reported median time to neutrophil and platelet repopulation ranging widely between 12 and 32 and 41 and 105 days, respectively [14]. In view of this, approaches for ex vivo expansion of a single UCB unit to achieve cell numbers for a successful adult transplantation have been widely sought after. In this review, we present emerging data from preclinical and clinical studies of UCB expansion with recently developed agents (including cytokine combinations, proteins, and chemicals) in combination with novel strategies, such as the use of mesenchymal stromal cell (MSC) coculture and bioreactors. Additionally, methods to enhance homing of UCB to facilitate hematopoietic recovery, as well as the crucial cellular pathways controlling HSPC survival and proliferation, will also be discussed. Although some preclinical data will be reviewed, including animal models using severe combined immunodeficiency (SCID) mice, this paper will focus mainly on strategies that have been, or are close to being, brought to clinical trials.

RECENT STRATEGIES TO EXPAND UCB GRAFTS

Cytokines

In the past 2 decades, various cytokines have been identified to play an important role in regulating HSPC survival, proliferation, and differentiation [15,16]. Although numerous studies have attempted to use these factors for HSPC expansion in vitro, the optimal combination and concentration are yet to be established, possibly because of the limited desired effects of these cytokines alone on the target population. Today, HSPC expansion cytokine cocktails vary among the various preclinical and clinical protocols but they generally include 3 key factors: stem cell factor (SCF), thrombopoietin (TPO), and Flt-3 ligand (Flt-3L) [17,18]. This cytokine combination has been shown to maintain hematopoietic cell viability [19] and telomere length coupled with elevated telomerase activity [20], as well as to regulate expression of adhesion molecules and enhancers of stem cell expansion [21,22]. The use of other cytokines, such as erythropoietin, granulocyte colony–stimulating factor (G-CSF), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 11 (IL-11), macrophage colony–stimulating factor, and platelet-derived growth factor, is more variable and with a tendency to promote cell differentiation [17,18,23]. For example, IL-3 supports rapid total cell expansion in vitro but the expanded cells possess reduced in vivo hematopoietic regenerative potential [24]. An important expansion study by Levac et al. showed that when the serum-free media were supplemented with the potent cocktail of SCF, TPO, and Flt-3L, withdrawal of several differentiation-promoting cytokines, such as IL-3, IL-6, and G-CSF from the expansion