Stem Cell Biology and Regenerative Medicine

Karen Ballen *Editor*

Umbilical Cord Blood Banking and Transplantation

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Umbilical Cord Blood Banking and Transplantation

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Preface

The first transplantation using umbilical cord blood (UCB) was performed in 1989; the transplant was performed with an international collaboration led by Dr. Eliane Gluckman and colleagues in Paris for a child with Fanconi anemia. Since then, over 30,000 umbilical cord blood transplantations (UCBT) have been performed worldwide, and over 600,000 UCB units have been donated for public use. The first UCBT were given to children, but with a better understanding of the importance of cell dose, UCBT were extended to adults. UCB is an important graft source for the seventy percent of patients who do not have a matched sibling donor, and especially crucial for racial/ethnic minority patients who have a difficult time finding a matched unrelated donor. UCBT outcomes have improved over the last twenty-five years, with exciting advances in cord blood expansion, homing, and double UCBT. However, several challenges remain including poor immune recovery, which contributes to a high rate of infections.

In this volume, we explore the regenerative potential of UCB, including applications in neurologic and cardiovascular disease. In the sections of UCB banking, the authors discuss quality control, the use of maternal human leukocyte antigen (HLA) typing, and methods to measure UCB potency. In the sections on pediatric UCBT, we review results in hematologic malignancies, non-malignant hematologic disorders, and metabolic storage diseases. Data on adult UCBT in Europe, Asia, the US and the Middle East are presented. An in-depth understanding of immune recovery after UCBT is essential to preventing and treating infection, and two chapters are devoted to this topic. Expansion of UCB, improvement of UCB homing, intra-marrow injection of UCB, and combinations of UCB with other graft sources are novel strategies to improve UCBT outcomes. Finally, we compare results from UCBT with outcome data from haploidentical, related and unrelated donor transplants, and we compare outcomes between single and double UCBT and explore selection of the optimal cord blood unit.

Much has been accomplished in the field of UCB banking and transplantation, and the next five years should be exciting ones indeed!

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Dr. Karen Ballen received her MD at Dartmouth College and then completed an Internal Medicine internship and residency at Beth Israel Deaconess Medical Center, Boston, and a Hematology/Oncology fellowship at Brigham and Women's Hospital, Boston. She has held several attending physician appointments, including Transplant Physician, Brigham and Women's Hospital and Children's Hospital, Boston, MA; Director, Bone Marrow Transplant Unit, UMass Memorial Health Care, Worcester, MA; and Acting Division Director, Medical Oncology, UMass Memorial Health Care. Dr. Ballen is currently a Full Professor in the Department of Medicine, Harvard Medical School, and Director of the Leukemia Program, Massachusetts General Hospital, Boston, MA. Her research interests are focused on novel therapies for leukemia and transplantation. A particular area of specialty is cord blood transplantation for those patients who do not have matched related or unrelated donors. Dr. Ballen's research has focused on improving outcomes after cord blood transplantation, and she has led multiple clinical trials focusing on double cord blood transplantation in adults, cord blood homing strategies, graft vs. host disease prophylaxis, and infection prophylaxis. She has authored more than 100 papers in this field.

Chapter 1
Applications of Umbilical Cord Blood-Derived Stem Cells in Vascular Medicine

Wouter Van't Hof and Mary J. Laughlin

1 Background: Cell Therapy in Vascular Medicine

Over the past three decades, intensive preclinical research has guided incremental progress in development of technology to promote neovascularization in vascular medicine. Extensive studies in mammalian embryology [51, 56] and vascular biology [8, 27] has provided new insights into cellular and molecular biology that drives microvascular angiogenesis and vasculogenesis in response to ischemia. This emerging understanding has defined the basis for novel therapeutic strategies in vascular medicine including cell-based therapies.

Umbilical cord blood (UCB) has emerged as a critical source of cells for hematology and regenerative medicine applications. UCB has many advantages over adult-derived cells including easy access and availability, a higher frequency of transplantable stem cells, and higher proliferative capacity. In addition, UCB hosts a variety of adult stem cell populations and progenitor lineages. As illustrated in Fig. 1.1, UCB contains vascular endothelial progenitors, mesenchymal stromal cells (MSC), unrestricted somatic stem cells (USSC), and very small embryonic stem cells (VSEL), suitable for clinical applications in vascular modulation or repair via angiogenesis or vasculogenesis.

Angiogenesis is defined as the sprouting of new capillary vessels from preexisting mature endothelial cells. This process includes proliferation of endothelial cells within existing blood vessels, and their migration into interstitial spaces in response to ischemia. Vasculogenesis is defined as neovascularization mediated by circulating marrow derived endothelial progenitor cells (EPC) in situ. Hypoxia has been identified and confirmed to be the driving mechanism underlying neovascularization to restore adequate oxygenation to ischemic tissues. Tissue hypoxia initiates a cascade of events including release of angiogenic cytokines (including but not limited to vascular endothelial growth factor, VEGF, nitric oxide, fibroblast growth factor, and platelet-derived growth factor, PDGF), proliferation of vascular endothelial cells, and recruitment/retention of EPC. This remains a complex process of neovascularization

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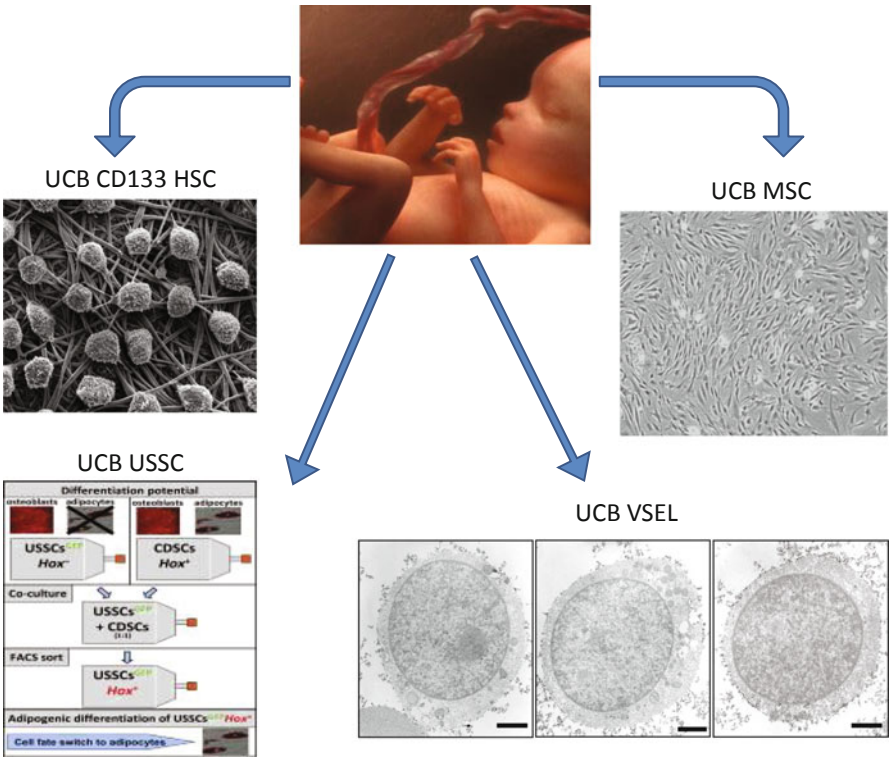

Fig. 1.1 Different subpopulations of umbilical cord blood-derived cells evaluated for clinical impact in vascular medicine. Umbilical cord blood (*UCB*) is a rich source for isolation of subpopulations of cells with properties attractive for cellular therapy in intervention with vascular disease. These UCB populations include CD133 positive hematopoietic progenitors (*UCB CD133*$^{+}$ *HSC*), containing vascular endothelial progenitor cells (*EPC*), mesenchymal stromal cells (*UCB MSC*), unrestricted somatic stem cells (*UCB USSC*), and very small embryonic stem cells (*UCB VSEL*).

which to date is incompletely understood and the subject of significant preclinical and clinical activity.

Human cell therapy clinical trials in vascular medicine have been initiated with an incorporation of the most current understandings of the role of vasculogenesis in neovascularization in the setting of tissue ischemia. Cell sourcing has included peripheral blood, bone marrow, adipose tissue, and UCB to augment microvascular angiogenesis in neurologic and cardiovascular clinical settings. The general tenet in the observations by many investigators is that paracrine factors secreted by transiently engrafted therapeutic cells mediate most, if not all, of the clinical benefits observed, providing a main rationale for use of allogeneic cell sources in vascular medicine applications [7, 47]. These paracrine effects appear to inhibit apoptosis of ischemic cells in situ and enhance neovascularization supporting tissue regeneration.

While the autologous approach of utilizing the patient's own cells has the advantage to avoid an immune rejection in an immune-competent host, it has also several disadvantages. Patients presenting with acute myocardial infarction or stroke may experience significant morbidity with attempted large volume marrow harvest or attempted cytokine-induced marrow stem cell mobilization. Moreover, a majority of patients with cardiovascular disease are of advanced age and increasing evidence points towards reduced potential and regenerative capacity of stem cells with increasing age [48, 83]. But most importantly, cytokines released during and immediately after myocardial ischemia or stroke are crucial for homing and integration of regenerative cells. Thus, the ready availability of an off-the-shelf cellular product at or shortly after the time of infarction would be ideal. However, this is not feasible in a routine and timely manner in an autologous setting, as it requires collection of the patient's bone marrow, stem cells purification, expansion, and testing prior to infusion back into the patient. Therefore, a vital protocol for generalized future cellular therapies is to develop off-the-shelf allogeneic cellular products from independent donors. UCB stem cells certainly facilitate this approach and evaluation of various UCB subpopulations in this context is reviewed below and relevant examples of preclinical evaluations of UCB-derived stem cells for treatment of vascular defects in a variety of neurological or cardiovascular diseases is summarized in Table 1.1.

2 Vascular Modulation by UCB Stem Cell Populations

2.1 UCB-Derived Prominin-1 ($CD133^+$) Hematopoietic Stem Cells

UCB is an abundant and rich source of stem cells, including $CD133^+$ EPC. Within this cell compartment, EPC positive for CD133 and hematopoietic progenitor cell antigen (CD34) are of particular interest for studies directed at therapeutic vasculogenesis [90, 6, 4]. Early studies by Peichev et al. [62] described this rare subpopulation of $CD133^+$ cells, comprising only 1–2 % of bone marrow-derived mononuclear cells. This population co-express CD34, endothelial-specific markers such as vascular endothelial growth factor receptor-2 (VEGFR-2/KDR), E-selectin, and vascular endothelial (VE)-cadherin, and common hematopoietic markers such as c-kit, aminopeptidase N (CD13), platelet endothelial cell adhesion molecule (PECAM), and chemokine receptor type 4 (CXCR4) which directs migration to stromal cell-derived factor 1 (SDF-1) and VEGF. CD133 is absent in mature endothelial cells, such as human umbilical vein endothelial cells (HUVEC). VEGFR-2-knockout mice embryos die without mature endothelial or hematopoietic cells [75]. These observations support the hypothesis that the $CD133^+$ cell pool includes the hemangioblast, the common precursor of hematopoietic and endothelial lineages. Early reports by Asahara and other groups indicated that these cells differentiate into endothelial cells after short-term culture [63]. Phase I/II clinical trials have supported use of $CD133^+$

Table 1.1 Summary of relevant preclinical studies using UCB-derived cells in neurological and cardiovascular disease

Indication	Disease model	Cell therapy product	Administration	Outcomes	Reference
Stroke	Transient middle cerebral artery occlusion in rats	Human UCB $CD133^{+}$EPC	IV, 10M cells, 24 h after occlusion	MRI at days 1, 7, and 14 after occlusion, of transplanted cell accumulation in stroke-affected hemispheres, significantly faster stroke volume reduction and higher magnitude of endogenous proliferation, angiogenesis, and neurogenesis in cell-treated animals	[34]
Spinal cord injury	Balloon catheter compression-induced spinal cord injury in dogs	Hu Ad MSC, BM MSC, WJ MSC, UCB MSC	Spinal cord injection, 6M MSC, 1 week post injury	8 weeks post injection locomotion improvements, smaller lesions, and increased numbers of surviving neuronal fibers, fewer microglia and reactive astrocytes in all groups. More nerve regeneration/ protection, inflammation reduction with UCB MSC	Ryu 2012 [71]
Amyotrophic lateral sclerosis	G93A SOD1 mice	Human UCB MNC	IV, 1–2.5M cells, weekly dosing	Mice receiving 1 million cells presymptomatically or 2.5 million cells symptomatically displayed significantly delayed functional deterioration, increased lifespan and higher motor neuron counts than control mice. Astrocytes and microglia significantly reduced in cell-treated groups	Garbuzova-Davis 2003 [26]
Alzheimer's disease	PSAPP mouse model of AD, at 7 months of age, Tg2576 AD mouse	Human UCB MNC	IV, 100K cells per mouse, 2x per month for 2 months, monthly for 4 months	Marked reduction in Abeta levels/beta-amyloid plaques and associated astrocytosis following multiple low-dose UCB infusions. Reduced cerebral vascular Abeta deposits in the Tg2576 AD mouse model, associated with suppression of the CD40-CD40 L interaction	[58]

Table 1.1 (continued)

Indication	Disease model	Cell therapy product	Administration	Outcomes	Reference
AMI	Porcine, ligation, circumflex coronary artery	UCB USSC	Left ventricle injection, 13M cells, directly after ligation	Transplantation of UCB MSC significantly improved LV function, prevented scar formation as well as LV dilation, 8 weeks post ligation	[28]
Hind limb ischemia	Right femoral artery ligation in NOD/SCID mice	Hu UCB $CD133^+$ EPC, BM $CD133^+$ EPC, BM MNC	IV (intracardiac), 0.5M cells, immediately after ligation	At 28 days, highest perfusion ratios seen in UCB $CD133^+$ EPC dosed animals, with statistically higher capillary density, reduced severe digit necrosis and increased engraftment in BM	[25]
Limb ischemia	Femoral artery ligation in athymic nude mice	Hu UCB MSC	IM, 1.3M cells, immediately after ligation	Hind limb salvage 60 % in animals treated with UCB MSC. Cells detected in the arterial walls of the ischemic hind limb in UCB MSC group	[40]

G93A glycine 93 changed to alanine, *SOD1* superoxide dismutase 1, *AD* Alzheimer's disease, *NOD/SCID* nonobese diabetic/severe combined immunodeficiency, *UCB* umbilical cord blood, *CD133+* Prominin-1, *EPC* endothelial progenitor cells, *MSC* mesenchymal stromal cells, *BM* autologous bone marrow, *USSC* unrestricted somatic stem cells, *Ad* adipose, *WJ* Wharton's Jelly, *PSAPP PS* mutation (M146L), *APP* mutation (Tg2576)

EPC in cardiovascular applications [77, 78]. Taken together, these data support the efficacy of $CD133^+$ cells in mediating vasculogenesis in response to ischemia, and lay the basis for current phase II human studies.

$CD133^+$ cells purified from hematopoietic tissues are not only enriched mostly in hematopoietic stem/progenitor cells but also contain some EPC and very small embryonic-like stem cells. Thus $CD133^+$ cells, which are akin to $CD34^+$ cells, are a potential source of stem cells with broader use in regenerative medicine. The lack of convincing donor-derived chimerism in the damaged organs of patients treated with these cells suggests that the improvement in function involves mechanisms other than a direct incorporation of the transplanted $CD133^+$ cells into the damaged tissues [44]. We and others hypothesized that $CD133^+$ cells secrete several paracrine factors that play a major role in the positive effects observed after treatment and tested supernatants derived from these cells for the presence of such factors. We observed that $CD133^+$ cells and $CD133^+$ cell-derived microvesicles (MVs) express mRNAs for several anti-apoptotic and pro-angiopoietic factors, including kit ligand, insulin growth factor-1 (IGF-1), VEGF, basic fibroblast growth factor (bFGF) , and interleukin 8 (IL8). These factors were also detected in $CD133^+$ cell-derived conditioned media (CM). More important, the $CD133^+$ cell-derived CM and MVs chemically attracted endothelial cells and displayed pro-angiopoietic activity both *in vitro* and *in vivo* assays. This observation should be taken into consideration when evaluating clinical outcomes from purified $CD133^+$ cell therapies in regenerative medicine [65].

To date, a number of vasculogenesis techniques such as gene therapy and use of growth factors are under evaluation in clinical trials. However, these interventions are limited by the age-related diminution in adults of the resident population of vascular endothelial cells competent to respond to the provided or induced angiogenic growth factors. Additionally, vascular endothelial cell function may limit the efficacy of patient-derived progenitor cells in mediating neovascularization [11, 32, 82]. This supports the concept that an exogenous source of EPC, rather than autologous patient-derived cells, may be optimal for cellular therapeutics intended to enhance vasculogenesis and collateralization around stenosed or occluded vessels to relieve ischemia. Human leukocyte antigen (HLA)-matched UCB-derived EPC offer distinct advantages as a cell source, including greater potential lifespan and reparative proliferation, relative to existing models of therapeutic angiogenesis derived from patient peripheral blood or marrow.

Our animal model studies [25] have shown that UCB derived $CD133^+$ EPC exhibit robust vasculogenic functionality compared with bone marrow-derived cells in response to ischemia (see Table 1.1). *In vivo*, neovascularization capacity has been directly correlated with SDF-1 and CXCR4 expression, suggesting that UCB-derived EPC may be more potent in neovascularization than autologous bone marrow (BM)-derived EPC [31, 89]. While our results showed the two sources to have equivalent potential, it is likely that engraftment and neovascularization of human cells in mice is suboptimal and that lack of cross-reactivity, or low cross-reactivity between crucial murine factors and the human cells may mask the full potential of the UCB EPC. Preclinical studies to date by this group and others demonstrated that augmentation

of endogenous microvascular collateralization in vascular injury rodent models is beyond that directly attributable to anatomic incorporation of infused human EPC into the murine vascular endothelium [52]. These observations point to paracrine effects and/or direct cell-cell interactions mediating vasculogenic activity elicited by the infused human EPC in response to inflammatory signals and cytokines released from the ischemic region [5]. Hypoxia is a known powerful inducer of VEGF and its receptors, as well as bFGF and angiopoietins, that in concert may contribute to vascularization via paracrine and autocrine signaling [81]. The therapeutic benefit of infused EPC may lie in augmentation of vasculogenesis via inflammatory signals elicited by stromal and hematopoietic cells present in situ rather than direct anatomic localization of injected cells.

2.2 UCB-derived MSC

MSCs isolated from UCB are similar to MSC derived from bone marrow as multipotential stem cells. They are capable of self-replication and differentiation along several pathways including adipogenic, osteogenic, and chondrogenic lines [29], although adipocyte differentiation may be more restricted in UCB MSC [68]. UCB-derived MSC, however, exhibit more robust proliferative capacity [39] compared to bone marrow or adipose tissue sourcing, and an enhanced ability to differentiate along neuronal pathways [20]. Preclinical studies of spinal cord injury comparing UCB versus adipose, marrow, or Wharton's jelly sourcing reveals that UCB-derived MSCs induced significantly more nerve regeneration and anti-inflammatory activity [70].

UCB MSC can be easily obtained from mononuclear cells using standard techniques and phenotype of these cells is similar to that of marrow-derived MSC [74]. MSCs have been proposed to exert trophic activity via secretion of factors [9] that provide immunosuppressive effects and to promote regeneration of injured tissue [37]. These attributes render MSC ideal for cell-based therapies. UCB MSC share potent immunosuppressive properties as seen for marrow-derived MSC. Allogeneic UCB MSC administration in humans is safe and demonstrates efficacy to relieve peripheral vascular ischemia [40]. In four patients with Buerger's disease (thromboangiitis obliterans), subcutaneous injection of one million UCB MSC proximal, adjacent to lesions resulted in loss of ischemic pain in affected extremities as soon as between 5 h and 12 days after transplant. Necrotic skin-lesion healing was observed within 4 weeks, together with increased number and size of digital capillaries in follow-up angiography and reduced vascular resistance in the affected extremities [40]. UCB MSCs can be induced *in vitro* to acquire angiogenic and vasculogenic properties and contribute to vascular growth in vivo [69], rendering these allogeneic cells ideal for further human studies [18, 85].

2.3 Unrestricted Somatic Stem Cells (USSC)

UCB derived USSC were first described by Kogler et al. [42] as a rare pluripotent CD45 and HLA class II-negative population that grew adherently and can be expanded to 10^{15} cells without loss of pluripotency. Under appropriate conditions, USSC can be differentiated into osteoblasts, chondroblasts, adipocytes, hematopoietic, and neural cells including astrocytes and neurons. In vivo differentiation of USSCs along mesodermal and endodermal pathways was demonstrated and no tumor formation was observed. USSC lack HLA class II and co-stimulatory molecules and similar to MSC are immunosuppressive in mixed lymphocyte cultures. Preclinical studies have confirmed the efficacy of human USSC to increase capillary density, improve left ventricular function, and prevent scar formation after acute myocardial ischemia [28, 35].

2.4 VSEL Cells

UCB has also been reported to contain a very rare population of pluripotent cells characterized by their small size (3–5 μm; slightly smaller than erythrocytes). These VSEL cells exhibit high ratios of nuclear to cytoplasmic volume and high presence of nuclear euchromatin, and display a phenotype pattern of $CD133^+$, $CD34^+$, $CD45^-Lin^-$, $CXCR4^+$ cells. VSEL display migratory responses to SDF-1, and express the embryonic transcription factors OCT4, SSEA-4, and Nanog at both the mRNA and protein level [45]. Based on these properties, VSEL have been proposed as putatively pluripotent stem cells of high potential interest for regenerative medicine [16]. They were observed to differentiate into cells from all three germ layers including cardiac and endothelial lineages. The absolute number of circulating VSELs in human peripheral blood is very low (1–2 cells/μl) and they mobilize into the peripheral blood of humans after acute myocardial infarction [65] and stroke [67]. In vivo preclinical models demonstrate VSEL capability to attenuate cardiac dysfunction and remodeling supporting clinical potential.

3 UCB Stem Cell Therapeutic Applications in Neurologic Vascular Medicine

3.1 Stroke

Stroke is the third leading cause of death and the most common cause of permanent disability in adults worldwide. Angiogenesis may contribute to recovery after stroke via remodeling of the damaged tissue and promoting neurogenesis [54]. Currently thrombolytic therapy with intravenous tissue plasminogen activator (tPA), although

effective, is given to less than 5 % of stroke patients due to its narrow therapeutic window (3–4.5 h) and risk of intracranial hemorrhage.

Stroke, hypoxia, and resulting neuronal death leads to proliferation of neural precursors in the subventricular zone, olfactory bulb, and hippocampus [43]. These anatomic areas termed "neurovascular niches" consist of neuroblasts, astrocytes, and neural stem cells located within a rich microvascular network [55]. The neuronal precursor responses however are in general not sufficient to fully restore tissue damage. The administration of UCB-derived therapeutic cells via systemic or local administration has produced functional recovery in animal stroke models [13, 21, 60, 72, 84, 87]. As UCB is a readily available "off-the-shelf" cellular therapy, these cells can be made available in a timely and effective manner to manage patients with acute stroke, and as such potentially alleviating current limitations in tPA- intervention protocols.

Further studies have indicated that stem/progenitor cells derived from human UCB improve vasculogenesis, neurogenesis, and functional recovery in murine and rat stroke models. UCB derived $CD133^+$ EPC have been demonstrated to improve angiogenesis and neurogenesis in a middle cerebral artery occlusion (MCAO) rat model. Animals were subjected to transient MCAO and 24 h later injected intravenously with 10 million UCB $CD133^+$ EPC. MRI performed at days 1, 7, and 14 after the insult showed accumulation of transplanted cells in stroke-affected hemispheres and revealed that stroke volume decreased at a significantly higher rate in cell-treated animals. Immunohistochemistry analysis of brain tissues detected the administered cells in the stroke-affected hemispheres only and indicated that these cells may have significantly affected the magnitude of endogenous proliferation, angiogenesis, and neurogenesis. The authors concluded that transplanted cells selectively migrated to the ischemic brain parenchyma, where they exerted a therapeutic effect on the extent of tissue damage, regeneration, and time course of stroke resolution [34]. These findings of efficacy of UCB-derived cells in the enhancement of microvascular vasculogenesis and amelioration of neurologic deficits after middle cerebral artery occlusion in vivo have been confirmed by other investigators [15]. Unlike current thrombolytic therapy that requires treatment within the first few hours after a stroke, UCB cell therapies are effective in these preclinical studies up to 48 h after the thrombotic event [57].

3.2 Neurodegenerative Diseases

In vitro studies of UCB-derived MSC demonstrate that these cells have a very high neurogenic differentiation potential compared with other sources of MSC [20]. In addition, purified UCB $CD133^+$ cells when exposed to retinoic acid differentiate into neuronal (astrocytes and oligodendrocytes) and glial cells that express neuronal markers including tubulin βIII, neuron specific enolase, neuronal nuclear antigen (NeuN), microtubule-associated protein-2, and the astrocyte-specific marker glial fibrillary acidic protein [36]. In animal models of amyotrophic lateral sclerosis (ALS), Alzheimer's and Parkinson's disease, observable behavioral improvement

has been observed in animals treated with UCB cells compared to control animals [12, 22, 23, 26, 58]. Because of the potential of UCB for procedural simplicity and robust, long-term benefits, this type of cellular therapy is expected to become a major adjuvant modality to current interventions for treatment of degenerative neurological disorders including Alzheimer's disease [41, 50]. Currently there are three human phase I/II studies ongoing, testing intracerebral or intravenous infusion of UCB MSC in patients with Alzheimer's-type dementia (NCT01297218, NCT01696591, NCT01547689).

Since the concept of "vascular niche" was advocated, "neuroangiogenesis," named for the overlapping mechanisms between neurogenesis and angiogenesis, has been intensively pursued in stem cell research [61]. Further published work has established that the vasculature and nervous system interact with each other by sharing similar signaling pathways implicated in cell differentiation, growth, and migration towards their targets [10]. Thus the use of novel drugs or growth factors, alone or in combination with stem cells delivered to a specific area, would be expected to have potential in halting and reversing neurodegeneration.

Therapy for Parkinson's disease during the past two decades has been rapidly advancing towards cell-therapy interventions. The first human embryonic (mesencephalic tissue) stem cell transplantation into the striatum of Parkinson's patients was performed in 1987 [2, 14, 17, 64, 88]. Since then, clinical trials performed on this chronic neurodegenerative disorder have provided evidence that the grafted cells can functionally integrate and induce symptomatic improvement [49, 53, 76]. These first observations are promising as there is no evidence to suggest that the disease process compromises the survival of the grafts [76]. Although stem-cell therapy holds considerable promise as a therapeutic regimen for Parkinson's disease, several technical issues need to be optimized including malignant potential of embryonic stem cells producing teratomas in study animals. Use of UCB as an alternative graft source may circumvent the logistical and ethical issues surrounding the use of fetal cells for stem cell therapy [79, 30]. Interestingly, UCB MSC have been demonstrated to inhibit glioblastoma multiforme proliferation, whereas adipose derived MSC cause a stimulatory effect [3], suggesting superior safety with use of UCB MSC in neuronal disease settings.

3.3 Spinal Cord Injury

UCB $CD34^+$ stem cell treatment in animal models of spinal cord injury is associated with recovery of hind limb function [46, 59, 71, 91]. Kamei et al. [38] compared *ex vivo*-expanded human cord blood-derived $CD133^+$ cells with freshly isolated $CD133^+$ cells as well as corresponding $CD133^-$ control mononuclear cells in respect to their ability to promote spinal cord repair using *in vitro* assays and cell transplantation into a mouse spinal cord injury model. *In vitro*, expanded cells as well as fresh $CD133^+$ cells formed EPC colonies, whereas $CD133^-$ cells formed no EPC colonies. In vivo, the administration of fresh $CD133^+$ and expanded cells enhanced angiogenesis, astrogliosis, axon growth, and functional recovery after injury.

In contrast, the administration of $CD133^{-}$ cells failed to promote axon growth and functional recovery, but moderately enhanced angiogenesis and astrogliosis. In addition, high-dose administration of expanded cells was highly effective in the induction of regenerative processes at the injured spinal cord.

4 Applications of UCB Stem Cells in Cardiovascular Medicine

Cardiovascular disease is a major cause of morbidity and death, causing 60 % of all deaths in the USA. There is a significant unmet need for therapeutic improvement for patients that are refractory to revascularization intervention, those who present with diffuse occlusive disease, and those who redevelop arterial occlusions after revascularization procedures. Early evidence linked the level of circulating marrow-derived EPC, characterized by expression of early hematopoietic stem cell markers CD133 and CD34, with the occurrence of cardiovascular events and death from cardiovascular causes [33, 73, 86]. The molecular and cellular mechanisms underlying marrow-derived EPC recruitment and differentiation within ischemic tissues are poorly defined. Previous theories suggested that vasculogenesis in the embryo was derived from endothelial progenitors, whereas angiogenesis in the adult resulted only from division of differentiated endothelial cells. However, current studies suggest that marrow-derived EPC are recruited to ischemic tissues and stimulate vasculogenesis in the adult.

Numerous preclinical studies have demonstrated efficacy of transplantation of EPC in restoring blood flow and improving cardiac function in animal models of ischemia (see examples in Table 1.1). The extent of engraftment is low and does not account for the magnitude of effect. This laboratory work has prompted phase I/II clinical trials utilizing individual patient-derived BM, culture-derived EPC, or whole BM uncultured mononuclear cells (MNC) infused or injected locally in attempts to augment vasculogenesis in response to ischemia. Many cell types have been tested and shown to increase the functional recovery of the heart after ischemia either by vasculogenesis and/or releasing proangiogenic and antiapoptotic factors in a paracrine manner that promote cardiomyocyte repair.

Clinical studies in humans have primarily used marrow-derived cells and recent meta-analyses [1] demonstrated that bone marrow cell transplantation is associated with physiologic and anatomic improvements beyond conventional therapy. Improvements include elevated left ventricular ejection fraction, reduced infarct size, and reduced left ventricular end-systolic volume. These observations support the concept that direct intracoronary injection of marrow-derived hematopoietic stem cells may be optimal for cellular therapeutics to enhance vasculogenesis and collateralization around blocked/narrowed vessels to relieve ischemia. However, cell therapy is in its early stages and several questions remain unanswered, including the optimal cell type, and timing and route of delivery. Clinical use of autologous marrow-derived cells is restricted due to significant logistic issues related to collection and processing of marrow-derived cells for each individual patient. Moreover,

the reduced population of resident vascular endothelial precursor cells in adults competent to respond to an available level of angiogenic growth factors limits the efficacy of autologous EPC. As discussed above, these logistics and biology of aging factors [24] as well as the findings that paracrine factors secreted by transiently engrafted cells mediate most of physiologic and anatomic benefits, has engendered enthusiasm for the use of allogeneic cell sources in cardiovascular medicine.

Crude mononuclear cell preps from adult bone marrow or mobilized peripheral blood have been isolated and expanded *ex vivo* in culture to generate EPC for clinical use in therapeutic angiogenesis. It is yet unknown upon administration which specific cell population within these heterogeneous cell cultures will home to sites of vascular injury and promote neovascularization. Therefore more recent clinical studies have focused on enriched stem cell infusions, including autologous bone marrow-purified $CD133^+$ injected either via intracoronary injection or via intramyocardial injection in patients with coronary artery ischemia. Investigators have hypothesized that $CD133^+$ EPC, rather than mature terminally differentiated endothelial cells, are the critical cell population. These cells home to sites of vascular injury in response to critical inflammatory and ischemic signals and mediate new vessel formation primarily via stromal cells and endothelial cells present in situ in the ischemic vascular bed [19]. Current studies are now focused on direct comparison of various cell types including MSC and EPC. Completion of these early stage studies is needed to confirm whether EPC may be optimal to enhance neovascularization and ventricular contractility [80].

5 Conclusions

UCB is an ideal source of therapeutic cells for clinical applications as the vast cryopreserved inventory allows HLA matching to minimize immune rejection. UCB cells are not tumorigenic and are easy to obtain and amplify. Early studies in vascular medicine have identified UCB stem cells to be effective in cellular therapeutic approaches. Preclinical and clinical evaluations support the main therapeutic hypothesis of benefit being dependent chiefly on trophic effects driven by specific soluble factors produced by UCB stem cells in response to the encountered pathology or microenvironments. Permanent engraftment does not appear to be a critical parameter for benefit, reducing the stringency for immunological matching of the UCB cells. Allogeneic use of UCB stem cells may overcome the age-dependent limitations in numbers or recruitment responses of endogenous stem cells in adults. These findings make UCB therapy for vascular indications fully compatible with existing UCB banking strategies. The next critical milestones on the development path are the outcomes of ongoing clinical Phase I and II studies that will define the most appropriate disease target and utility of UCB stem cells and progenitor populations.

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Chapter 2
Regenerative Potential of Cord Blood

Jessica M. Sun and Joanne Kurtzberg

1 Introduction

The field of regenerative medicine is dedicated to the study of repairing, replacing, or regenerating damaged human cells, tissues, or organs to restore or establish normal function [1]. This could be approached through numerous strategies, from stimulating endogenous processes to repairing damaged tissue to deriving or transplanting entire organs to replace those that are beyond repair. Though the field is currently in its infancy, regenerative medicine is predicted to be one of the most important disciplines in the next decade, with applications in a wide variety of conditions. Potential cells that could serve as source materials for regenerative medicine and cellular therapies include hematopoietic stem and progenitor cells derived from bone marrow (BM) or umbilical cord blood (CB), placental and amniotic fluid and tissues, mesenchymal stromal cells (MSCs), skin cells, and other organ-specific cells that could be engineered to perform reparative functions. This chapter will explore some of the potential regenerative applications for which CB could serve as a valuable source of cells.

2 Umbiblical Cord Blood (UCB) as a Source of Stem Cells for Regenerative Applications

Human CB is rich in highly proliferative stem and progenitor cells mobilized by placental signals promoting homing to developing organs [2, 3]. CB is readily available, can be collected noninvasively without risk to the mother or infant donor, and can be cryopreserved for several decades for future use. Compared to stem cells obtained from adult BM, CB stem cells are less mature and therefore have longer telomeres and greater proliferating potential [4]. They are also less immunogenic and less likely to transmit infections via latent viruses. In more than 25 years of use in allogeneic,

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unrelated hematopoietic stem cell transplant (HSCT), CB has not been shown to cause any teratomas or solid tumors. CB is often discarded as medical waste with the placenta after birth. Recently, induced pluripotent stem (iPS) cells have been isolated from CB with simpler methods and greater efficiency as compared to adult cell sources [5–7].

CB is a well-established source of stem cells for hematopoietic rescue after myeloablative HSCT. In addition, CB also contains nonhematopoietic stem cell populations with the ability to differentiate into numerous cell types throughout the body. In particular, the CB-derived unrestricted somatic stem cell (USSC) first described by Kogler et al. is a nonhematopoietic multipotent cell with the ability to differentiate into several lineages in vitro and in vivo [8]. USSCs can give rise to cell types from all three germinal layers, including osteoclasts [8], hepatocytes [9], and neurons [10], among others. CB-derived stem cells can also differentiate into MSCs [11], chondrocytes [12–14], osteocytes [13–18], adipocytes [14–17], neural cells [14, 15, 18–20], myocytes [21], hepatocytes [14, 15], pancreatic cells [22], cardiomyocytes, skin cells, and endothelial colony forming cells (ECFCs).

CB donor-derived tissue-specific cells have been identified in multiple organs in both animals and humans after HSCT, including the liver [19], lung, pancreas [19, 23], skeletal muscle [24], and brain [13]. In addition, transplanted lineage-negative human CB cells with high aldehyde dehydrogenase activity ($ALDH^{hi}Lin^{-}$) have been detected in several nonhematopoietic tissues in mice, including the liver, lung, kidney, heart, pancreas, cartilage, brain, and retina [19]. While CB cells have the ability to differentiate into tissue-specific cells and integrate into host organs, there is growing evidence that their therapeutic effects may stem more from their ability to initiate tissue repair by activating host cells via paracrine effects. Nonetheless, these observations indicate that transplanted CB cells are capable of repopulating more than just the hematopoietic system [25, 26]. This may be due to the presence of a true embryonic-like stem cell in CB or small numbers of committed tissue-specific, nonhematopoietic progenitors.

The pluripotential nature of CB, as well as the relative ease of collection, processing, testing, and storage, make it an attractive source of cells for regenerative medicine applications across many disciplines, including neurology, cardiology, orthopedics, endocrinology, and others. In this chapter, numerous preclinical, animal, and human studies evaluating the use of CB and CB-derived products across a wide range of clinical conditions will be reviewed.

3 Cord Blood (CB) Therapies in Neurological Diseases

Neurologic impairment can result from acquired injuries, genetic conditions, or neurodegenerative diseases of unclear etiology. Recovery from neurological injuries is typically incomplete and often results in significant and permanent disabilities. Currently, most available therapies are limited to supportive or palliative measures, aimed at managing the symptoms of the condition. Since restorative therapies targeting the underlying cause of most neurological diseases do not exist, cell therapies

targeting anti-inflammatory, neuroprotective, and regenerative potential hold great promise. CB cells can induce repair through mechanisms that involve trophic or cell-based paracrine effects or cellular integration and differentiation. Both may be operative in CB therapies for neurologic conditions, and there are numerous potential applications of CB-based regenerative therapies in neurological diseases, including genetic diseases of childhood, ischemic events such as stroke, and neurodegenerative diseases of adulthood.

Multiple in vitro studies have demonstrated that neurons, astrocytes, oligodendrocytes, and microglia can be derived from CB cells via gene transfection, ex vivo culture with growth factor supplementation, and/or the use of chemical agents [20, 27–34]. Neural differentiation has been documented in phenotypic and functional assays. The phenotype of the derived cells has been characterized by gene arrays [35] and the expression of standard neural-specific markers and proteins. Additionally, functional characteristics have been demonstrated through the presence of voltage- and ligand-gated ion channels with the ability to conduct electrical activity, indicating the development of functional characteristics of neurons [31].

The mechanisms of repair are expected to vary between indications, and several possibilities have been hypothesized [36]. Transplanted cells may deliver trophic factors that provide anti-inflammatory and neuroprotective effects and enhance the survival potential of host cells [37–40]. They may increase the plasticity of the injured brain by enhancing synaptogenesis, angiogenesis resulting in neovascularization, endogenous repair mechanisms, and migration and proliferation of endogenous neural stem cells [41–43]. Stem cells may also migrate, proliferate, and differentiate into "replacement" neuronal and glial cells and play a role in remyelination [44]. Additionally, many neurologic diseases involve activation of proapoptotic signal transduction, which could be harnessed to attract cells to brain lesions in those diseases. Thus, CB-derived cells could also potentially act as a vehicle to deliver neuroprotective and restorative factors in a targeted way toward damaged brain tissue.

3.1 Genetic Brain Diseases in Children

As discussed in greater detail in the chapter by Dr. Prasad, allogeneic transplantation of human CB in patients with certain genetic lysosomal and peroxisomal storage diseases is effective in preventing or ameliorating the associated neurological damage [45–48]. The engraftment of donor cells into a patient with an inherited metabolic disease provides a constant source of enzyme replacement, thereby slowing or halting the progression of disease. Patients with these diseases, ranging in age from newborns to young adults, transplanted early in the course of their disease derive extensive benefits from the transplant procedure, which both extends life for decades and greatly improves neurologic functioning [49–51]. Clinical and pathological observations from these patients provide additional support for the concept that CB cells can repair nonhematopoietic tissues.

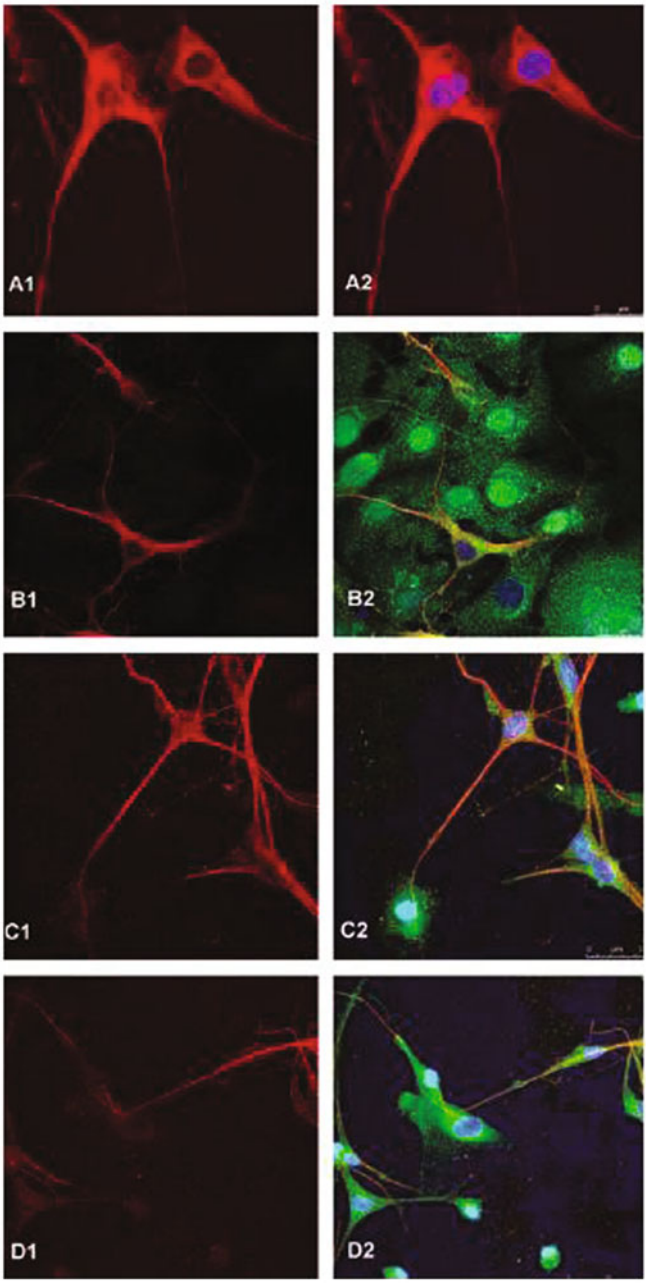

Fig. 2.1 In vitro functional assay of myelination of shiverer mouse neurons by cryopreserved cord-blood-derived oligodendrocyte-like cells (*O-cells*). Shiverer neurons cocultured with O-cells were co-stained for BT3 (*Texas Red*) and MBP (*fluorescein isothiocyanate*). Controls stained positive for BT3 (*panel A1*) but not MBP (*A2*). When cocultured with O-cells for 1 week, BT3 (*B1*) and MBP (*B2*) were expressed. Z-stacked projection after 3 weeks in culture demonstrated BT3 expression (*C1, D1*) and close association between BT3-expressing neuronal cells and MBP-expressing cells (*C2*), with MBP expression along axonal processes (*D2*)

Autopsy studies in humans who died after intravenously administered, sex-mismatched BM and CB transplant have confirmed the engraftment of donor cells throughout the brain months after transplantation [52–54]. Most engrafting cells were nonneuronal microglial cells, but donor-derived neurons, astrocytes, and oligodendrocytes have been identified. Globoid bodies, the pathological perivascular signature of Krabbe disease, were not detected in a patient transplanted for early infantile Krabbe disease at 3 weeks of age who died of unrelated causes at 5 years of age [54]. Based on these observations, our group hypothesized that CB contained cells capable of differentiating into oligodendrocyte-like ("O-cells") and microglial-like cells. We subsequently cultured and expanded O-cells from fresh and cryopreserved CB after 3–4 weeks in tissue culture supplemented with neurotrophic growth factors [20, 28]. These cells grow as an adherent population that, after 21 days in culture, express surface antigens found on oligodendrocytes (O1, O4, Proteolipid Protein (PLP), Myelin Basic Protein (MBP)) and microglia (CD45, CD116), make corresponding RNAs, and myelinate shiverer neuron axons in an in vitro potency assay (Fig. 2.1). They also constitutively produce IL-6 and IL-10 and retain the ability to produce lysosomal enzymes in culture after manufacturing. Intrathecal dosing in immunodeficient newborn mice showed the best distribution of O-cells in the central nervous system. A phase I trial administering these cells intrathecally 1 month after a standard HSCT from the same CB donor is planned. This trial is one example that the availability of well-characterized, screened, and HLA-typed CB coupled with its vast differentiation potential makes it an attractive source of stem cells for applications in tissue repair and regeneration, particularly in the central nervous system.

3.2 Ischemic Injuries

Observations using CB to treat children with genetic conditions led to the hypothesis that CB might also be beneficial in patients with brain injury. Accordingly, CB cells have been investigated in preclinical models of stroke, neonatal hypoxic-ischemic encephalopathy (HIE), traumatic brain injury, and spinal cord injury. These injuries are typically characterized by immediate damage to all neural cell types within the affected region. Therefore, therapeutic strategies might involve methods to promote cell survival and repair or regeneration of the affected areas, potentially via anti-inflammatory effects, neurogenesis, synaptogenesis, and/or angiogenesis after the injury has been sustained.

Numerous animal models have demonstrated both neurological and survival benefits of CB cells in the setting of stroke, ischemia, intracranial hemorrhage, and spinal cord injury [55–61]. Neuroprotection [55], neovascularization [43], and neuronal regeneration [62] have all been demonstrated in various models. For example, in HIE, a neonatal rat model has been developed by unilateral carotid artery ligation on day seven of life. Without intervention, these animals universally develop severe cerebral damage and contralateral spastic paresis. Meier and Jensen administered human CB mononuclear cells to these animals intraperitoneally 1 day after the hypoxic event, showing that the cells migrate to the area of brain damage and persist

for at least 2 weeks. Although the extent of morphologic injury on gross pathology was not altered, animals who received CB mononuclear cells did not develop spastic paresis, indicating functional recovery [57]. In a baby rabbit model of HIE [63], Tan demonstrated that labeled human CB cells reached the brain within 24 h, persisted for at least a week, and decreased the degree of brain damage on magnetic resonance imaging (MRI). In severely affected animals, CB administration improved gross motor function in a short-term functional assay [64]. Additionally, Ballabh and colleagues developed a rabbit model of intraventricular hemorrhage (IVH) by administering glycerol intraperitoneally to premature rabbit pups [65]. In this model, IVH is followed by the development of hydrocephalus and subsequent white matter demyelination. Intraventricular administration of human CB cells 24 and 72 h after glycerol failed to prevent the hydrocephalus, but did reduce subsequent demyelination (Ballabh, personal communication, 2014).

The therapeutic potential of intravenous infusions of autologous CB is currently being investigated in young children with cerebral palsy, HIE, and traumatic brain injury. In a safety study, we treated 184 infants and children with cerebral palsy (76 %), congenital hydrocephalus (12 %), and other brain injuries (12 %) with intravenous autologous CB infusions [66]. Patients were treated in the outpatient clinic through a peripheral IV after a single dose of Tylenol, Benadryl, and Solumedrol. Approximately 1.5 % of patients experienced hypersensitivity reactions (i.e., hives and/or wheezing) during the CB infusion that resolved after discontinuation of the infusion and outpatient medical management. With more than 3 years of follow-up, no additional adverse events have been reported, indicating that the procedure is safe. Parental reports of improved function were common, but it was difficult to know whether these improvements were directly related to the infusion of CB cells. Thus, a randomized, double blind, placebo-controlled study is in process to determine the efficacy of this approach. In this study, children ages 1–6 years are randomly assigned to the order in which they receive CB and placebo infusions, each given 1 year apart (Fig. 2.2). Motor, cognitive, and imaging studies are performed at baseline and 1 and 2 years to evaluate any differences between CB and placebo groups. The primary endpoint is improvement in motor function on standardized scales. A similar study of allogeneic CB and erythropoietin was conducted in Korean children with cerebral palsy [67]. They reported greater improvements in cognitive and select motor functions in children who received CB and erythropoietin versus controls. There was no CB-only group for comparison.

In the USA, CB is being investigated in clinical trials for children with cerebral palsy, neonatal hypoxic ischemic encephalopathy, stroke, traumatic brain injury, and autism (NCT01072370, NCT01700166, NCT01988584, NCT01251003, NCT01638819). Studies administering CB cells intravenously or intrathecally are being conducted in children with brain injury in other countries as well. Of note, intrathecal administration of allogeneic CB-derived cells, mostly MSCs, has been performed for a variety of neurologic conditions in a few small studies, primarily in China. In general, side effects are reported to be minor and transient, most commonly including fever, headache, and dizziness [68–71]. Efficacy cannot be determined at this point.

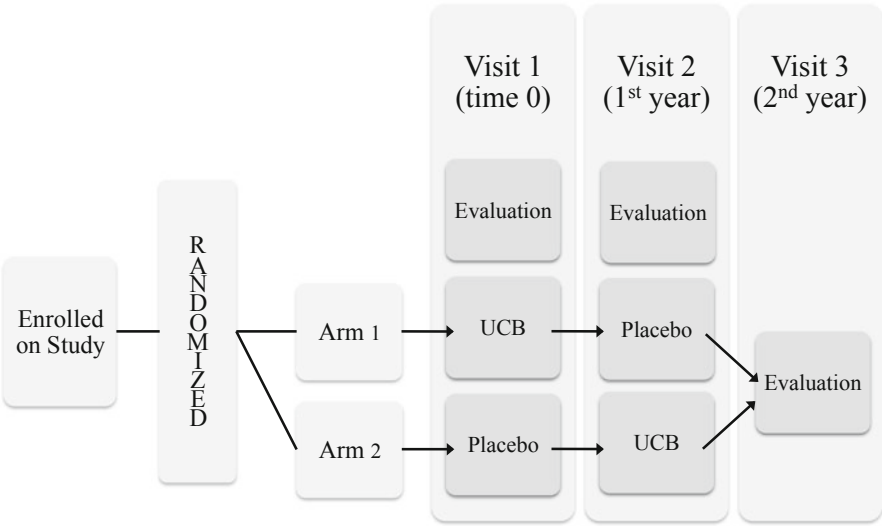

Fig. 2.2 Study design of a randomized, double blind, placebo-controlled trial of an intravenous infusion of autologous CB in children with cerebral palsy. Endpoints include changes in functional status (gross motor, fine motor, speech, cognition), quality of life, and neuroimaging (brain MRI with DTI, fibertracking, and connectivity analyses [139])

In phase I trial of newborns with hypoxic ischemic brain injury at birth conducted at Duke University, fresh, noncryopreserved autologous CB processed on Sepax 1 (Biosafe, Geneva) was infused in 1, 2, or 4 doses within the first 72 h of life in babies with moderate-to-severe encephalopathy qualifying for systemic hypothermia [72]. These babies were compared to a concomitant group of babies who were cooled at Duke but did not receive CB cells. Infusions were found to be safe in these critically ill babies, and babies receiving cells had increased survival rates to discharge (100 % vs. 85 %, $p = 0.20$) and improved function at 1 year of age (74 % vs. 41 % with development in the normal range, $p = 0.05$). A phase II randomized trial is currently in development. If this therapy improves the outcome of babies with significant birth trauma, there could be potential implications for the current model of CB collection and banking. In order to make this therapy available to all eligible babies, at a minimum all obstetric providers would need to be trained in CB collection, CB would need to be routinely collected at at-risk deliveries, and centers would need to either process and infuse the CB or transfer the babies and their CB to centers that have the ability to do so. Alternatively, CB collection could become a routine practice at every delivery. Units could then be stored for a limited time until it is clear if the baby will need it. If not, the autologous CB units could then be discarded, used for research or other regenerative applications, or, if appropriate consent and testing were obtained, transferred to the public registry.

Most human studies of stem cells in adults who have suffered a stroke have utilized autologous BM cells [73, 74]. Though no safety concerns have been identified, the studies are too small to reliably assess efficacy, and investigations of clinical

endpoints are currently underway. However, as the majority of adult stroke victims are elderly and critically ill following their injury, a CB-derived off-the-shelf therapy is an attractive alternative to autologous BM as it would avoid the need for a potentially risky BM harvest in these critically ill patients. In addition, these frequently elderly patients may also have decreased progenitor cells and other conditions that may limit the functionality of their own BM cells [75, 76]. A few clinical trials of CB cells given intravenously or intraparenchymally into the brain (NCT01700166, USA; NCT01884155, Korea; NCT01673932, China) are accruing patients at the present time but no results have been published to date.

3.3 Neurodegenerative Diseases

Cellular approaches to neurodegenerative diseases have been studied most extensively in Parkinson's disease, in which degeneration of primarily nigrostriatal dopaminergic neurons results in motor symptoms such as resting tremors, rigidity, and hypokenisia. Cell-replacement strategies have been attempted in over 300 patients with Parkinson's disease using intrastriatal implantation of fetal mesencephalic tissue. While several open-label trials suggested clinical benefit, two double-blind studies did not find a significant effect [77, 78]. However, some patients have demonstrated durable improvements including the ability to withdraw dopaminergic medication, and clinical benefit has been seen preferentially in less-disabled patients. Given the limitations of using human fetal tissue, interest has grown in generating dopaminergic neurons from other cell sources. Neurons that express dopamine-related genes and demonstrate the ability to synthesize and release dopamine have been successfully derived from CB stem cells in vitro [29, 30]. In hemiparkinsonian rats, unmodified human umbilical cord MSCs injected into the striatum can improve behavioral symptoms, and this effect is enhanced by adenovirus-mediated VEGF modification of the cells [79]. These studies indicate that CB has potential as a source of stem cells for cellular replacement strategies in Parkinson's disease.

CB cells have been evaluated in in vitro and in vivo models of Alzheimer's disease. Transgenic mice treated with CB-MSCs show a reduction in both microglial activation and beta-amyloid deposits, the pathologic signature of the disease [80]. CB-treated mice also demonstrate decreased cognitive impairment in functional assays [81] and an extended lifespan [82]. Although the mechanism is not entirely clear, it is possible that the CB cells mediate the microglial response to beta-amyloid deposits, promote beta-amyloid phagocytosis, and/or prevent apoptosis of host cells. Neurostem®, a CB-MSC product, has been investigated in a phase I trial in Korea, though results of that study have not yet been published.

3.4 Autism

Emerging data suggest that autism is caused by a complex interaction of genetic and environmental conditions, resulting in abnormal brain functioning early in life.

Recently, stem cell therapy has become appealing as a potential therapy to many within the autism community. In a mouse model of autism, intraventricular administration of human adipose-derived stem cells resulted in decreased repetitive movements and improved social activity [83]. Clinical trials of autologous BM and CB in children with autism are being conducted in the USA (NCT01638819), Mexico (NCT01740869), China (NCT01343511) [84], and India (NCT01974973, NCT01836562) [85]. Due to the heterogeneity in both etiology and symptomology of autism, identifying appropriate subjects and outcome measures remain particularly challenging.

4 Cord Blood (CB) Therapies in Cardiovascular Diseases

Regenerative medicine may have a role in multiple types of cardiac disease, including ischemic damage, heart failure, and even engineering replacement heart valves [86]. Research, primarily utilizing human BM-derived cells, has thus far focused on attempting to minimize the effects of myocardial infarction (MI), most commonly via intracoronary injection of stem cells. Numerous animal models have shown that BM-MSCs and/or MSCs can improve myocardial perfusion, reduce infarction scar size, and decrease left ventricular remodeling [87–89]. In human studies, over 2600 patients with acute or chronic ischemic heart disease have been safely treated with autologous BM cells. A meta-analysis of 50 such studies concluded that BM-MSCs not only improve left ventricular function, infarct size, and remodeling in patients with ischemic heart disease, but also result in an increased in rate of survival and decreased rates of recurrent MI and stent thrombosis [90]. For acute MIs, the therapy seems to be more efficacious when given early (i.e., within 7 days of MI), though benefits were still seen when cells were administered 7–30 days after the ischemic event. A dose-effect was also demonstrated, with $< 40 \times 10^6$ cells resulting in no improvement. A phase III randomized controlled study of autologous BM-MSCs after acute MI is planned (NCT01569178). An allogeneic BM-MSC product (Prochymal; Osiris) was also shown to be safe when delivered intravenously after acute MI, and is currently under investigation in a phase II study (NCT00877903).

CB stem cells could offer advantages over patient-derived stem cells, including longer lifespan and proliferation potential and avoidance of cytokine mobilization and harvesting procedures in patients who are inherently at increased cardiovascular risk. CB-derived cells have been successfully differentiated into cardiomyocytes in vitro [91], and human CB-derived cardiomyocytes have been demonstrated in the ventricles, septum, and Purkinje fiber system of preimmune fetal sheep after intrauterine transplantation [8] and in the myocardium of rats after intracoronary injection [92]. However, direct replacement of damaged myocardium by the differentiation and engraftment of CB or other hematopoietic cells is unlikely to be the main mechanism by which stem cells can aid in repair. In rodent and porcine models of acute and chronic MI, transplanted CB cells demonstrate improvement in cardiac function despite surviving at most 2 months [93–96]. This suggests that CB cells

can modulate the myocardial response to injury, thereby inducing preservation or regeneration of host myocardium and ultimately resulting in decreased scar formation and improved functional recovery. Potential mechanisms of action include the release of cytokines and growth factors that promote cytoprotection and neovascularization, activation of host cardiac stem cells to regenerate, and/or recruitment of stem cells from other tissues (i.e., BM) to differentiate into replacement cardiomyocytes. CB-MSCs are being investigated in clinical trials for dilated cardiomyopathy (NCT01739777, Chile) and ischemic disease (NCT01946048, China).

Regardless of the cell source, there are site-specific challenges for cellular regenerative techniques in cardiovascular diseases. The heart is subject to high blood flow velocity, so the majority of stem cells administered intravascularly can easily be swept away in the circulation before they are able to take hold in the desired location. Additionally, therapeutic cells may have decreased survival when delivered to an ischemic environment. Techniques to enhance cell homing, recruitment, and retention in the damaged area may improve the effectiveness of the delivered cells.

Stem cell therapies are also under investigation for critical limb ischemia, which occurs as a result of severe peripheral vascular disease. Asahara first demonstrated the existence of circulating hematopoietic lineage endothelial precursors (EPCs) that express the surface markers CD34, CD133, and Flk-1/KDR, can differentiate into mature endothelial cells in vitro, and contribute to vessel formation after transplantation [97]. Given their close relationship and numerous shared surface markers with hematopoietic stem cells, it is difficult to distinguish the two cell populations. In preclinical models, BM or CB cell administration has resulted in improved vascularization and symptoms [98–101]. Human studies have been conducted with BM-derived cells, showing improvement in symptoms and capillary density [102–105]. Intramuscular CB cell administration for limb ischemia has been reported in a few small case reports [106, 107] in which the therapy was well tolerated and some patients demonstrated improvement such as healing ulcers. However, the samples are too small to draw conclusions regarding efficacy, and a larger study is underway (NCT01019681, USA). Methods of administration have included intramuscular and intra-arterial injections, and the cell dose, number of doses, and reported duration of effect vary.

5 Cord Blood (CB) Therapies in Bone and Collagen Diseases

Musculoskeletal problems including bone defects and nonunions, avascular necrosis, and arthritidies are common, and they continue to increase along with the elderly population, the obesity epidemic, and orthopedic advances that allow for limb-sparing procedures. Autologous bone grafting is a standard treatment approach in multiple scenarios, but it requires harvesting bone from another location (often the posterior iliac crest) and suffers from decreased regenerative potential of the graft in elderly patients. Several bone substitutes, synthetic or natural, have been utilized, but they typically decrease the rate of osteogenesis and carry all the risks of implanted foreign bodies. Therefore, cellular therapies have elicited great interest for use in bone and cartilage regeneration.

While bone has an inherent capacity for effective healing, there are many situations in which this feature is impaired. Therefore, manipulation of the microenvironment is likely to play an essential role in promoting healing, with cellular supplementation being essential, but not sufficient for repair. Effective bone repair is likely to require: (1) a biocompatible scaffold that mimics the natural bone extracellular matrix niche, (2) osteogenic cells to produce the bone tissue matrix, (3) morphogenic signals that help to direct the cells to the desirable differentiation, and (4) sufficient vascularization to meet nutrient supply and clearance needs of the growing tissue.

BM cell preparations have been investigated to promote bone healing for over 20 years [108]. Several studies have demonstrated bone healing in nonunions after autologous BM-MSC grafting [109–111], though it has not yet become a routine practice. Osteocel® Plus is an off-the-shelf allograft cellular bone matrix product containing BM-MSCs and osteoprogenitor cells combined with demineralized bone matrix and cancellous bone. It has been utilized successfully in spinal [112–114], hindfoot, and ankle [115] fusion procedures and is commercially available.

CB-MSCs can differentiate into osteoblasts and chondroblasts [15, 18, 116], and a CB-derived product might be able to support bone regeneration by enhancing both angiogenesis and bone mineralization. In a rat models of localized critical bone defects, CB-USSCs on a collagen I/III and beta-tricalcium phosphate scaffold [117] and MSCs seeded on calcium phosphate cement have demonstrated bony reconstitution [118]. Improvement in bone formation in large bone defects has also been seen with CB-MSCs mixed with beta-tricalcium phosphate in a canine model [119]. At the UC-Davis Regenerative Medicine Laboratory in California, equine umbilical cord tissue is collected and banked, and BM-, adipose-, and CB-MSCs are being studied for bone regeneration purposes in racehorses. Cartistem®, an allogeneic-unrelated CB-derived MSC product, is currently being evaluated in a human phase II/III study for treatment of microfractures in patients with articular cartilage defect or injury (NCT01733186). In the future, one can envision tissue-engineering techniques combining certain scaffolds with particular cell types and cytokines tailored to the specific orthopedic condition.

Interestingly, after standard CB transplantation (or enzyme replacement therapy) in children with mucopolysaccharidoses, little effect is seen in the numerous bony and cartilaginous manifestations of the disease. The pathophysiology of skeletal deformities in the mucopolysaccharidoses is not well understood, and it is possible that the enzyme deficiency is not solely responsible for the widespread disruption in normal bone formation. Treatment of these disease manifestations is likely to require a different approach than that applied to localized, traumatic bone injuries.

Recently, stem cell therapy for the treatment of epidermolysis bullosa, a mucocutaneous blistering disease caused by a mutation in type VII collagen, has also been explored. In a mouse model of the disease, purified $CD150^{+}CD48^{-}$ cells from the BM of wild-type mice transplanted into newborn affected animals migrated to the site of skin lesions, produced collagen VII protein, prevented blister formation, and resulted in improved survival [120]. Wagner et al. subsequently transplanted six children with epidermolysis bullosa with allogeneic BM or CB and reported partial correction of the collagen VII deficiency and variably improved skin and mucosal

integrity [121]. Additional alternative therapies, including recombinant collagen injections and gene therapy, are also under investigation for this disease. However, these studies provide evidence that hematopoietic stem cells, including those from CB, may have a role in collagen-related diseases.

6 Cord Blood (CB) Therapies in Diabetes

In type 1 diabetes, insulin-producing pancreatic islet β-cells undergo T cell-mediated autoimmune destruction, resulting in lifelong insulin dependence. Therefore, in order for a therapy to successfully eliminate the need for exogenous insulin therapy and the numerous sequelae of long-standing diabetes, it would not only have to preserve or regenerate lost β-cells but also modulate the immune response to prevent destruction of the replacement β-cells. CB-derived cells may have a role in both of these strategies.

CB-derived pancreatic islet insulin-expressing cells have been identified in CB recipients at autopsy after sex-mismatched CB transplantation for unrelated diseases [23]. In contrast, donor-derived islet cells were not detected in recipients of sex-mismatched BM transplantation [122]. The production of β-cells capable of producing insulin in vitro from CB-MSCs has been well documented and reproducible [22, 123]. However, their ability to respond appropriately to varied glucose levels is debatable. Some animal models have shown improved blood glucose levels and survival after administration of CB-MSCs or CB-derived insulin producing cells [124, 125]. Haller et al. performed autologous CB infusion in 24 children with type 1 diabetes aged 3–6 years, demonstrating that it is safe in young children [126]. Median time from diagnosis of diabetes to CB infusion was 3 months, and median TNCC infused was 1.88×10^7 cells/kg. Despite autologous CB infusion alone [127] or in conjunction with supplementary vitamin D and docosahexaenoic acid (DHA) [128], β-cell function continued to decline as evidenced by decreasing C-peptide levels (reflects endogenous insulin production) and increasing insulin requirements. However, an increase in Tregs was observed at 6 months post infusion, suggesting a potential immunomodulatory effect.

7 Genetically Modified Cord Bood (CB) Cells

Induced pluripotent stem cells (iPSCs) have been created from a variety of human cell sources via viral, plasmid, or recombinant protein reprogramming to a pluripotential embryonic-like state. These cells could potentially be derived from the patient in need of them, thereby avoiding a host immune response by using an autologous cell source. However, it is unclear if patient or disease-specific aged iPSCs are the ideal source of cells, as they may contain mutations in their genomes that could cause a later malignancy. Therefore, the ability to create iPSCs from younger HLA-matched cells is an attractive alternative. iPSCs have been isolated from CB with simpler

methods and greater efficiency as compared to adult cell sources [5–7]. Nonetheless, there is much work to be done to eliminate oncongenic factors, increase efficiency, and develop methods to produce the number of cells that are likely to be required for clinical applications.

Additionally, CB cells could be used as a source of hematopoietic stem cells for gene therapy techniques, particularly for genetic blood cell diseases. Initial efforts at autologous HSCT using genetically modified stem cells were attempted for primary immune deficiencies without any chemotherapy conditioning, including adenoside deaminase-deficient severe combined immunodeficiency (ADA-SCID) [129–131]. This approach led to transient, low-level gene correction and no clinical benefit. However, improvements in gene transduction techniques and the addition of non-myeloablative conditioning with busulfan have resulted in durable engraftment of gene-corrected cells [132–135]. There have been no reports of cancer or lympho-proliferative disease in gene therapy-treated patients with ADA-SCID, in contrast to those treated for other immunodeficiencies such as X-linked SCID and Wiskott-Aldrich syndrome [136–138]. As work continues to identify less oncogenic vectors, an alternative approach of gene correction is also under investigation. In this method, cells are supplied with donor DNA complimentary to the necessary site that contains the missing sequence. An agent is used to create a double-strand DNA break close to the target site, and then the donor DNA sequence is inserted at the break site as the cell repairs the break with its endogenous repair mechanisms. This allows the inserted genetic sequence to reside at the intended site and thereby hopefully be regulated as intended. CB cells could be useful as a vehicle for such gene therapy techniques as they continue to be refined.

8 Summary

Regenerative medicine is a field with enormous potential to impact the treatment of diseases that affect millions of people and conditions for which there are currently no effective therapies. CB is an attractive source of multipotent stem cells for these applications. In addition to its ability to differentiate into numerous cell types, one of the main advantages of CB compared to other cell sources is its practicality. CB can be easily collected after most births without risk to the donor, can be cryopreserved for later use, and is not a socially or politically controversial resource. In order to capitalize on these features and to make regenerative therapies available to the population at large, allogeneic products that can be utilized without the need for high-dose chemotherapy or long-term engraftment will be necessary.

If CB develops a significant role in regenerative medicine as expected, it could not only affect the way numerous diseases are treated but could also promote a shift in the current paradigm of public and private CB banking. CB collection might become the standard of care for all deliveries, provided by hospitals and obstetric providers. Parents may become more inclined to bank their baby's CB privately. Public banks might therefore need to develop mechanisms to reserve stored units for the donor

family for a few years, and then list them on the public registry if the family did not require their use. Smaller units, not typically stored by public banks and less likely to be sufficient for standard allogeneic HSCT, might also have to be banked for a number of years and may be useful for regenerative medicine applications.

Cellular therapies are much more challenging to fully characterize, develop, and refine than traditional pharmaceuticals. Current FDA regulations for drug development and manufacturing are not directly applicable to or relevant for cellular products. Many questions remain unknown, including ideal cell source, route of administration, dose and dosing regimen, timing, and role of immunosuppression in these therapies. Risks of adverse events differ for the use of unmanipulated or manipulated products as well as autologous versus allogeneic cell sources. The field of regenerative medicine has exploded in recent years and considerable progress has been made at the preclinical level. Several early proof-of-concept studies are underway but no studies have progressed to the level of a phase II/III clinical trial. Thus, there is still much to be learned and tested before regenerative medicine approaches become routine therapies in the clinic.

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Chapter 3
Quality Control in Cord Blood Banking

Monica B. Pagano and N. Rebecca Haley

1 Regulations

1.1 Historical Overview

In 1995, the FDA published the first document concerning the regulation of placental/umbilical cord blood stem cell products intended for transplantation or further manufacture into injectable products [1]. In subsequent years, the FDA proposed a tiered approached to cell and tissue regulation, focusing on five areas: (1) preventing the use of contaminated units; (2) preventing the improper handling of products; (3) ensuring clinical safety and effectiveness; (4) labeling accurately; and (5) monitoring and communicating with the industry through registering and listing products with the FDA [2]. For these purposes, three Final Rules were proposed:

The First proposed rule was the Registration Rule: 21 CFR Part 1271 Subparts A and B, "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment, Registration and Listing." The final rule was published in 2001.

The Second proposed rule was the Donor Eligibility Rule: 21 CFR Part 1271 Subparts C, "Suitability Determination for Donors of Human Cellular and Tissue-Based products [3]." This rule involves donor testing. The final rule was published in 2004, and became final May 25, 2005 [4]. Regulations regarding screening and testing HCT/P donors and labeling were reviewed and finally approved in 2007 [5].

The Third proposed rule was the cGTP rule: 21 CFR Part 1271 Subparts D, "Current Good Tissue Practice for Manufacturers of Human Cellular and Tissue-Based Products: Inspection and Enforcement [6]." The final rule was published in 2004, and became final in May 25, 2005 [7].

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1.2 Regulations of Unrelated HPC Cord and HPC Apheresis

In 2006, the FDA's Center for Biologics Evaluation and Research (CBER) made a series of recommendations to manufacturers, suggesting that they apply for a biologic license (BLA) under Title 21 CFR Part 601 for placental/umbilical cord blood products that are minimally manipulated, unrelated and intended for use in hematopoietic and immune reconstitution in patients with abnormal hematopoietic systems. Products that are more than minimally manipulated, as in the case of units that undergo ex vivo expansion, or are used for a different indication including regenerative medicine, require an Investigational New Drug (IND) application [8].

HPC, Cord Blood intended for unrelated allogeneic use is considered a drug under the Federal Food, Drug and Cosmetic (DF&C) Act, and applicable regulations include 21 CFR Parts 201 (labeling), 202 (prescription drug advertising) and 210-211 (Current Good Manufacturing Practice Regulations—cGMP). cGMP apply to the methods, control and facilities to manufacture HPC, Cord Blood. These requirements are intended to prevent the introduction, transmission or spread of communicable diseases. Additionally, HPC, Cord Blood is considered a biologic product under the Public Health Service (PHS) Act, and applicable regulations include 21 CFR Parts 600 (biological products, general) and 610 (biological products standards).

Lastly, HPC, Cord Blood is considered Human Cells, Tissues and Cellular- and Tissue-based Products (HCT/Ps), with regulations contained in 21 CFR 1271 - Subpart C, Donor eligibility and Subpart D, Current Good Tissue Practice (cGTP). Overall, cGTP regulations are more general and apply to several tissues, whereas cGMP regulations are more specific to prepared pharmaceutical products and include allogeneic HPC, Cord Blood.

For HPC, Cord Blood units intended for related allogeneic (first- or second-degree relative) transplants, all subparts of rule 21 CFR 1271 apply, and when intended for autologous use, the same rules apply, with the exception of Subpart C (donor eligibility), which is optional.

1.3 Standards for Cellular Therapy Services, AABB

The Cellular Therapy Standards Program Unit (CT SPU), as part of the Standards Program Committee from the AABB, published the sixth edition of the *Standards for Cellular Therapy*. These standards are meant to provide guidance in developing policies, processes and procedures for safe collection, storage and administration of cellular therapy components. The requirements contained in the standards must be met in order to obtain AABB accreditation.

1.4 Standards for Cord Blood, FACT

NetCord and the Foundation for the Accreditation of Cellular Therapy (FACT) have recently published the fifth edition of the *International Standards for Cord Blood*

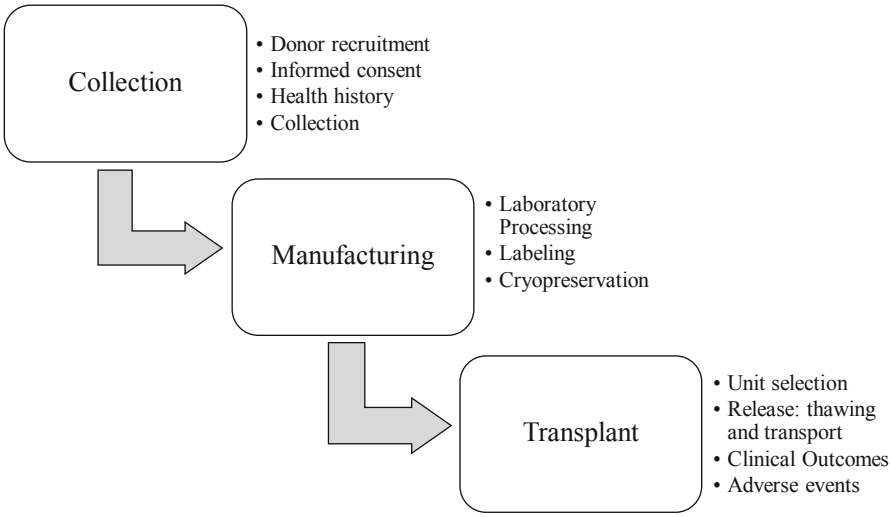

Fig. 3.1 HPC, Cord Blood processing steps. Precise steps are required in each of these areas to assure safety, purity, potency and identity.

Collection, Banking, and Release for Administration. NetCord is the international cord blood banking branch of EuroCord, an international registry for the European Group for Blood and Marrow Transplantation (EBMT). FACT was founded by the American Society for Blood and Marrow Transplantation (ASBMT) and the International Society for Cellular Therapy (ISCT). The objective of the *Standards* is "to promote quality medical and laboratory practices throughout all phases of cord blood collection, banking, and release for administration to achieve consistent production of quality placental and umbilical cord blood units for administration."

2 HPC, Cord Blood Unit General Requirements for Unrelated, Allogeneic Collections

Cell therapy laboratories should develop standard operating procedures (SOPs) with a detailed description of the manufacturing steps as outlined in Fig. 3.1 [8]. The laboratory should also define the specifications that must be met for an unrelated, allogeneic HPC, Cord Blood unit to be acceptable for clinical use. These specifications include:

- Collection requirements

The donor mother's consent must be obtained before the HPC, Cord Blood collection. Only the baby's mother need sign the consent, but it is wise to have a policy addressing objections by other family members. The collection must be performed under agreement with a qualified healthcare facility if the collection is

done in that facility, by trained personnel using a qualified collection supplies. In order to achieve a component with an acceptable cellular dose, the volume of blood collected must be at least 50 mL, but smaller units ($\geq$ 40 mL) may be accepted. The larger the cell count of the collected unit, the more likely it will be chosen for a cell source for transplantation. Due to the low distribution rate of lower cell count units (below 10×10^8 total nucleated cells) many public cord blood banks do not incur the expense of processing the smaller units.

- Maternal sample requirements

The maternal sample used for infectious disease testing must be correctly labeled with two unique identifiers; must be collected within seven days of HPC, Cord Blood collection; and must be tested within the time frame specified by the qualified test method used.

- Delivery and health history

The baby must be free from observable congenital abnormalities and malformations, including metabolic disorders, chromosomal abnormalities and structural anomalies. The unit should be collected from normal deliveries where the infant is normal, in good health, and there is no evidence of infection in mother or baby. Additionally, the unit cannot be collected in cases of multiple births or when from pre -term deliveries ($\leq$ 37 weeks) [9].

2.1 HPC, Cord Blood Bag Requirements

A qualified, approved bag must be used and cannot be damaged. It must be labeled to include the type of product (HPC, Cord Blood) and two donor identifiers (mother's full name, plus mother's date of birth or medical record number).

- Packaging requirements

After the unit has been collected, it must be packaged and transported to the manufacturing facility. The transportation method from the collection area or facility to the cord blood laboratory must be controlled and validated according to the bank's specifications.

3 Quality-Control Program

The quality-control program (QCP) evaluates a process in progress. It must oversee the validation, implementation and monitoring of processes to ensure each product's adequate performance; it also investigates errors and suggests corrective actions. The Guidance for Industry specifies the QCP responsibilities, including:

- Approve or reject the in-process materials, packaging, labeling and CBU.
- Review records to ensure that no errors have occurred or to investigate any errors.

- Approve or reject procedures that affect the identity, strength, quality and purity of the HPC Cord Blood.
- Review and approve written procedures and process control, as well as any changes to these procedures.
- Review and approve laboratory control mechanisms, including specification, standards, sampling plans, test procedures and any changes in them.
- Review and approve UCB production and control records to determine compliance.
- Conduct an internal quality audit for management review.

3.1 Process Verification and Validation

Process validation is defined as establishing documented evidence that a specific process will consistently result in a product meeting its pre-determined specifications and quality attributes. Process verification is defined as confirmation by objective data that specified requirements have been met. The FDA provides specific regulatory requirements for process verification and validation in 21 CFR sections 1271.220(c), 1271.230 (d) (2), 211.110(a). Specifically, all the manufacturing steps must be performed in a way that does not cause contamination or cross-contamination and prevents the introduction, transmission and spread of communicable disease. Pre-cryopreservation processing usually involves volume reduction by depletion of red blood cells and plasma, followed by a validated controlled-rate freezing that ensures the preservation of viability and potency and allows the acceptable recovery of cells. The cryopreservation and thawing processes must be validated to preserve potency and maintain at least 70 % viability. In order to process cord blood within 48 hours of collection; time limits must be established for each phase of production; and the time between freezing and storage must be minimized. Regulations prohibit pooling units during manufacturing. Processes need to be verified by inspections and testing, or should be validated and approved according to established procedures. Validation protocols must include the evaluation of accuracy and specificity, precision (repeatability), linearity and range, system suitability and robustness.

3.2 Laboratory Controls

The Guidance for Industry for the Biologics license application presents information about the tests to be performed and the expected results in order to provide information about the safety, purity, potency and identity of the HPC, Cord Blood.

3.2.1 Safety: Infectious Disease, Sterility and Hemoglobin Testing

Infectious disease testing for relevant communicable diseases, as defined by CFR 21 1271, must be performed using a peripheral blood sample from the mother within

7 days of collection. Relevant communicable diseases include Hepatitis B (HBV), Hepatitis C (HBV), Human Immunodeficiency Virus (HIV), Creutzfeldt-Jakob disease (CJD), *Treponema pallidum*, Human T-lymphotropic virus, type I (HTLV-1) and Human T-lymphotropic virus, type II (HTLV II). Units must also be tested for cytomegalovirus (CMV) and labeled with the corresponding result, but a positive result does not disqualify the unit for transplantation. CMV is not a relevant communicable disease according to regulations, but may be important in planning treatment of the transplant recipient. Microbiologic testing able to detect bacteria and relevant fungi should be performed on a sample of the HPC, Cord Blood unit before cryopreservation, and culture results must be negative to release a unit for an unrelated, allogeneic transplantation.

All tests must be performed using appropriate FDA-licensed, -approved or -cleared donor screening tests. Relevant communicable disease test results must be negative, with the exception of non-treponemal tests for syphilis. A positive syphilis screening test must be followed by a negative confirmatory test to accept an HPC, Cord Blood unit for transplantation. Additionally, donors will be rejected when there is a concern for donor plasma dilution that may affect the results of communicable disease testing. Clinical situations in which donor plasma dilution is suspected include the infusion of more than 2 L of blood or colloids within 48 hours before the specimen collection, or of 2 L of crystalloids within 1 h of the specimen collection.

Directed donor units with incomplete testing or positive test results may be released by approval of the Medical Director, the requesting physician and the recipient or his/her guardian. These units must be labeled with a biohazard legend indicating the communicable disease risks.

A hemoglobin screening test on a sample of the HPC, Cord Blood unit must be performed to determine the donor's hemoglobin, to test for thalassemia and sickle cell anemia.

3.2.2 Potency: Total and Viable nucleated cells. Viable CD34 Positive Cells

A well-known factor that restricts the use of HPC, Cord Blood for transplant in adults is the limited number of cells on a single HPC, Cord Blood. Currently, unit selection for transplantation is based on the dosing of total nucleated cells (TNC) and the degree of Human Leukocyte Antigen (HLA) match. Quite often, clinicians face the challenge of having to choose between a better cell dose and a better degree of HLA match. Several clinical studies have evaluated transplant clinical outcomes using potency measures of $CD34^+$ cell dose, colony forming units assays (CFU)] and degree of HLA match, as outlined in other chapters.

The Guidance for Industry for the Biologics license application establishes that the minimum number of nucleated cells in the HPC, Cord Blood unit will be at least 5×10^8 total nucleated cells; at least 85 % of the nucleated cells will be viable; and a minimum of 1.25×10^5 $CD34^+$ cells are present in the HPC, Cord Blood unit after processing [8].

Different methods exist to determine $CD34^+$ cells content, which result in variability between institutions. Single-platform $CD34^+$ testing is done by doing a direct count of viable $CD34^+$ cells compared to a set number of fluorescent beads. The count from this method does not depend on the TNC count, and only viable cells are counted. Double-platform $CD34^+$ testing is done by determining the percentage of cells that are $CD34^+$; usually only viable cells are counted and reported and that percentage is then multiplied by TNC. Results of single- platform $CD34^+$ counts have been reported to be lower than double-platform $CD34^+$ counts on post-thaw samples. This may be because washing of the cells in the double platform $CD34^+$ method concentrates the cell fraction by removing some of the non-viable cells.

CFU assays are performed to determine the hematopoietic growth potential of cells in the bone marrow, peripheral blood and cord blood. Early studies with CFU assays evidence the hematopoietic potential for HPC, Cord Blood, by demonstrating the capability of these cells to differentiate into lineage committed colonies, including BFU-E (erythroid burst forming unit), CFU-GM (colony forming unit granulocyte-macrophage) and CFU-GEMM (colony forming unit granulocyte-erythrocyte-macrophage-megakaryocyte) [10]. The CFU assay involves culturing the cells in a semisolid methylcellulose-based medium supplemented with growth factors. Cells grow forming different type of colonies, which can be classified based on their morphologic characteristics and type of cells. This assay is highly operator dependent and not easy to reproduce across institutions, which could explain the conflicting results obtained when evaluating the correlation between the CFU assay and engraftment [11, 12]. A recent study evaluating more than 400 pediatric HPC, Cord Blood transplants concluded that the CFU dose, obtained pre and post cryopreservation, was a strong predictor of engraftment [13]. Overall, CFU assays are performed before cryopreservation and the results are used for selecting units.

3.2.3 Identity: Histocompatibility, ABO and Rh Testing

HLA testing must be performed in a laboratory accredited by CLIA and ASHI (American Society for Histocompatibility and Immunogenetics) for initial and confirmatory typing of HPC, Cord Blood units. Typing should include intermediate resolution—for HLA class I (A and B) loci and allele-level typing for HLA class II (DRB1) loci [8, 14]. HLA confirmatory typing should be performed in the potential recipient and the HPC, Cord Blood unit. Confirmatory testing in the HPC, Cord Blood unit should be performed using a contiguous segment attached to the unit. ABO and Rh blood groups should be determined and recorded.

3.2.4 Stability Testing

A stability program should be developed to evaluate potency, integrity and sterility of products stored at appropriate temperatures and specific time intervals. Post-thaw nucleated cell counts, % recovery of pre-freeze cell counts, numbers of viable

$CD34^+$ cells remaining and the % recovery and post-thaw CFU assays are important measures in this analysis. These results must be used to determine suitability of storage conditions and unit expiration dates.

4 Clinical Outcomes

The Center for International Blood and Marrow Transplant Research (CIBMTR) is the entity charged with gathering post-transplant outcome data in the United States by the Health and Human Resources Administration. Each public cord blood bank that distributes units through the National Cord Blood Coordinating Center and each transplant center that receives the units must agree to share anonymous data through this reporting center. The cord blood bank receives information on the clinical outcome of its distributed units through this agency. Transplant clinical outcomes of patients receiving HPC, Cord Blood are the critically important quality indicator. Communication with the transplant center, as well as investigation of infusion reactions, infection transmission, failure or delayed engraftment is relayed through the coordinating center along with requests to investigate the unit processing details to determine if these events were related to manufacturing-related problems.

5 Financing

Private and public cord blood banks operate financially in different ways. Private banks charge a fee to the newborn's parents to collect, process and store HPC, Cord Blood units. These units belong to the corresponding family for their future use, and cannot be released to the general population. The percentage of use of private inventories for transplantation is less than 1 % [15]. In contrast, public banks cover the collection, processing and storing costs of donating units and the newborn's parents are not charged any fees. The HPC, Cord Blood units from public banks are available to the general population through national and international registries. The percentage of use of public banks, including our center experience, is approximately 7 % [16]. HPC, Cord Blood units help to close the gap of suitably matched units for racially and ethnically diverse patients. Some qualifying public cord blood banks receive federal funding to help build a diverse national inventory. However, the price of an individual cord blood unit is high enough to be a financial constraint, especially for double cord blood transplants, which are often required in adult transplant situations.

Not long ago, cord blood was considered a biological wastage, and now, its therapeutic value in stem cell transplantation has been proven. Federal regulations and laboratory quality systems are essential to ensure a safe and potent product. Current efforts are focused in improving cell dosage by developing expansion techniques or combination of two or more units, and in defining degree of HLA matching to ensure successful engraftment while decreasing recurrence of the disease and GVHD.

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Chapter 4
Maternal HLA Typing and Cord Blood Unit Choice

Andromachi Scaradavou

1 Background

The fetus inherits one human leukocyte antigen (HLA) haplotype from the father (inherited paternal antigens, IPA) and one from the mother (inherited maternal antigens, IMA; scheme in Fig. 4.1). During pregnancy, bidirectional transplacental trafficking of cells exposes the fetus to the maternal cells, expressing both the IMA as well as noninherited maternal antigens (NIMA), resulting in the development of NIMA-specific responses. Further, fetal cells enter the maternal circulation and the mother gets sensitized to the IPA of the fetus [1, 2].

1.1 Fetal Tolerance to NIMA

The so-called NIMA effect has been studied extensively, and the proposed mechanism described by Mold et al., [3] is the development of CD4 + CD25 + Fox + regulatory T cells that suppress fetal responses specifically to NIMA. The presence of regulatory T cells has been implicated in the role of NIMA in related kidney [4] and related hematopoietic stem cell transplants [5]. Recent studies also indicate that these regulatory T cells are responsible for suppressing the expansion of donor alloreactive cells that cause graft versus host disease (GvHD). Importantly, though, they do not abrogate the cytotoxic effect of CD8 + cells, and, therefore, do not affect their graft versus tumor function [6]. Another mechanism, proposed by Mommaas et al. [7], is that cord blood (CB) carries NIMA-specific cytotoxic CD8 + T cells, which can be

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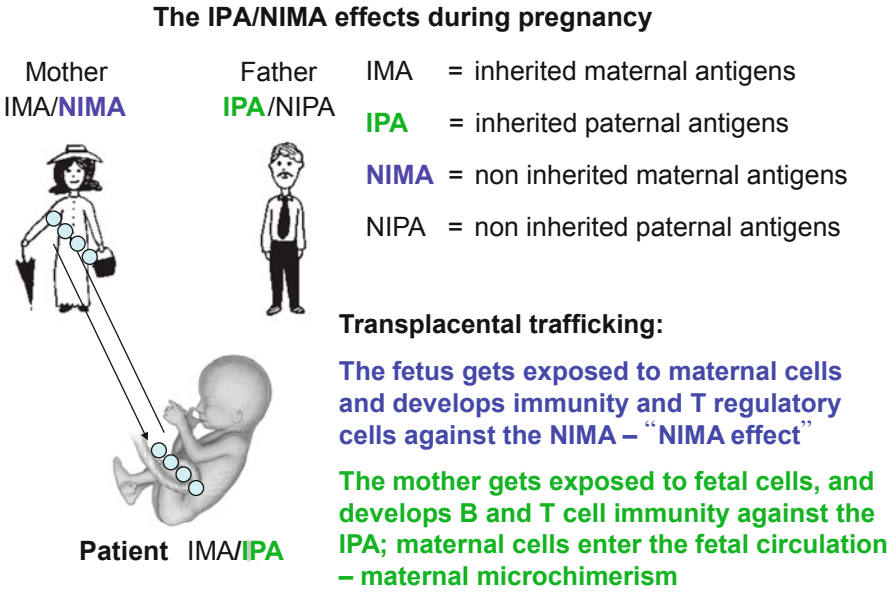

Fig. 4.1 Scheme of fetal-maternal interactions during pregnancy. (Courtesy of Prof. J. J. van Rood)

present at birth or can be generated after *ex vivo* priming, that are capable of lysing NIMA-specific targets *in vitro*. Additional evidence for the presence of cytotoxic and regulatory CD8 + cells in CB comes from the study of van Halteren et al., [8] who identified CD8 + cells in the offspring against maternal minor H-antigens.

1.2 Maternal Microchimerism in CB

Small numbers of maternal cells can be detected in fetal tissues [9], as well as in CB samples [10]. Some of the maternal cells are memory lymphocytes and can persist for a long time. These maternal T cells have been exposed and sensitized to the IPA expressed on the fetal calls that enter in the maternal circulation. The presence of the anti-IPA cells may be responsible for the superior outcomes of haploidentical T cell-depleted transplants from maternal donors, in comparison to those from paternal donors [11]. In unrelated CB transplants, anti-IPA-sensitized maternal cells are transplanted with the CB into the recipients.

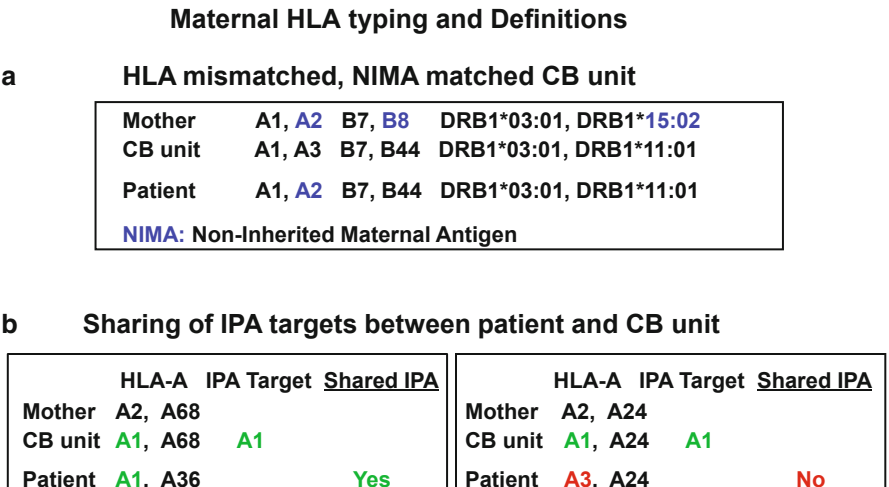

Fig. 4.2 **a** Example of human leukocyte antigens (*HLA*) MM, *NIMA* M cord blood unit (*CBU*): The HLA assignments of CBU and mother are shown; NIMA are shown in *blue.* The patient has one HLA-A locus MM with the CBU (CB has A3 while patient has A2); however, the NIMA at the locus "matches" the patient's MM antigen (A2). **b** Example of shared or not shared *IPA* targets: The HLA assignments of CBU and mother are shown for the HLA-A locus; the IPA can be inferred, i.e., the CB HLA-A antigen not present in the mother are shown in *green* (A1). Maternal cells have been sensitized to the IPA target. Patient and CBU match at A1, so the patient "shares" the IPA target of the maternal cells. In contrast, in the other example the patient does not have A1, so the sensitized maternal cells have no target in the recipient

2 Fetal-Maternal Interactions Improve Unrelated CB Transplant Outcomes

2.1 Improved Outcomes with NIMA-matched CB Transplants

The first study to evaluate the impact of fetal exposure to NIMA on the outcome of unrelated CB transplants was published in 2009 by van Rood et al. [12]. The hypothesis was that exposure to NIMA during fetal life would have an effect on transplant outcomes in cases where there was a NIMA match between recipient and CB donor (example shown in Fig. 4.2). This was evaluated in 1121 patients with hematologic malignancies that received single unit CB grafts from the New York Blood Center's (NYBC) National Cord Blood Program (NCBP).

Patients were assigned in three groups: (1) those with 0 HLA mismatched (MM) grafts ($N = 62$, 6 % of total); (2) those with HLA matched (M), NIMA M grafts ($N = 79$, 7 % of total); and (3) those with HLA M, NIMA MM grafts ($N = 980$). Of

note, the NIMA matching was assigned retrospectively, so matches happened only by chance; the CB grafts were not selected based on NIMA at the time of transplant. The analysis showed statistically significant improvements in transplant-related mortality (TRM) for the NIMA M grafts ($p = 0.034$ for all patients and $p = 0.012$ for those older than 10 years) compared to those of NIMA MM. Furthermore, overall mortality and treatment failure for HLA MM, NIMA M grafts were significantly improved ($p = 0.022$ and 0.002, respectively, for patients above 10 years), and engraftment was improved, particularly for patients who received lower cell dose grafts. Overall, outcomes of one HLA MM, NIMA M grafts were similar to those of zero MM (i.e., 6/6 HLA matched) grafts. Importantly, post-transplant relapse tended to be lower in patients with acute myeloid leukemia (AML) that received one HLA MM, NIMA M CBUs. There was no increased incidence of GvHD in recipients of HLA MM, NIMA M grafts [12].

A subsequent study of Rocha et al. [13] aimed to confirm the superior outcomes of HLA MM, NIMA M CB grafts. Using a smaller patient cohort and a different analytic approach, the authors compared the results of 48 HLA MM, NIMA M single unit CB grafts to those of 118 patients who received HLA MM, NIMA MM CBUs; all recipients had hematologic malignancies. This study also assigned NIMA matches retrospectively. The frequency of NIMA M CB grafts was 8.5 % among the 508 eligible patients. Importantly, in this study also, the TRM was lower after NIMA M grafts (RR = 0.48, $p = 0.05$); consequently overall survival (OS) was shown to be higher after NIMA M CB transplants: 5-year probability of OS was 55 % after NIMA M grafts versus 38 % after NIMA MM units ($p = 0.04$). Outcomes of one HLA MM, NIMA M transplants were not shown separately in this analysis to allow direct comparison with the previous study [12]. No effects on engraftment, incidence of GvHD, or relapse were detectable in this dataset [13].

Transplants with HLA MM, NIMA M CB grafts were not associated with adverse effects in either study. Further, using different analytical approaches, these two large retrospective studies showed a beneficial role for NIMA M CB grafts, leading to significant improvement in post-transplant survival. The findings indicate that, in the absence of a fully matched donor, HLA MM, NIMA M CB grafts can be the graft of choice for patients with hematologic malignancies.

2.2 Improved Outcomes with CB Units that Share IPA Targets with the Recipients

Another important biological aspect of the fetal-maternal interactions is the presence of maternal microchimerism in the fetus, and in the CB. van Rood et al. hypothesized that the IPA-sensitized maternal cells transplanted with the CB may have an effect on outcome when the patient has the same antigen as the IPA, so that the maternal cells would have an IPA "target" (example shown in Fig. 4.2). In those cases, patient and CB donor "share" an IPA target for the maternal cells.

A total of 845 recipients with AML or ALL (acute lymphoblastic leukemia) and the NYBC CBUs they received were retrospectively assigned in two groups, those with

shared IPA targets at 1, 2, or all 3 HLA loci ($N = 751$), or those with no shared IPA targets ($N = 64$), representing 6 % of the total patient-unit pairs [14]. All recipients received single unit grafts. The two groups were similar in regards to patient and disease characteristics and TNC doses. The incidence of acute GvHD grade III and IV was not different among the groups. On the contrary, there were significantly lower relapse rates in the group of HLA MM but shared IPA grafts. In particular, relapse reduction was most significant in patients receiving one HLA MM CB graft with shared IPA target (HR = 0.15, $p < 0.001$). The strong graft-versus-leukemia effect was mediated by the maternal microchimeric cells and it was independent of other HLA associations [14–16].

This was the first study to provide evidence for an immunological mechanism for the reduced relapse rates after unrelated CB transplants. Further, the findings support avoiding CBUs with no shared IPA targets, if possible, for patients with hematologic malignancies.

2.3 Future Studies

Although the precise mechanisms remain unclear, the existing work related to NIMA effects on transplantation supports conducting more studies. To overcome the low frequency of NIMA M CB grafts occurring just by chance and involving the more common HLA antigens [13, 17], in prospective clinical trials, preferential selection of NIMA M CBUs will need to be implemented, so that the number of patients that can be evaluated can increase substantially, in a relatively short time. Such a strategy requires HLA typing of the CB donors' mothers, so that NIMAs can be assigned prior to final CBU selection.

Another important consideration is the evaluation of NIMA effects in CB grafts that have two HLA MM, but both are NIMA M (2 HLA MM, 2 NIMA M) since these were not evaluated in the retrospective studies. Furthermore, the effect of the NIMA on the outcomes of the large proportion of patients receiving double unit CB grafts needs to be analyzed. Finally, although the NYBC preliminary analysis did not show any effect for patients with nonmalignant diseases, a larger cohort may be needed for a definitive evaluation.

3 CB Phenotypes Including NIMA—"Virtual" Phenotypes

Despite the size of the worldwide CB inventory (estimated to be approximately 650,000 by the World Marrow Donor Association (WMDA) [18]), still the majority of patients receive HLA MM grafts. Including the NIMA in the search algorithm allows for the selection of "permissible" HLA MM that lead to superior clinical outcomes.

Figure 4.3 illustrates the potential impact of including the NIMA in the CB search: By substituting a single NIMA in the CB phenotype, we can generate six potential

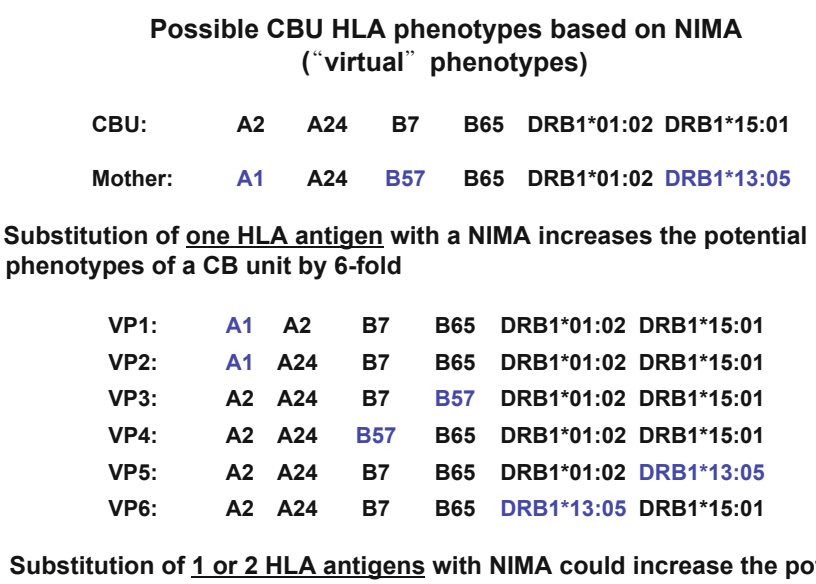

Fig. 4.3 Impact of including the noninherited maternal antigens (*NIMA*) in searches: generation of alternative ("virtual" phenotypes) from a single cord blood unit (*CBU*) by substituting one *NIMA*. *HLA* human leukocyte antigens

new phenotypes. The new phenotypes have been named "virtual" phenotypes by the Leiden group. Further on, by substituting one or two NIMA antigens, we may increase the number of potential phenotypes to 18. And by substituting one, two, or three NIMA, the maximum potential phenotypes that can be generated can be as many as 28. Although this is not the case in every mother-CB pair (because of homozygosity or shared HLA antigens between them), it is evident from the example that the number of potential ("virtual") phenotypes that can be derived from a single CBU by including the NIMA is fairly large. Further, in search simulation studies, the number of patients that could find optimal matches using the NIMA-generated phenotypes improves significantly [19]. The numerical improvement of the probability of finding donor grafts by including the NIMA in CB searches is under evaluation [19], but overall, such a strategy increases substantially the probability of finding optimal, "virtual" matches for the patients.

4 CB Unit Selection Considering the HLA Typing of the CB Donor's Mother

The strategy of the NCBP to perform maternal HLA typing prior to releasing any CBU for transplantation has enabled the retrospective analyses and evaluations of the effects of the fetal-maternal interactions on patient outcomes. These analyses

Patient - CBU NIMA and IPA assignments

Patient Search							
HLA typing	A	A	B	B	DRB1	DRB1	
Patient	01:01	68:02	15:03	58:02	11:01	15:03	
CBU	02:02	68:02	15:03	58:02	07:01	15:03	HLA MM
Mother	02	23	81	58	07:01	11:01	NIMA match
		68	15			15:03	shared IPA targets

Interpretation:

CBU has 2 HLA MM with patient, in HLA-A and -DRB1

Shared IPA targets are present at HLA-A, -B and -DRB1

NIMA match at HLA-DRB1

Fig. 4.4 Example of a patient's search: By reviewing the various 4/6 matched cord blood units (*CBU*s) and respective mothers' human leukocyte antigens (*HLA*) assignments a CB graft was selected that had two HLA M with the patient (in -A and -DRB1); one noninherited maternal antigen (*NIMA*) M (at -DRB1) and shared inherited paternal antigen (*IPA*) targets (at HLA-A,-B and DRB1). *CWD* common and well-documented, *MM* mismatched
Note: the HLA typings are shown as search determinants or CWD

used HLA donor-recipient matching at the antigen level (including the "splits") for HLA-A and -B and allele level typing for HLA-DRB 1. The effects on OS and TRM were significant even without considering HLA-C or class I allele level matching [20, 21].

It becomes, therefore, increasingly important to include the maternal HLA typing in the CBU selection criteria. To accomplish that, maternal DNA sample and informed consent at the time of collection have to be present.

To select NIMA M CBUs and/or identify CBUs that share IPA targets with the patients (example shown in Fig. 4.4), two strategies can be used:

a. Registry-based search
b. Individual patient search

Bone Marrow Donors Worldwide (BMDW) has now implemented a search strategy for HLA MM but NIMA M CBUs based on the maternal HLA typings provided by the NCBP and other European CB banks. In addition to the regular search, an option exists to identify NIMA M CBUs (at this point, the search identifies CBUs with all mismatches being NIMA M; [22]).It is clear that such an approach requires large numbers of already HLA-typed maternal data and modification of the search algorithms so that the maternal typings are included. Further, given this growing database, ongoing analyses are addressing the probability of finding "virtual" matches based on the race/ethnicity of the donor and patient, and various degrees of HLA MM [23].

These analyses will also guide selection of the maternal samples to be typed: For example, priority will be given to maternal sample of CBUs with high total nucleated cell counts (TNC) and/or those of ethnic minorities.

On the other hand, an individual patient-search strategy would work as follows: A search will need to be run to identify the potential CB matches for a patient. If there are no fully matched CBUs, then the 5/6 M and 4/6 M CBUs of interest will be selected, based on TNC cell dose and/or other selection criteria that the transplant center is using. Maternal HLA typing will have to performed (for example at the time of CBU HLA confirmatory typing) to evaluate for grafts with shared IPA targets and/or NIMA M among the MM CBU selected. In these cases, the frequency of haplotype or allele in the donor's race is important to estimate the probability of finding a NIMA M [17]. It is clear that such a search may take some time to complete if several CBU/maternal typings will need to be evaluated. The final selection will depend on whether there are any CBU with NIMA M and/or IPA shared targets, and appropriate TNC cell dose.

In our opinion, both strategies need to be implemented in parallel, for the field to move forward.

The cost of maternal HLA typing adds to the overall expense of the search. However, particularly for large numbers of samples, methods exist that allow for fast and relatively inexpensive HLA assignments. Overall, the cost effectiveness of typing the maternal samples has to be assessed by counteracting the multiple expenses of adding large numbers of new, HLA diverse, CBUs to the inventory (for the CB Banks), or the costs related to post-transplant complications and treatment of potential patient relapses (for the transplant centers).

5 Summary

Unrelated CB is increasingly used as alternative stem cell source for patients requiring hematopoietic reconstitution, particularly those of ethnic minorities. Despite the expanding worldwide inventory, still, the majority of patients receive MM CB grafts. Selecting those with "permissible" MM and superior graft-versus-leukemia effect improves the overall transplant outcomes.

There is an increasing body of evidence that fetal-maternal interactions affect the outcome of CB transplants. By HLA typing the CB donor's mother, we have ways to identify grafts with NIMA M and/or shared IPA targets with the recipients that enhance the overall efficacy of unrelated CB transplantation.

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Chapter 5
Optimizing Donor and Cord Blood Unit Selection for Banking and Transplantation

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1 Introduction

It was first recognized more than 30 years ago that umbilical cord blood was a rich source of hematopoietic stem cells (HSCs) and progenitor cells [1, 2]. Initially, Broxmeyer et al., through a series of critical experiments, demonstrated that the HSCs in cord blood showed high proliferative potential, could successfully repopulate hematopoiesis in murine models, and tolerated cryopreservation and thawing with efficient recovery of HSCs [3, 4]. Serial testing of samples from these early collections have since demonstrated that cord blood cryopreserved for nearly 25 years still retains sufficient clonogenic potential after thawing [5]. Based on this early work, the first cord blood transplant (CBT) was performed in France in 1988 using a matched sibling donor in a 5-year-old boy with Fanconi's anemia [6]. The transplant was successful and the recipient is currently healthy with full donor chimerism > 25 years post transplant. This early success led to subsequent CBT using related donors. The first unrelated donor cord blood transplantation (UCBT) was performed in 1993 in a child with leukemia [7]. Since that time, cord blood has become a standard source of donor HSCs for transplantation.

To meet the increasing need for cord blood donors, public banks were established. Currently, over 160 public cord blood banks exist worldwide leading to an inventory of $> 600{,}000$ cord blood units (CBUs) [8]. Advantages of cord blood include low donor risk, ease of procurement, decreased risk of graft-versus-host disease (GvHD), and more lenient requirements for human leukocyte antigen (HLA) matching due to the immunologic naivety of the cells. With less stringent matching, more patients lacking fully matched related or unrelated donors are able to find an appropriate CBU especially in non-Caucasian patients who are less likely to find an unrelated adult donor through registries [9]. Disadvantages of cord blood include slower engraftment, delayed immune reconstitution, and increased rates of graft failure as compared to other donor sources[10, 11], although this discrepancy has lessened in

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more recent series [12, 13]. Engraftment delays and graft failure are felt, in large part, to be due to loss of potency in some CBUs after thawing.

Cord blood potency, defined as the likelihood of a given CBU to engraft in a timely fashion, is influenced by factors associated with the donation, processing, potential changes occurring during cryopreservation and storage, and the recovery of the cells at the time of thawing for transplantation. Patient-related factors, such as concurrent infections or marrow dysfunction due to their underlying disease, may also influence the speed or probability of engraftment, but is beyond the scope of the current review. In this chapter, we explore methods of assessing CBU health and potency, review how certain cord blood characteristics are associated with outcomes after transplantation and discuss methods being explored to improve assessment of cord blood potency.

2 Total Nucleated Cell Content

It became evident in early reports of related and unrelated CBT that a higher infused total nucleated cell count (TNCC), adjusted for patient body weight, was a critical determinant of engraftment and survival [7, 14, 15]. In a review of 143 patients receiving related or unrelated CBT, Gluckman et al., on behalf of Eurocord group, observed that patients infused with $> 3.7 \times 10^7$ cells/kg were more likely to engraft with a trend towards improved survival [14]. The following year, Rubinstein confirmed those findings in a series of 562 primarily pediatric patients receiving UCBT with CBUs provided by the Placental Blood Program at the New York Blood Center. They observed that patients receiving CBUs with precryopreservation TNCC $< 2.5 \times 10^7$/kg experienced poorer outcomes, specifically, slower engraftment and increased transplant-related mortality (TRM) [15]. These retrospective studies supported the concept that a "minimum" TNCC is required for successful engraftment and outcomes after UCBT. It therefore became standard practice to select the closest HLA-matched donor CBU that could deliver the highest TNCC with a minimum acceptable precryopreservation TNCC of $\geq 2.5 \times 10^7$/kg although higher thresholds have been suggested [11, 16, 17]. For younger children, finding CBUs that could deliver adequate cell doses did not present a major challenge, but it was quickly realized that only a small proportion of banked CBUs contained sufficient TNCC to deliver an adequate cell dose to a larger child or adult.

The majority of CBTs performed before 2000 occurred in pediatric patients. In the first prospective UCBT trial (the Cord Blood Transplantation Study, COBLT) that enrolled pediatric patients with hematological malignancies from 1999 to 2004, children received CBUs with a median precryopreservation and infused TNCC of 5.1×10^7 and 3.9×10^7 cells/kg, respectively. The majority of patients received a cord blood graft mismatched at 1–3 HLA loci. The cumulative incidence of neutrophil engraftment (defined as achieving 3 consecutive days of an absolute neutrophil count of $> 0.5 \times 10^9$/L) in this cohort was 0.79 by day 42 post transplantation. Higher precryopreservation TNCC ($\geq 5.1 \times 10^7$/kg) was associated with increased and faster

engraftment [18] although the rate of primary graft failure remained higher than other donor sources. These early reports supported the role of umbilical cord blood as an acceptable HSC donor for transplantation when a closer matching related or unrelated living donor was not available, especially in pediatrics. Subsequent series have confirmed the association between higher TNCC and improved outcomes in pediatric patients with malignant and nonmalignant diseases [13, 19, 20].

The early experience using single unit UCBT in adults was not as promising. In adults, who are inherently larger than children, the TNCC doses established in the pediatric experience were more difficult to achieve using a single CBU. In three of the initial reports describing adult patients and their outcomes after UCBT, patients received comparably lower infused TNCCs (medians: 1.5–2.3 $\times$ 10^7 cells/kg) [10, 21, 22]. The overall outcomes for adult patients after UCBT showed slower engraftment and higher TRM as compared to bone marrow transplant recipients [10, 21, 22]. In the prospective COBLT study, the cumulative incidence of neutrophil engraftment and overall survival (OS) at 6 months after UCBT for adults with hematological malignancies were 0.66 (95 % confidence interval, 95 %CI, 0.48–0.79) and 0.30 (95 %CI, 0.14–0.46) [23], respectively. Additional retrospective registry studies of UCBT outcomes in adults have showed improved outcomes as long as adequate TNCC cell doses were delivered [24–26]. However, with a finite cell dose available in an individual CBU, that was often inadequate to meet these dosing targets using a single CBU, strategies were developed to compensate for this limitation.

3 Efforts to Increase the Available TNCC

The two major clinical strategies to increase the available TNCC dose for transplantation are double UCBT and *ex vivo* expansion. Double UCBT was first pioneered in adults at the University of Minnesota where two donor CBUs were given sequentially to a single patient undergoing UCBT to increase the cell dose for transplantation [27, 28]. This approach increased the survival of adults undergoing UCBT by increasing the probability of engraftment, shortening time to engraftment and reducing TRM although increased incidence of acute GVHD was also noted. While both units could be detected in a fraction of patients in the first few weeks post transplant, the vast majority of patients had durable engraftment from only one CBU before 3 months. Predicting the persisting unit after double UCBT has proved to be more difficult. Early studies failed to demonstrate any association between the engrafting unit and the TNCC, $CD34^+$, or $CD3^+$ cell doses cryopreserved or infused, the degree of HLA matching, or the order of infusion. More recently, in a series of 84 adult double UCBT recipients, Barker observed that the dominate cord had higher $CD3^+$ content and percentage of postthaw viable $CD34^+$ cells [29]. Conversely, Moore concluded in a murine model that cord dominance occurred through a graft-versus-graft immune interaction mediated by $CD34^-$ cells [30]. In spite of the observation that one unit dominated as the engrafting unit, outcomes were improved with a disease-free survival of 57 % (95 % CI, 35–79 %) at 1 year [28]. More recent reports of double

UCBT indicate that it is a viable option in adults when an adequately dosed single unit is unavailable [31, 32].

In cooperation with the Children's Oncology Group and the Pediatric Blood and Marrow Transplant Consortium, the Blood and Marrow Transplant Consortium recently completed a randomized study comparing outcomes after adequately dosed single versus double UCBT in children with hematological malignancies (BMT CTN 0501) [33, 34]. The median TNCC for the single versus double cohorts was 4.8×10^7 versus 8.9×10^7/kg, respectively. Nearly half of the patients in both cohorts received grafts mismatched at two HLA-loci. Early results indicated no benefit of double UCBT as compared to single unit UCBT at 1 year post transplantation with additional analyses ongoing. Higher grade III–IV acute GvHD was seen in recipients of double UCBT ($p = 0.03$). There was no differential protection against relapse although it was low in both groups (12 and 14 % for single and double UCBT, respectively). As both cohorts received adequate total TNCC (defined as $> 2.5 \times 10^7$/kg), this study did not address the concept of the "minimal TNCC" required for successful engraftment. Results of double UCBT and the use of *ex vivo* expansion are discussed in greater detail in accompanying chapters.

4 HLA Matching and Interaction with TNCC Cell Dose

The role of HLA matching has also been established as an important prognostic feature both independently and in combination with the TNCC dosing. In a retrospective analysis of 550 UCBTs for hematologic malignancies reported to the Eurocord Registry, Gluckman et al. suggested that HLA mismatching could be overcome by higher TNCC [35]. In a large retrospective series of pediatric patients receiving either UCBT or matched unrelated donor transplant for leukemia, Eapen et al. further analyzed the interaction between HLA matching and TNCC cell dose [13]. Although no differences were observed for fully matched or 2-antigen mismatch UCBT, higher cell dose (defined as $> 3.0 \times 10^7$/kg) in 1-antigen mismatched UCBT showed improved neutrophil engraftment compared to lower cell dose with the same mismatch. Improved engraftment kinetics and overall rates were seen in matched as compared to mismatched (1 or 2 loci) UCBT. More recently, Barker et al. analyzed a cohort of 1,061 patients [36]. Those receiving a 6/6 matched CBU regardless of TNCC had lower TRM, followed by patients receiving a 1-mismatched unit delivering a TNCC $> 2.5 \times 10^7$/kg or a 2-mismatched unit with a TNCC of $> 5.0 \times 10^7$/kg. There was no difference in survival outcomes in patients receiving a 1-mismatched unit with a TNCC of 2.5–4.9 $\times 10^7$/kg compared to patients receiving a 2-mismatched CBU that delivered a TNCC of $> 5.0 \times 110^7$/kg. Atsuta et al. observed that the association between HLA and TNCC impacted pediatric patients but not adult patients outcomes after UCBT [37]. Therefore, the interplay between HLA matching and TNC dosing is complex and warrants further investigation to better define how to prioritize in donor selection.

5 CD34$^+$ Cell Content

A higher CD34$^+$ cell dose has also been identified as an important predictor of outcomes after UCBT. Early on, Wagner et al. observed in a series of 102 primarily pediatric patients that infused CD34$^+$ doses $> 1.7 \times 10^5$/kg were associated with improved engraftment, TRM, and survival [38]. Additional studies have confirmed the importance of sufficient CD34$^+$ cell dosing [39–43]. As such, some transplant centers utilize the CD34$^+$ cell dose in CBU selection recognizing that significant interlaboratory variability exists [44–46]. In a series of 435 patients receiving a single donor UCBT after myeloablative conditioning at our institution, we observed that postthaw CD34$^+$ dose was a significant predictor of neutrophil engraftment in multivariate analysis ($P = 0.04$), but to a lesser degree than post-thaw colony-forming units (CFU) ($P < 0.0001$). Furthermore, CD34$^+$ dose was not associated with platelet engraftment, but was weakly associated with OS at 180 days post-transplantation [47]. As a surface marker of HSCs, the presence of CD34$^+$ cells in a given CBU does not assess how healthy those cells may be. Therefore, there has been interest in measuring the viable CD34$^+$ content. Scaravadou et al. investigated the impact of the percentage of viable CD34$^+$ measured post-thaw and subsequently infused in adult patients receiving double UCBT. They observed that the higher viable CD34$^+$ cell dose correlated with the unit that ultimately engrafted [48] and with the speed of engraftment [49]. With the interlaboratory difficulties encountered measuring the total CD34$^+$ content of grafts, especially on a post-thaw sample, specifically significant interlaboratory variability, it is unclear whether the viable CD34$^+$ will be a useful and technically practical measure in donor selection.

6 Impact of Potency

Despite attempts to increase the cell dose available for transplantation, some CBUs will still fail to engraft. In a study of 159 children transplanted for inherited metabolic diseases at our institution, graft failure occurred in 21 % of patients despite administration of very high cell doses (median: 9.7×10^7cells/kg) from the cord blood graft [41]. In this study of "high cell dose recipients," both precryopreservation and post-thaw graft parameters of the CBU transplanted were correlated with clinical transplant outcomes. TNCC, CD34$^+$ cells, and CFU from the cryopreserved and post-thaw data were correlated with survival, neutrophil and platelet engraftment. The parameter that best correlated with both engraftment ($p < 0.0001$) and survival ($p = 0.01$) was post-thaw CFUs measured on the infused CBU [41].

7 Impact of CFUs

The importance of CFU dosing was previously recognized in a report from Rubinstein in 2001 showing that precryopreservation CFU dose was more closely correlated with engraftment of both neutrophils and platelets than TNCC [50]. Wall et al. observed

in 153 pediatric patients enrolled in the COBLT study that the postthaw CFU counts and yield was the most important predictor of both engraftment and OS at 2 years [51]. Other studies have observed that, specifically, the CFU-granulocyte-macrophage (CFU-GM) subtype was associated with the kinetics of both neutrophil and platelet engraftment [52, 53]. More recently, we extended the findings of Prasad et al. [41] in a larger cohort of primarily pediatric patients receiving single UCBT after myeloablative conditioning [47]. We investigated the impact of precryopreservation and postthaw data including TNCC, $CD34^+$, and total CFU dosing on the clinical endpoints of survival, neutrophil and platelet engraftment. Higher CFU dosing was the only precryopreservation graft characteristic predictive of neutrophil ($p = 0.0024$) and platelet engraftment ($p = 0.0063$) in multivariate analysis. Likewise, postthaw CFU content was the best predictor of neutrophil and platelet engraftment (both $p < 0.0001$; Fig. 5.1). This is not surprising given that the CFU assay requires viable cells to grow colonies to yield positive results and, as such, indirectly measures the health or potency of a CBU. While CFU growth prior to cryopreservation has been included as a potential measure of potency in the US Food and Drug Administration guidelines for licensure, the CFU assay has several issues that currently preclude its widespread use in donor selection. Similar to measuring $CD34^+$ content, there are issues with standardization between laboratories [54, 55]. Additionally, it is a time-consuming assay that provides results weeks later. Development of an alternate measure of potency more rapidly obtained would be a significant addition to the banking and transplant communities.

8 The Cord Blood Apgar (CBA) Score

We then hypothesized that a composite analysis of multiple graft characteristics would provide a more sensitive and specific method to assess the potency of an individual cord blood graft. To this end, we retrospectively identified recipients of myeloablative single unit UCBT at our institution. With this dataset, we then created and retrospectively validated a novel scoring system, named the Cord Blood Apgar (CBA) score, to optimize umbilical cord blood graft selection for transplantation. The score was defined as the weighted summation of selected precryopreservation or post-thaw graft characteristics with the weight based on the magnitude of the hazard ratio in the univariate analysis of neutrophil engraftment. Each transplanted CBU was assigned three scores: a precryopreservation score using precryopreservation characteristics, a post-thaw score using values measured on the actual CBU at the time of thawing for transplantation, and a composite score based on combined precryopreservation and postthaw graft characteristics. Applying the CBA to the dataset, it was found that patients transplanted with a CBU that scored in the top quartile for any of the three scoring components were the most likely to engraft (all $p < 0.0001$) with shorter median times to engraftment. In multivariate models that considered the CBA along with 15 clinical characteristics, all three components of the CBA were the strongest predictors of neutrophil engraftment in their respective models

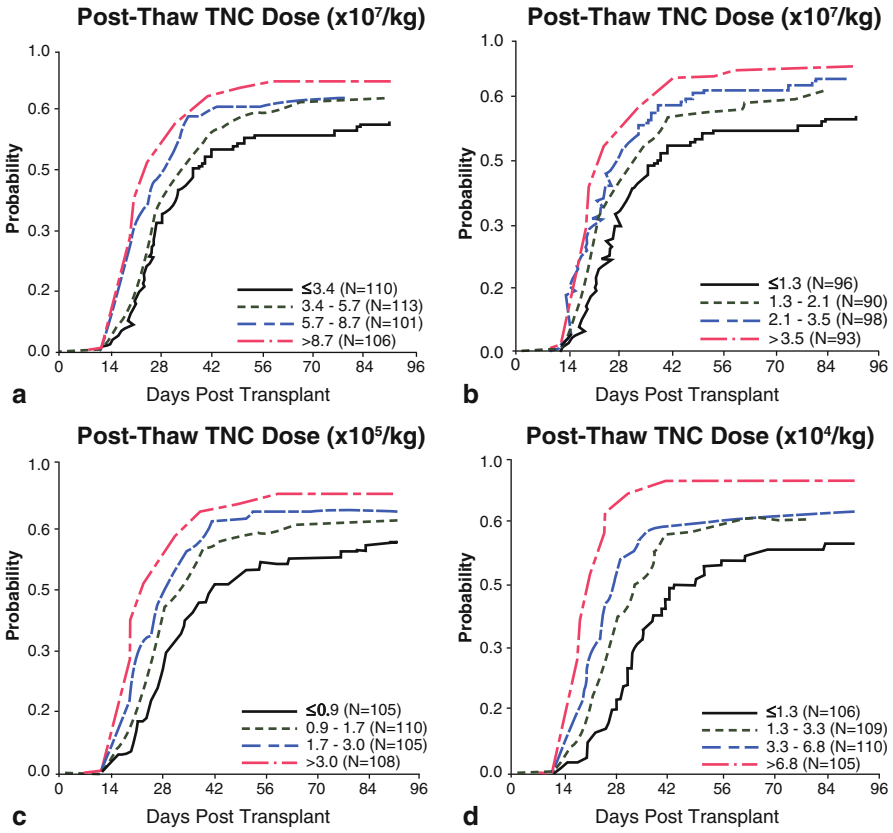

Fig. 5.1 Impact of post-thaw graft characteristics on the probability of neutrophil engraftment. Probability plots are shown for each of the four quartiles. Panels **a**–**d** depict the impact of post-thaw *TNCC* total nucleated cell count ($\times$ 10^7/kg recipient weight), *MNC* mononuclear cell ($\times$ 10^7/kg recipient weight), CD34 + ($\times$ 10^5/kg recipient weight), and *CFU* colony-forming units ($\times$ 10^4/kg recipient weight) doses, respectively, on neutrophil engraftment. (Used with permission. Page et al. [47])

(all $p < 0.0001$; Fig. 5.2). Thus, the CBA strongly predicted engraftment and is a promising tool for selecting CBUs for transplantation. Currently, work is underway to address the limitations of the CBA in its current form. Specifically, the CBA relies on the CFU assay that has issues with standardization and is time-consuming. Furthermore, the postthaw data, which best assesses potential damage incurred by the cord blood graft during cryopreservation, storage, and thaw, is unavailable at time of donor selection. To address these shortcomings, our laboratory has developed a potency assay that can be performed at the time of confirmatory testing using a segment attached to a cryopreserved CBU. The flow cytometry-based assay measures expression of aldehyde dehydrogenase (ALDH), an intracellular enzyme found in high concentration in HSCs. Cells scoring positive ($ALDH^{br}$) in this assay are viable and likely to correlate with stem cell content of a graft. In bone marrow, peripheral blood stem cells (PBSCs), and thawed CBUs, $ALDH^{br}$ activity strongly correlated

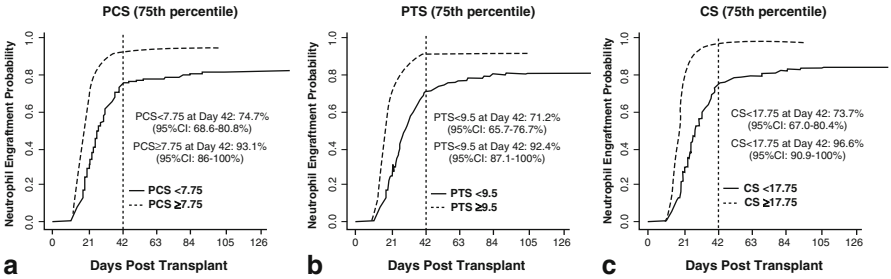

Fig. 5.2 Impact of CBA score on the probability of neutrophil engraftment in the overall data set. Probability plots are shown for scores above and below the 75th percentile. (**a**) Impact of Pre-cryopreservation Score (PCS) using the 75th percentile as the cutoff on neutrophil engraftment in the overall data set. (**b**) Impact of the Post-thaw Score (PTS) on neutrophil engraftment in the overall data set using the 75th percentile. (**c**) Impact of the Combined Score (CS) on neutrophil engraftment using the 75th percentile as cutoff. (Used with permission. Page et al. [68])

with CFUs and with speed of engraftment in transplant recipients [56]. These data suggest that ALDH^{br} content of a CBU may predict potency. In addition to ALDH^{br}, the assay enumerates CD34$^+$, CD45$^+$, glycophorin A +, viability (7-AAD$^+$), and plates CFUs from the thawed segment in advance of the transplant without sacrificing the entire unit. Preliminary studies demonstrated high correlation of ALDH^{br} measured on the segment with CFUs and with engraftment [57]. Based on these findings, the segment assay has strong potential as a surrogate for postthaw measurements to assess potency of a potential CBU graft and to increase the robustness of the CBA.

9 Increasing the Potency of Banked CBUs

Since the establishment of the first public cord blood bank at the New York Blood Center in 1992, there has been an effort by cord blood banks to expand the yield and cellular content of donor collections. In general, there are main two approaches: Identify donations likely to yield a successful collection or enhance collection techniques. We have recently analyzed characteristics of more than 5,200 CBUs that were collected and processed at the Carolinas Cord Blood Bank (CCBB), a public unrelated donor cord blood bank [58]. The CCBB accepts donations from mothers delivering healthy babies ≥ 34 weeks of gestation who meet donor screening and other technical specifications. We used CFUs as the primary biomarker of potency combined with CD34$^+$ and TNCC content as single and composite measures of quality. In multivariate analysis, decreased time from collection to processing (< 10 h), younger gestational age of the donor (< 38 weeks), increased birth weight (> 3500 g), and race/ethnicity were all highly predictive of CBUs with higher potency. While younger gestational age (34–37 weeks) was associated with lower postprocessing TNCC, these collections from younger babies were more potent than collections from babies born closer to term (38–40 weeks) as demonstrated by enriched CFUs and CD34$^+$ cell content. Importantly, cord blood collected from postterm infants (> 40 weeks) had lower CFUs and CD34$^+$ cell content overall. Our results confirmed

the findings of others who have described a positive correlation between increased birth weight and postprocessing TNCC $CD34^+$, or CFU content [59–63]. Based on these results, we recommended that banks prioritize collections from younger infants likely to have higher CFUs and $CD34^+$ cells recognizing that it is also likely to have a slightly lower postprocessing TNCC.

After adjusting for other variables, the cord blood baby donor's race/ethnicity was a strong independent predictor of the quality and potency of CBUs confirming and extending the results of others [62–65]. Most notably, Caucasian infants were more likely to have CBUs with higher potency compared to African-American infants. Interestingly, when we examined the concentration of CFUs, $CD34^+$, and TNCC by adjusting for the volume of cord blood collected, CBUs from Caucasian infants had higher numbers of circulating cells compared to African-American infants despite similar collection volumes. The potential impact of these findings could lead one to prioritize collections from Caucasian infants. However, this would only make sense if race/ethnicity matching or the potential for better HLA-matching had no impact on transplantation outcomes. Prasad et al. showed that race matching was associated with improved survival in a cohort of 159 infants and children undergoing UCBT for inherited metabolic diseases [41]. Ballen et al., on behalf of the Center for International Blood and Marrow Transplant Research (CIBMTR), investigated the impact of race/ethnicity in adults with leukemia who received myeloablative conditioning followed by a single unit UCBT. In multivariate analysis, Black patients had inferior OS as compared to either white or Hispanic patients. However, outcomes were comparable for patients who received well-matched (5 or 6/6 loci) or with adequate TNCC ($> 2.5 \times 10^7$/kg) regardless of race [25]. Therefore, these results suggest that the public inventory should contain adequate high quality and potent CBUs addressing a wide racial diversity and HLA repertoire. To achieve this, it will be critical to enhance recruitment of African-American donors and increase resources to collect higher numbers of CBUs from this population.

Collection-related variables that could potentially be modified to increase CBU quality were also examined including increased collection volumes and shortening time to processing. Larger-volume CBUs were more likely to have higher potency overall compared to smaller CBUs regardless of delivery type. Currently, many banks receive collections from distant sites and, therefore, delays in processing due to travel may exist. Results of the COBLT study indicated that viability, post-TNCC, and $CD34^+$ content remain stable at room temperature for > 48 h leading to the rule that cryopreservation of a processed CBU must begin within 48 h of collection [60]. Others have also demonstrated loss of cells when aliquots of CB are tested from 24–96 h after collection [66, 67]. However, our analysis revealed found small but significant losses of CFUs, $CD34^+$ cells, and TNCC that occur in units processed at least 24 h after delivery compared to those processed less than 10 h from collection (Fig. 5.3; [58]). These results have impacted standard operating procedures in the Carolinas Cord Blood Bank by altering the schema used to prioritize processing of CBUs within 24 h of collection. This information could also be incorporated into a clinical and technical scoring system, thereby extending the CBA scoring system, to identify the most potent CBUs.

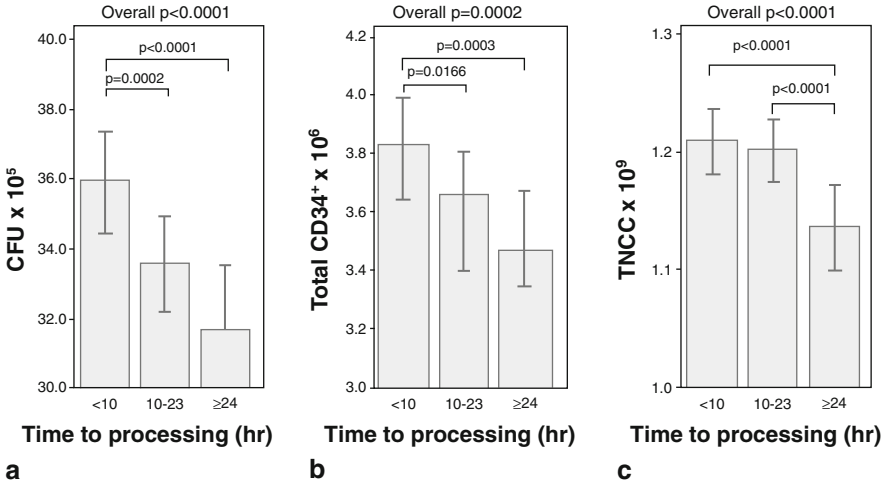

Fig. 5.3 Impact of time to processing on CFU, CD34 $^{+}$, and post-TNCC content. In a through c, the adjusted mean CFUs (**a**), CD34 $^{+}$ (**b**), and post-TNCC (**c**) by time to processing is presented after adjusting for infant race/ethnicity, sex, gestational age, birth weight, collection volume, delivery type, and maternal age. Only significant *p* values are shown. Whisker plots represent the 95 % CIs. *CFU* colony-forming units, *TNCC* total nucleated cell count. (Used with permission. Page et al. [58])

10 Future Directions

As the fields of cord blood banking and transplantation have matured into an established therapy, focus has turned to refining the use of cord blood for hematopoietic stem cell transplantation and developing novel indications in the emerging field of regenerative medicine reviewed in an accompanying chapter. Further understanding of the stem cell biology and mechanisms supporting engraftment will also inform these efforts. Success of these therapies relies heavily on the availability of reliable sources of healthy and potent CBUs. Therefore, in our own laboratory, we are focusing efforts on validating the segment-based assay as a measure of potency and the further development of the CBA scoring system. The ultimate goal is to accurately assess potency of potential donor CBUs that, in turn, may improve overall outcomes of UCBT and other life-saving therapies.

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Chapter 6
Cord Blood Transplantation for Pediatric Hematologic Malignancies: Indications, Mechanisms, and Outcomes

Heather E. Stefanski and Michael R. Verneris

1 Introduction

In the early 1980s, Hal Broxmeyer, Judith Bard, and the late Edward Boyse discussed umbilical cord blood (UCB) as a potential source of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs). The discovery that UCB could be cryopreserved with functional HPCs gave impetus to perform the first UCB transplant for a patient with Fanconi anemia in 1988 [1]. Recently, the 25th anniversary of the first UCB transplant was celebrated; to date, over 30,000 UCB transplants have been performed throughout the world [2–4].

UCB confers several advantages over adult HSC sources from either the bone marrow (BM) or peripheral blood (PB). These include the lack of donor attrition, absence of collection risk to the mother or infant donor, and reduced transmission of blood-borne infectious diseases (such as human cytomegalovirus). The wait for adult donor transplantation can take as long as 3–4 months due to the process of donor identification, confirmatory typing, infectious disease testing, and scheduling. In contrast, the UCB unit has undergone all pretransplant testing at the time of collection and the only rate-limiting step to transplantation is the delivery of the UCB unit and any patient-associated factors. Thus, the ability to rapidly secure a UCB unit has been extremely beneficial in treating leukemia, since it spares patients exposure to additional (and potentially unnecessary) pretransplant chemotherapy in order to ensure remission while waiting for coordination of an adult donor [5]. Perhaps the single most important difference between UCB and adult stem cell sources is the ability to cross histocompatibility barriers without cell manipulation. Thus, while allele level matching at human leukocyte antigens (HLA)-A, -B, -C, and -DRB1 are necessary for adult donor transplantation, the matching requirements for UCB are much less stringent. Using intermediate level (serologic) HLA typing for HLA-A and -B and high-level (allele) matching for HLA-DRB1, up to two antigen mismatched UCB units can be safely transplanted, provided adequate cell dose [6]. Thus, the

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ability to use mismatched grafts with equivalent rates of acute graft-versus-host disease (aGVHD) as adult stem cell sources makes it possible to identify stem cell grafts for the majority of individuals [6]. Recent studies suggest that more precise HLA matching (using allele level typing) can improve outcomes by lowering non-relapse mortality (NRM) [7]. However, mismatched grafts may be beneficial since they may lead to increased T and NK cell alloreactivity and graft-versus-leukemia (GVL) reactions (discussed below).

2 The Early Days: Cell Dose as a Major Determinant of Transplant Outcomes

A requirement for successful outcomes following any type of allogeneic transplantation is hematopoietic engraftment. However, a major limitation to the use of cord blood is the availability of sufficient numbers of HPCs and HSCs within the UCB unit, and this directly impacts the probability and speed of engraftment [2, 3, 8]. For instance, in 1996 Kurtzberg and colleagues analyzed the outcomes of 25 patients with a variety of different diseases who had received UCB transplantation from unrelated donors [9]. They found HSC recovery in 23 patients and the overall 100-day survival rate was 64 %. The number of nucleated cells infused per kilogram of the patient's body weight correlated directly with the rate of myeloid engraftment [9]. In 1997, a Eurocord registry analysis reported the results of 143 related and unrelated UCB transplants performed at 45 centers [10]. They found that improved neutrophil engraftment occurred when the total nucleated cell (TNC) dose was above 3.7 × 10e7/kg. Overall survival (OS) at 1 year was 73 % in recipients of HLA matched UCBT and 33 % in recipients of cord blood mismatched for one or more HLA antigens [10]. In 1998, Rubinstein and colleagues reported transplant outcomes of 562 patients undergoing UCBT. Patients were transplanted at a number of different centers, but all had received New York Blood Center cord blood units [6]. The most significant graft characteristic for ensuring engraftment was the TNC dose. UCB grafts that were mismatched for up to two HLA antigens were found to be effective stem cell sources in patients without HLA identical related donors [6]. While the above work established that MNC dose/kg was important in engraftment, these findings could be interpreted in a number of ways since increased MNC dose could reflect increased engraftment due to increased numbers of stem cells, increased dose of lymphocytes (which facilitate engraftment), or both. A study by Wagner and colleagues partially addressed these issues and demonstrated that the UCB CD34 cell dose was key parameter in the rate of engraftment. There was a high probability of survival in recipients of UCB grafts that were disparate at no more than two HLA antigens and when the graft contained at least 1.7 × 10e5 CD34 + cells/kg, supporting the concept that MNC is reasonable surrogate for stem cell content and that this (stem cell content) was what drove engraftment [11].

In 2000, Rocha and colleagues reported the analysis of 113 recipients of UCB from HLA-identical siblings compared to 2,052 recipients of HLA-identical sibling BM from 1990–1997 [12]. Multivariate analysis demonstrated a lower risk of aGVHD

Table 6.1 Multivariate analysis of risk of relapse

Reference	HSC source	2	Risk of relapse	(95 % CI)	*p* value
Eapen [13]	HLA matched BM	116	1	Reference	Reference
Marrow vs. UCB	Mismatched BM	166	0.77	(0.51–1.16)	0.2171
In leukemia	HLA matched UCB	35	0.68	(0.35–1.32)	0.2524
–	One antigen mismatched UCB	157	0.67	(0.43–1.02)	0.0593
–	Two antigen mismatched UCB	267	0.54	(0.36–0.83)	0.0045
–	*HSC source*	*N*	*Risk of relapse*	*(95 % CI)*	*P* value
Verneris [20]	Single UCB	84	1	Reference	Reference
sUCB an dUCB in leukemia	Double UCB	93	0.5	(0.2–1)	0.04
–	*CR status*	*N*	*Risk of relapse*	*(95 % CI)*	*P* value
Ruggeri [37]	CR1–CR21	149	1	Reference	Reference
sUCB in ALL	CR3	21	4.25	(3.56–18.69)	0.001
–	*MRD status*	*N*	*Risk of relapse*	*(95 % CI)*	*P* value
Ruggeri [37]	MRD −	96	1	Reference	Reference
sUCB in ALL	MRD +	74	2.15	(1.2–3.9)	0.001

UCB umbilical blood cord, *HSC* hematopoietic stem cell, *HLA* human leukocyte antigens, *sUCB* single umbilical blood cord, *dUCB* double umbilical blood cord, *ALL* acute lymphoblastic leukemia, *MRD* minimal residual disease, *CI* confidence interval, *BM* bone marrow

(relative risk, 0.41; $p = 0.001$) and chronic graft-versus-host disease (cGVHD; relative risk, 0.35; $p = 0.02$) among recipients of cord blood transplants. OS was not statistically significant between the two groups. This data established a lower incidence of both aGVHD and cGVHD in recipients of HLA-identical sibling UCB.

Eapen and colleagues used registry data to assess disease-free survival (DFS) in children with acute leukemia following either UCB or BM transplantation [13]. In this study, 503 children received UCB and 282 received BM as the stem cell source (Table 6.1). Similar 5-year DFS in patients was seen in patients that received either one or two antigen mismatch UCB compared to BM recipients. While NRM was a competing risk for relapse, the rates of relapse were lower after two antigen HLA mismatched UCB transplants. Overall, there was equivalent DFS in BM and UCB, strongly supporting the assertion that at adequate doses, one or two antigen mismatched UCB yields equivalent outcomes as the "gold standard" BM in children with acute leukemia [13].

Between January 1999 and May 2003, a prospective National Institutes of Health (NIH)-sponsored trial of unrelated donor UCB transplantation was performed (cord blood transplantation study, COBLT) [14]. In this phase 2 study, 191 pediatric patients diagnosed with hematologic malignancy were transplanted with one UCB unit. The study also found a critical impact on cell dose since a precryopreserved TNC

dose of greater than 2.5 × 10e7/kg of body weight resulted in engraftment. The relative incidence of relapse at 2 years was 19.9 % (95 % confidence interval, CI, 14.8–25 %), which compares favorably to a number of studies using BM [15, 16]. In a subgroup analysis from this study, patients who showed evidence to reactivity to viral antigens early after transplantation had a significantly better DFS ($p = 0.001$) which was due to reduced relapse ($p = 0.003$), directly linking immune recovery to GVL reactions [17].

3 Double UCB Transplant and Potential Advantages in Leukemia

The above studies showed that UCB is an appropriate alternative stem cell source in treating hematologic malignancies. They also demonstrate the importance of cell dose in hematopoietic recovery and survival after UCB transplant. The fixed cell dose is particularly limiting for adolescents and adults where the number of cells in a single unit may be inadequate for engraftment. One approach to overcome the fixed cell dose in a UCB unit has been to *ex vivo* expand the UCB unit (please see chapter by De Lima). However, the concern for loss of pluripotency during expansion required the development of strategies to ensure safety. Pioneering work by Barker and Wagner at the University of Minnesota tested a dual unit transplant approach. In this pilot study, 23 patients were transplanted with two partially matched (nonmanipulated) UCB units following myeloablation [18]. Strikingly, at D + 100 after transplantation, only a single unit was detected in most patients. Given this adult cohort, the expected probability of engraftment was higher and time to engraftment was significantly more rapid than historical data, [9] suggesting that either transiently increasing the stem cell dose or immune graft-versus-graft reactions influences engraftment. These initial studies of "double UCB transplantation (dUCBT)" showed that the procedure was safe and effective, leading to the widespread use of UCB in older children and adults.

Subsequent studies in dUCBT showed an increased incidence of aGVHD compared to single unit recipients [19]. MacMillan and colleagues analyzed 265 consecutive patients receiving transplants with UCB graft composed of one ($n = 80$) or two ($n = 185$) units and determined the incidence of aGVHD. The incidence of grade III–IV aGVHD was similar between cohorts. However, grade II–IV aGVHD was higher among double UCBT recipients (58 vs. 39 %, $p < 0.01$).Given this, and the possibility of graft-versus-graft interactions following dUCBT, we hypothesized that dUCB transplants would have lower rates of leukemia relapse. To test this, single UCB transplant recipients were compared to those that received dUCBT in a retrospective, single center study [20]. In this study, 177 acute leukemia patients that had received an UCB graft composed of either one (47 %) or two (53 %) partially HLA-matched unit(s) were examined (Table 6.1). The choice to receive single or dUCBT was based on weight of the patient and the number of MNC in the UCB unit, such that if the patient was assessed to have a single unit that was inadequately sized, a dUCBT was performed. The incidence of relapse for all patients was 26 %

(95 % CI, 19–33 %). In multivariate analysis, patients in first and second complete remission who received dUCBT had significantly lower relapse compared to single UCB recipients (RR = 0.5, $p < 0.03$). Leukemia-free survival was 40 % (95 % CI, 30–51 %) and 51 % (95 % CI, 41–62 %) for single- and double-unit recipients, respectively ($P = 0.35$).This data suggested that double-unit UCB transplants had better GVL effect.

Based on the above findings, a prospective randomized trial compared single to double UCB transplants for children with hematologic malignancies. Preliminary results reveal no survival advantage or advantage in engraftment in double UCB transplant recipients, as compared to those transplanted with a single UCB unit [21]. Recipients of two units had a higher incidence of aGVHD, but relapse risk was unchanged [21]. Therefore, if an adequate cell dose is available in a single UCB unit, there is no indication for double UCBT in children.

4 Who to Transplant and Outcomes of UCB Transplant

A number of studies have evaluated the feasibility of utilizing UCB as a stem cell source in instances when matched sibling donor is not available. Eapen and colleagues used registry data to assess DFS in children with acute leukemia following either UCB or BM transplantation [13]. The goal of this analysis was to compare the outcomes of UCB to BM and to assess the relative effect of UCB HLA match and their potential interaction on DFS. In this study, 503 children received UCB transplants versus 282 children received BM as the stem cell source. The 5-year DFS in patients that received either one or two antigen mismatch UCB was similar to BM recipients. Transplant-related mortality rates were higher after one (relative risk, RR, 1.88; $p = 0.0455$) or two antigen (RR 2.31, $p = 0.0003$) HLA-mismatched UCB transplant. Interestingly, while NRM is a competing risk for relapse, rates of relapse were lower after two antigen HLA mismatched UCB transplant recipients. Thus, these investigators were able to demonstrate similar DFS in BM and UCB. Additional studies have revealed a significant interaction between the infused cell dose and HLA mismatching where rates of NRM can be reduced with a higher cell dose [22]. These studies strongly support the assertion that at adequate doses, one or two antigen mismatched UCB yields equivalent outcomes as the "gold standard" BM in children with acute leukemia [13].

Although the majority of children and adolescents with acute lymphoblastic leukemia (ALL) can be cured with multimodal chemotherapy, patients with very high risk ALL or recurrent disease have a worse prognosis [23−26]. Current definitions of very high risk ALL include induction failure, severe hypodiploidy (< 44 chromosomes), certain translocations (t9;22,q34;q11), mixed-lineage leukemia (MLL) gene rearrangements, and identifiable intrachromosomal amplification of chromosome 21 (iAMP21). With these cases, transplantation should be considered in first complete remission (CR1) [27].

Infant leukemia is an aggressive constellation of diseases that have a dismal prognosis. The best treatment for such patients is debated, but transplantation in the

early stages of the disease should be strongly considered. A study from the Japan infant leukemia study group showed favorable results with transplantation using either sibling or unrelated donors [28]. The 3 year OS and DFS for infants undergoing transplantation was 58.2 and 43.6 % respectively, comparing favorably to nearly all chemotherapy protocols for this disease. For recipients of unrelated donor transplantation, there was no difference in outcomes between patients receiving UCB or adult donor stem cell sources.

While myeloproliferative diseases are uncommon in the pediatric population, juvenile myelomonocytic leukemia (JMML) is one such disease. To date, no chemotherapy regimen has been curative and allogeneic transplantation is the only curative option. Recently, a retrospective analysis was performed on 110 JMML patients who received UCB transplant [29]. The 5-year DFS rate was 44 %. In multivariate analysis, factors predicting better disease-free survival rate were age < 1.4 years at diagnosis, 0–1 HLA disparities in the donor/recipient pair and a karyotype other than monosomy 7. Thus, given that HLA disparities are common in UCB transplantation, this stem cell seems ideal for patients with JMML.

A number of studies have shown the importance of transplantation as consolidation therapy for children with acute myeloid leukemia (AML). In 2001, Woods et al. first demonstrated that allogeneic-related donor HCT improved outcome compared to children treated with chemotherapy alone [30]. At the University of Minnesota, we investigated whether allogeneic HCT with best available donor in CR1 would abrogate the poor outcomes associated with high risk AML in children and young adults treated with chemotherapy [31]. We reviewed 50 AML patients (ages 0–30 years) who received a myeloablative allo-HCT between 2001 and 2010. Thirty-six patients (72 %) were high risk (HR) (FLT-3 ITD, 11q23 MLL, monosomy 5 or 7, induction failure, or refractory disease), the majority of which received UCB as a stem cell source. The outcomes for these patients were no different than patients transplanted with sibling marrow (OS (72 vs. 78 %, $p = 0.72$), leukemia-free survival, LFS (69 vs 79 %, $p = 0.62$), relapse (11 vs. 7 %, $p = 0.71$), or TRM (17 vs. 14 %, $p = 0.89$)). Thus, transplantation with UCB in CR1 abrogates the high risk factors seen in children and young adults with high risk AML. The Eurocord group reported 95 children receiving UCB transplant for AML [32]. Most patients received a one or two HLA antigen-mismatched UCB transplants. Poor prognosis cytogenetic abnormalities were identified in 29 cases. The 2-year cumulative incidence of relapse was 29 ± 5 % and was higher in patients with advanced disease. Similar to the above study, children with high-risk cytogenetic features had similar DFS compared with other patients (44 ± 11 vs. 40 ± 8 %). Thus, UCBT is a viable therapeutic option for children with very poor-prognosis AML and who lack an HLA-identical sibling.

Although rare in the pediatric population, myelodysplastic syndrome (MDS) is otherwise incurable and therefore transplant is aggressively pursued for nearly all pediatric patients. We have previously shown that DFS was improved in patients that had a shorter time from diagnosis to transplant and in those patients that did not receive pretransplant chemotherapy [33]. Madureira and colleagues studied the outcomes of 70 children with MDS who received single unit UCB transplantation after myeloablative transplant from 1995–2005 [34]. The 3-year DFS was 50 % for transplantations after 2001 compared with 27 % for the earlier period ($p = 0.018$).

After 2001, transplantation was performed earlier (within 6 months of diagnosis) and used UCB units with higher cell dose, possibly explaining these findings. DFS was highest in patients with monosomy 7 (61 %) compared with other karyotypes (30 %), ($p = 0.017$). These data again support the assertion that use of a rapidly available stem cell product (UCB) may be beneficial for patients with high risk hematological malignancies, such as MDS.

5 Remission Status

There are a number of disease-specific factors that are associated with increased risk of relapse after UCB transplant. These include disease type, patient age, and CR status prior to transplantation [15, 35]. We have recently shown that children with advanced B-precursor ALL, > CR3) have inferior outcomes compared to those transplanted in CR2 (30 vs. 75 %, $p = 0.04$) [36]. Moreover, patients that were in CR2 and had a short duration of their first remission had a higher risk of relapse (50 vs. 34 %, $p = 0.06$).

Similar findings have been shown using retrospective registry data by Ruggeri and coworkers at the Eurocord registry [37]. Children with ALL were transplanted after myeloablative conditioning with a single UCB unit ($n = 170$) at varying disease stages (43 % in CR1, 45 % in CR2, and 12 % in CR3; Table 6.1). The cumulative incidence of relapse at 4 years was 30 %. Those transplanted early in their disease course had the lowest risk for disease progression (i.e., CR1 and CR2; HR = 0.3, $p = 0.002$). Probability of LFS for the whole cohort at 4 years was 44 % (56, 44, and 14 % for patients transplanted in CR1, CR2, and CR3, respectively ($p = 0.0001$)). These analyses support transplant early in the disease course, but a randomized intention to treat study comparing chemotherapy to transplant has not been performed in children with relapsed ALL.

In AML, similar principles apply. There are markedly inferior outcomes for pediatric AML patients transplanted not in CR, but whether or not the outcomes are different for cord versus adult donors is not known. In the Eurocord analysis, the 2-year DFS was 42 ± 5 % (59 ± 11 % in CR1 vs. 50 ± 8 % in CR2 vs. 21 ± 9 % for children not in CR) [32]. At the University of Minnesota, we also found interesting results in our study comparing single versus double UCB transplants regarding remission status [20]. In multivariate analysis, relapse was higher in advanced disease patients (> CR3; RR = 3.6; $p < 0.01$), with a trend toward less relapse in recipients of two UCB units (RR = 0.6; $p = 0.07$). However, relapse was lower for early stage patients (CR1–2) who received two UCB units (RR 0.5; $p < 0.03$). Although it is known that transplantation in CR1 and CR2 is associated with less relapse risk, this analysis suggests an enhanced graft-versus-leukemia effect in acute leukemia patients after transplantation with two partially HLA-matched UCB units [20]. Preliminary data of outcomes of 242 patients undergoing UCB transplant at the University of Minnesota also showed the importance of remission status. Patients with AML who were CR2 or greater had a much higher incidence of relapse compared to CR1 (Hazard Ratio CR2/CR2 + = 13.29).

6 Minimal Residual Disease and Transplant Outcomes

Recently, the presence of minimal residual disease (MRD) prior to transplantation has been identified as a major risk for relapse. Numerous studies now show that detectable levels of MRD using sensitive testing methods, such as flow cytometry or PCR, identify patients with a high likelihood for posttransplant relapse. In a landmark study by Bader and colleagues, 91 patients with ALL undergoing myeloablative adult donor transplantation were studied. MRD was detected using PCR against a clonotypic B-cell receptor gene and was defined as $> 10^4$ leukemic cells in the BM at the time of transplant [38]. Patients with pretransplant MRD had a significantly higher rate of relapse (57 % vs. 13 %, $p = 0.001$) and an inferior disease-free survival 27 versus 60 % [38]. To date, only two studies have examined the impact of pretransplant MRD in UCB recipients. Ruggeri and colleagues show that the presence of MRD was a strong predictor of relapse (HR = 0.4, $p = 0.01$; Table 6.1) [37]. We recently conducted a retrospective, single center review examining the outcomes of 86 patients with B precursor ALL transplanted with UCB who had fluorescence-activated cell sorting (FACS)-based MRD testing prior to transplantation. In patients with MRD detected before transplant, the relapse rate at 1 year was higher compared to those that did not have detectable MRD (30 vs. 13 %, $p = 0.02$) [39]. These studies demonstrate the importance of this new methodology and predict that it will continue to guide management of children prior to transplantation. As well, MRD + patients represent a high-risk group where novel approaches to relapse prevention could be tested, such as rapid tapering of immune suppression or targeted therapies.

7 Graft Content, GVHD, GVL, and Immune Reconstitution Post-UCB Transplant

There are fundamental differences between UCB and other stem cell sources. For instance, essentially all UCB units are cryopreserved while the opposite is the case for adult donors. While this provides a degree of flexibility in favor of UCB, it also introduces additional variables that do not apply to other types of transplantation. For instance, there is significant variability in postthaw viability of clinical units [40]. This variable (postthaw viability) has significant impact on UCB engraftment, but less is known about whether this also influences GVHD and GVL reactions after transplantation [40].

There are also dramatic differences between the various stem cell sources and the cell dose and the type of cell infused. Significantly fewer cells are delivered with UCB transplant (> fivefold fewer lymphocytes) than BM transplant. Moreover, in contrast to BM or PB from adult donors the vast majority of UCB T cells are naïve. Similarly, the NK cells in UCB and adult blood are distinct. UCB contains a higher fraction of the less mature $CD56^{bright}$ NK cells and fewer terminally matured NK cells than adult blood. Most studies suggest that UCB NK cells have lower cytotoxicity, but intact cytokine production [41]. Interestingly, following short incubation (24 h)

with IL-2 or IL-15, we and others have found that UCB NK cells rapidly acquire cytotoxicity comparable (or even higher) than adult NK cells, suggesting that once infused, these cells may behave similar in GVL reactions [41].

These differences between the lymphocytes in UCB and adult blood have led to concerns about the ability of UCB to mediate GVL effects. However, the incidence of leukemia recurrence following UCB transplantation is not different from that reported in recipients of BM or even PB [13]. Interestingly, high absolute lymphocyte counts (ALCs) early after transplantation (days 21–30) have been associated with lower rates of disease relapse following adult blood or marrow transplantation [42−44]. To address whether similar principals apply in the UCB setting, we examined the impact of early ALC at Day + 30 after transplantation. For patients with a Day + 30 ALC > 200, LFS was significantly better [45]. However, unlike most of the other studies in this area, UCB recipients with rapid count recovery did not experience less relapse. Instead, high ALC was an independent risk factor for significantly less NRM and a lower density of infections. Moreover, Parkman and colleagues evaluated pediatric leukemia patients transplanted with UCB and assess the development of antiviral immunity after transplant [17]. The presence of an antigen-specific response resulted in a dramatic relapse-free survival advantage ($p = 0.0001$), which was primarily due to a decrease in leukemic relapse ($p = 0.003$) [17]. In another study, thymic function was found to correlate with relapse [46]. In this study, two groups of children receiving either an HLA-haploidentical family donor or an UCB donor were evaluated for both relative and absolute beta-thymic receptor excision circles (TREC) values, which are a measure of postthymic, naïve T cells. Patients who ultimately relapsed after transplant had a significantly less efficient thymic function both before and 6 months after transplant, with especially low beta-TREC values, suggesting a relationship between early intrathymic T-cell differentiation and the occurrence of leukemia relapse [46]. These studies suggest that functional T cell immunity post transplant confers a survival advantage due to decreased leukemia relapse, possibly because of increased GVL.

The exact mechanisms of GVL are not well understood. Many have speculated that GVL varies based on a complex combination of factors that include the precise details of the leukemic clone, as well as the genetics of the donor and the recipient. One unique aspect of UCB transplantation is that MHC disparity nearly always occurs, perhaps leading to greater alloreactivity. Whether this MHC disparity is tolerated because of the relatively low dose of cells contained within the graft, the naïve status of the cells or because the cells have been present in highly tolerogenic environment (i.e., maternal-placental interface) is unknown. We speculate that because of this, the mechanisms of GVL may be different in the UCB compared to other stem cell sources. In support of this, a number of studies that have compared UCB to adult stem cell sources have demonstrated either equivalence or superiority of UCB compared to either adult BM or PB. [47] The vast majority of studies show very low rates of relapse; however, NRM is a competing risk factor and this is typically higher in cord transplantation. While UCB has the many positive attributes outlined above, there are a number of disadvantages, such as the inability to go back to the donor for additional lymphocytes for customized cell therapy such as those directed to either viruses or to

enhance GVL. A significant proportion of the units now being collected are stored in split bags (typically 20–80 bags). This would allow for customized therapies using the smaller fractions, while maintaining the majority of cells for transplantation. While this is not currently being done, we envision such applications in the future.

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Chapter 7
Results of Cord Blood Transplantation in Children with Nonmalignant Hematologic Conditions

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1 Introduction

Encompassing a heterogeneous group of disorders, the nonmalignant hematological diseases include congenital and acquired bone marrow failure (BMF) syndromes, primary immunodeficiencies (PIDs), hemoglobinopathies, and others. In these diseases, donor-derived cells are used to replace or repopulate a dysfunctional hematopoietic or immune system through hematopoietic stem cell transplantation (HSCT). Special considerations for these patients with nonmalignant conditions include minimization of the risk of acute and chronic graft-versus-host disease (GHVD) and late toxicities caused by the preparative regimen. Cord blood (CB) has been used as a source of donor hematopoietic stem cells (HSCs) for patients without fully matched related donors (MRDs) or matched unrelated donors (MUDs) increasing access to transplantation for some patients. However, because CB grafts contain lower numbers of total nucleated cells (TNCC), the use of CB in patients with resistance to engraftment or undergoing reduced intensity conditioning (RIC) for transplantation has been challenging. Other than the immune deficiencies, patients with other inherited hematological diseases have normal immune systems, which pose an additional challenge to engraftment after CB transplantation (CBT). In this chapter, we will describe the experience to date using CBT to treat nonmalignant hematopoietic diseases and efforts underway to improve these outcomes.

2 Inherited Bone Marrow Failure Syndromes

Inherited bone marrow failure (IBMF) syndromes are a diverse group of diseases that can affect single or multiple cell lineages leading to failure of production of one or several hematopoietic lineages. Many of the congenital BMF syndromes involve

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additional somatic abnormalities, chemotherapy and/or radiation sensitivity, or an increased risk of malignancies all of which may complicate the care of and influence the long-term prognosis for these patients.

2.1 Fanconi Anemia

Fanconi Anemia (FA) is characterized by congenital abnormalities, progressive BMF, and increased susceptibility to cancers [1]. Early attempts at HSCT in FA patients resulted in high transplant-related mortality (TRM) and poor overall survival (OS) [2, 3] predominantly due to the fact that FA patients are exquisitely sensitive to certain chemotherapy agents [4], irradiation [5], and have difficulties repairing tissue damage caused by GVHD [6]. Modifications reducing the intensity of conditioning regimens, specifically incorporating Fludarabine (Flu) and avoiding radiation, have dramatically improved the TRM [5, 7–10]. Recently, the European Group for Blood and Marrow Transplantation (EBMT) reported on outcomes of 795 FA patients transplanted with related or unrelated bone marrow and/or mobilized peripheral blood stem cells (PBSCs) spanning a 40-year timeframe [10]. Improved outcomes after HSCT were seen in younger (< 10 years) patients and those without evidence of leukemic transformation or abnormalities in the bone marrow [8, 10–13]. Independently, the development of GVHD was associated with increased rates of second malignancies [6, 14], which in turn, negatively impacted the long-term OS.

2.2 Related Cord Blood Transplantation Experience

Based on the success of the first related CBT (RCBT) in a young boy with FA [15], who remains healthy with full donor chimerism > 25 years later, the use of HLA-matched, nonaffected MRD CB has become a standard of care. Gluckman et al., on behalf of the Eurocord registry, described 36 FA patients among a larger cohort of RCBT recipients ($n = 596$). Overall, neutrophil engraftment occurred in a median of 22 days with a cumulative incidence (CI) of 91 % (± 2). The 4-year OS for nonmalignant patients, including FA, was 91 % (± 2) [16]. Comparatively, after related bone marrow transplantation (BMT), engraftment rates and OS for FA patients in recent reports exceeded 85 and 75 % at 5 years, respectively [5, 10, 17, 18]. These comparable outcomes justify the use of either marrow or CB from an MRD for transplantation in FA patients. When a CB unit (CBU) has been banked from a MRD, the available TNCC dose will guide the decision to use the CBU alone or in combination with marrow from the donor.

2.3 Unrelated Cord Blood Transplantation Experience

Unfortunately, most FA patients will not have a nonaffected MRD. Traditionally, bone marrow from MUDs has been used although CB is an attractive option for FA

patients given the lower incidence of GVHD. Early experience with unrelated CBT (UCBT) in FA patients, as described by Rubinstein et al., demonstrated difficulties with engraftment and higher TRM (Table 7.1) [19]. Gluckman et al., described similar issues in a retrospective review of FA patients who received UCBT from 1994–2005 ($n = 93$). In this report, the CI of neutrophil engraftment was only 60 % (± 5 %) at 60 days with a 3-year OS of 40 % (± 5 %). However, improved engraftment was seen in patients receiving Flu-based conditioning (72 vs. 42 %) and receiving TNCC $\geq 4.9 \times 10^7$/kg (50 vs. 25 %). These two factors along with negative cytomegalovirus (CMV) recipient status were also associated with significantly improved survival (Flu-based regimen 50 % vs. non-Flu regimen 25 %; higher TNCC 49 vs. lower TNCC 31 %; CMV negative 64 vs. CMV positive 26 %) [20]. Ruggeri et al., explored the use of double UCBT as salvage therapy for high-risk BMF syndromes (defined as prior graft failure or leukemic transformation) in a series of 14 patients (eight with FA). While the 2-year OS was 33 % (± 16 %) for the FA patients, this approach could be considered for FA patients who have undergone leukemic transformation or failed a prior transplant and otherwise have an extremely poor outcome [21].

3 Other Inherited Bone Marrow Failure Syndromes

In these rare diseases, previous HSCT experience for other IBMF syndromes has informed approaches to CBT such as timing, donor selection, and conditioning regimens. The timing of HSCT is an important consideration for certain IBMF syndromes. In severe congenital neutropenia (SCN), outcomes are improved when HSCT occurs prior to malignant transformation [22]. In Diamond-Blackfan anemia (DBA), transfusion-dependent patients are at increased risk of toxicity due to iron overload. Therefore, HSCT is recommended, for DBA patients failing medical management, when they are younger, well chelated or not heavily transfused [23, 24]. Higher graft failure rates have been seen in infants with malignant infantile osteopetrosis [25–29] which may be important when selecting donors. Conditioning regimens for these diseases have been influenced by the increased toxicity observed in patients with Dyskeratosis Congenita (DC; pulmonary/liver; [30–33]), Shwachman-Diamond Syndrome (SDS; cardiac [34]), and osteopetrosis (pulmonary hypertension/veno-occlusive disease or VOD/hypercalcemia; [25–29]). Using RIC approaches in DC has shown success but the role of CBT in this disease has yet to be defined [35, 36].

3.1 Related Cord Blood Transplantation

The literature describing RCBT for non-FA IBMF syndromes (including DBA, DC, congenital amegakaryocytic thrombocytopenia (CAMT), SCN, and SDS) is limited to individual cases included in small series [37, 38] or incorporated into larger analyses (overlap of patients is possible; Table 7.1 [39–43]. In the largest dedicated

CBT series, Bizzetto et al., on behalf of Eurocord, reviewed the outcomes of CBT recipients ($n = 64$) for non-FA IBMF syndromes after primarily myeloablative conditioning (80 %) from 1994–2008 [44]. Twenty patients, most commonly diagnosed with DBA ($n = 13$), received MRD CB grafts. Almost all RCBT patients engrafted with a CI by day 60 of 95 % (95 % confidence interval, *95 %CI* 85–100 %) and an estimated 3-year OS of 95 % (*95 %CI* 85–100 %). The results described for RCBT in these diseases demonstrate comparable outcomes to those seen after MRD BMT [23, 33, 43, 45]. While there are small numbers of IBMF patients transplanted with related CB, the chronic nature of these diseases and superior outcomes using MRDs justify recommendations for families to consider preimplantation diagnosis as an approach to increasing availability of matched CB donors for their affected child [46]. In addition, family or directed donor CB banking should be facilitated for families with an affected child or those families known to be at risk for conceiving a future child affected with an IBMF syndrome.

3.2 Unrelated Cord Blood Transplantation

The experience to date using UCBT for the non-FA IBMF syndromes has shown mixed results (Table 7.1). In their analysis of 44 UCBT recipients, Bizzetto et al., reported a CI of neutrophil engraftment of 55 % by day 60 with a high rate of primary graft failure (39 %). The OS at 3 years was 61 % (*95 %CI* 47–75 %) but improved OS was seen in younger patients (< 5-years old) receiving high TNCC doses ($\geq 6.1 \times 10^7$/kg) [44]. Several disease-specific trends were also observed within this cohort. All patients with DC, receiving UCBT, died (seven transplant-related causes, one unknown). As most patients in this series received myeloablative conditioning, regimen-related toxicity may have played a role in the high TRM observed in the DC patients. Subsequently, Dietz et al., described a RIC approach in six DC patients receiving unrelated HSCT (UCBT $n = 3$) [47]. Two of the three UCBT patients were alive at 12 months with one death secondary to infection. Longer follow-up is needed to fully evaluate the impact of an RIC approach in DC. In the eight patients with DBA, Bizzetto observed three deaths due to graft rejection and two toxicity-related deaths. Others have also seen challenges to engraftment in DBA [48–51]. At our institution from 1996–2011, we have transplanted six children with DBA using unrelated CB grafts after myeloablative busulfan (Bu)-based conditioning. All engrafted with full-donor chimerism. Four are alive, well, and are transfusion-free ranging from 3–16 years posttransplant. All these children received high TNCC grafts which may have overcome engraftment barriers seen by others [52].

Recently, Ruggeri et al., described the outcomes of 45 children with osteopetrosis who received UCBT from 1995–2012 as reported to the Eurocord registry [53]. Almost all received myeloablative conditioning (primarily Bu-based regimens) followed by infusion of CB grafts delivering high TNCCs doses. Despite this, 18 of the 45 patients experienced graft failure with only three of 18 surviving. VOD was seen in six patients (two VOD-related deaths). At 3 years, the OS for these children was 45 (± 8 %) [53]. Using RIC regimens followed by UCBT has largely been unsuccessful in this disease with only one survivor reported [54–56].

Table 7.1 Studies describing the use of cord blood transplantation to treat inherited bone marrow failure syndromes (Overlap of patients may occur)

Disease	Cord blood recipients reported in literature		Engraftment CI (95 % CI) or engrafted/total	Survival CI (95 % CI) or alive/total	Key citations
	Related	Unrelated			
Fanconi anemia		93	60 % (± 5) at day 60	40 % (± 5) OS at 3 years	Gluckman [20]
		35	64 % (44–84) at day 42	UNK	Rubinstein [19]
	8	8	5 of 8 RCBT; UCBT UNK	5 of 8 RCBT; UCBT UNK	Gluckman [41]
		8	5 of 8	3 of 8	Ruggeri [21]
	Total $n = 15$	Total $n = 147$			Also [39, 50, 112, 113]
Dyskeratosis congenita	2	6	1 of 2 RCBT; UCBT UNK	1 of 2 RCBT; 0 of 8 UCBT	Bizzetto [44]
		3	2 of 3	2 of 3	Dietz [47]
	Total $n = 3$	Total $n = 11$			Also [21, 41, 114]
Schwachman Diamond	1	1	1 of 1 RCBT; 1 of 1 UCBT	1 of 1 RCBT; 1 of 1 UCBT	Bizzetto [44]
		3	3 of 3	3 of 3	Vibhakar [115]
	Total $n = 1$	Total $n = 2$			Also [116, 117]
Diamond blackfan anemia	13	8	12 of 13 RCBT; UCBT UNK	13 of 13 RCBT; 3 of 8 UCBT	Bizzetto [44]
		6	6 of 6	4 of 6	McFarren [52]
	Total $n = 16$	Total $n = 20$			Also [19, 39, 41, 50]
Congenital amegakaryocytic thrombocytopenia	1	4	1 of 1 RCBT; 4 of 4 UCBT	1 of 1 RCBT; 4 of 4 UCBT	Mahadeo [38]
	3	13	3 of 3 RCBT; UCBT UNK	2 of 3 RCBT; 9 of 13 UCBT	Bizzetto [44]
	Total $n = 5$	Total $n = 20$			Also [40, 118–120]
Severe congenital neutropenia		4	4 of 4	2 of 4	Ferry [121]
	1	15	1 of 1 RCBT; UCBT UNK	1 of 1 RCBT; 10 of 15 UCBT	Bizzetto [44]
		5	4 of 5	5 of 5	Morio [98]
		4	3 of 4	4 of 4	Oshima [122]

Table 7.1 (continued)

Disease	Cord blood recipients reported in literature		Engraftment CI (95 % CI) or engrafted/total	Survival CI (95 % CI) or alive/total	Key citations
	Related	Unrelated			
	Total $n = 2$	Total $n = 28$			Also [37, 123–125]
Acquired severe aplastic anemia	92	9	6 of 9 91 % (89–93 %) at day 60[a]	77 % (50–100 %) 91 % (89–93 %) at 4 years[a]	Chan [59] Gluckman [16]
		4	2 of 4	3 of 4	Ruggeri [21]
		19	63 % (33–93 %) at day 42	UNK	Rubinstein [19]
		18	0 of 16	16 of 18	Liu [126]
		31	55 % at day 42 (36–70 %)	41 % (24–58) at 2 years	Yoshimi [127]
	Total $n = 97$	Total $n = 101$			Also [112, 128]
Osteopetrosis	Total $n = 0$	45 Total $n = 64$	27	45 % (37–53 %) OS at 3 years	Ruggeri [53] Also [19, 41, 50, 56, 129, 130]

CI cumulative incidence, *95 % CI* 95 % confidence interval, *RCBT* related cord blood transplantation, *UCBT* unrelated donor cord blood transplantation, *UNK* data is unavailable

[a] Refers to overall cohort for engraftment and nonmalignant cohort for survival data

4 Acquired Bone Marrow Failure Syndromes

Experience using CBT in acquired severe aplastic anemia (SAA) is more limited and was initially discouraging. In an early report of CBT outcomes by Rubinstein et al., SAA patients experienced high rates of adverse transplant events (i.e. autologous recovery, graft failure or death; CI of 80 % at 180 days) and poor survival [19]. The largest study of UCBT in SAA, reported by Eurocord and EBMT, analyzed 71 primarily pediatric patients with SAA transplanted with either a single (79 %) or double UCBT from 1996–2009. The CI of neutrophil engraftment by day 60 was only 51 % (± 6 %) with a 3-year OS of 38 % (± 6 %) [57]. Improved engraftment (58 vs. 33 %) and 3-year OS (59 vs. 49 %) was seen in patients receiving higher precryopreservation TNCC doses ($> 3.9 \times 10^7$/kg) as compared to those receiving lower doses, respectively [57]. This highlights the importance of higher TNCC dosing to potentially overcome engraftment barriers. Interestingly, a trend towards improved OS was seen in patients receiving a Flu-based RIC approach as opposed to myeloablative conditioning. All patients receiving TBI (12 Gy) died in this series [57]. Several small series have also described RIC regimens followed by UCBT with mixed results [58, 59]. To increase the available TNCC dose, Childs et al., investigated the co-infusion of a single CBU along with $CD34^+$-selected HSCs collected from a haploidentical relative in eight treatment-refractory pediatric SAA patients. All patients promptly engrafted (seven with CBU cells, one haploidentical cells). Early T-cell chimerism predominately indicated engraftment of the CB cells. Conversely, initial myeloid engraftment was from the haploidentical cells, but over time transitioned to CB engraftment. Seven of the eight patients are alive and transfusion-independent. One died 14-months posttransplantation due to infectious causes. They observed that the haploidentical cells shortened the time to neutrophil engraftment by providing a bridge until cord engraftment could occur [60]. Therefore, additional data is needed to optimize conditioning and graft selection, but well-matched CBUs delivering a high cell dose is a viable donor option for refractory SAA patients.

5 Hemoglobinopathies

Thalassemia major and sickle cell disease (SCD) are the most common hemoglobinopathies worldwide. While the major life-threatening complications associated with thalassemia are red blood cell transfusion dependence and the long-term effects of iron overload, the fundamental problem with SCD is vasculopathy and resulting tissue ischemia. Allogeneic HSCT is currently the only curative treatment and best outcomes occur if HSCT is performed early in life, before significant organ dysfunction has occurred. The first successful allogeneic HSCT for thalassemia and SCD were reported using bone marrow from MRDs in the early 1980s [61, 62]. Over the ensuing three decades, > 1500 patients with these conditions have undergone allogeneic HSCT, predominantly using MRDs, using Bu/Cyclophosphamide (Cy)-based myeloablative regimens, associated with a high disease-free survival

(DFS; 80–90 %). To refine criteria for patient selection, the Pesaro group established a risk classification to predict HSCT outcomes for children (< 18 years) with thalassemia defining three adverse risk factors: inadequacy of prior iron chelation, hepatomegaly, and portal fibrosis. Pesaro class I includes patients with no risk factors; Pesaro class II patients have 1–2 risk factors and Pesaro class III patients have all three risk factors. In earlier reports, the DFS for class I-II patients was 80–90 %, whereas that for class III was inferior at 50–60 % [63]. With modifications in the conditioning approach to increase immunosuppression but reduce regimen-related dose intensity for class III patients, improved outcomes comparable to lower risk groups were demonstrated [64].

The allogeneic HSCT experience for SCD is less extensive than that for thalassemia, but similar in principle and sufficient to prove its curative efficacy. Currently, widely accepted indications for HSCT include patients with moderate to severe SCD with one or more of the following: stroke, increased risk of stroke based on elevated transcranial doppler velocities, multiple acute chest syndromes, and multiple vaso-occlusive crises. DFS from American and European studies using predominantly Bu/Cy-based myeloablative regimens in MRD BMT ranges from 82–86 % [65–67]. Stabilization of vasculopathy and pulmonary function has been demonstrated after HSCT [68]. Gonadal toxicity remains a significant long-term concern with myeloablative regimens [69], however this risk can be partially offset with fertility preservation prior to chemotherapy in patients who have reached puberty.

While the results of HSCT from a MRD are excellent, the majority of these patients do not have an available MRD. As most of these patients will be unable to locate a fully HLA-matched adult donor on the donor registries, unrelated CB is being explored as an alternative donor source for these patients. However, the prospect of increased early TRM and long-term effects associated with unrelated HSCTs needs to be weighed carefully against the risks associated with the natural course of an individual patient's disease.

5.1 Related Cord Blood Transplantation

Since the first successful reports of RCBT in patients with hemoglobinopathies almost 20 years ago [70, 71], there has been significant interest in CB for these patients. To facilitate collections, a sibling donor CB bank was established in Oakland, California in 1998. Using related CBUs supplied by this bank, Walters et al., described the clinical outcomes of 22 patients with hemoglobinopathies (14 thalassemia, 8 SCD) who received myeloablative conditioning (Bu/Cy ± anti-thymocyte globulin, ATG) followed by RCBT. They observed a very good DFS (18 of 22 patients) although follow-up was limited to a median of 12.4 months [72]. Locatelli et al., on behalf of the Eurocord registry, described 44 RCBT recipients for either thalassemia (Pesaro class I-II patients $n = 33$) or SCD ($n = 11$) [73]. These patients also received Bu-based myeloablative regimens along with combinations of Cy, Flu, or thiotepa. The median infused TNCC was 4.0×10^7/kg. All patients survived in this multicenter

study, thus highlighting the safety of this approach. The 2-year event-free survival (EFS) was 90 and 79 % for SCD and thalassemia, respectively, with graft failure being the most common adverse event. Improved EFS was seen in thalassemia patients when hydroxyurea +/−thiotepa and/or Flu were added as opposed to Bu/Cy alone [64].

Recently, Locatelli et al., on behalf of Eurocord, compared the outcomes of 485 patients with hemoglobinopathies using either MRD CB or bone marrow grafts [74]. Survival was excellent regardless of donor source. They observed a 6-year DFS of 86 and 92 % after BMT in thalassemia and SCD patients, respectively. Comparatively, the 6-year DFS after RCBT was 80 and 90 % for thalassemia and SCD patients, respectively. Slower neutrophil engraftment was seen in the RCBT group, but also less GVHD confirming previous reports [40, 73]. Notably, no RCBT recipient experienced extensive chronic GVHD compared to 5 % of MRD BMT recipients [74]. Since graft-versus-leukemia effect is not required in these patients, the decreased risk of GVHD seen with MRD CB donors can be a very important factor when selecting the optimal donor for transplantation. Furthermore, directed donor CB banking should be encouraged in these families when nonaffected siblings are born.

5.2 Unrelated Cord Blood Transplantation

While the RCBT data is excellent, the use of UCBT for hemoglobinopathies is more challenging. These patients have an inherent increased risk of graft rejection due to various factors such as marrow hyperactivity to compensate for chronic anemia, alloimmunization resulting from multiple transfusions, and lack of prior chemotherapy exposure leaving the patient immunocompetent immediately prior to transplant. In addition, the lower cell dose available from a CB graft is less likely to outcompete autologous recovery. Successful case reports using UCBT in thalassemia patients have been published from a few centers (Table 7.2) [75–77]. Jaing et al., reported the outcomes of 35 pediatric patients with thalassemia undergoing UCBT after myeloablative conditioning (Bu/Cy/ATG). Most received a single CB graft (68 %) that was HLA-mismatched at 1–2 loci (83 %). Despite grafts delivering a high median TNCC (7.8×10^7/kg), the CI of engraftment was 70 % with six patients experiencing graft failure. A high incidence of grade II-III acute GVHD was also reported (80 %), although no severe acute and minimal chronic GVHD was seen. The 5-year OS and DFS were 88.3 and 73.9 %, respectively. While these results are encouraging, the GVHD and graft failure rates are significantly higher with a corresponding decrease in survival when compared with MRD grafts [78].

The challenges of UCBT in hemoglobinopathies were further highlighted by several recent studies [79, 80]. Ruggeri et al., on behalf of Eurocord, the Center for International Blood and Marrow Transplant Research (CIBMTR) and the National Cord Blood Program, recently reported the outcomes of UCBT in 51 children with either thalassemia ($N = 35$) or SCD ($N = 16$). Most patients (76 %) received myeloablative

Table 7.2 Studies describing the use of cord blood transplantation to treat hemoglobinopathies[a]

Disease	Cord blood recipients reported in literature		Engraftment CI (95 % CI) or (Engrafted/Total)	Survival CI (95 % CI) or (Alive/Total)	Key citations
	Related	Unrelated			
Thalassemia	9		55 % at day 60	44 % EFS at 1 year; 88 % OS at 1 year	Fang [76, 131]
	14		13 of 14	12 of 14 ESF	Walters [72]
	66		90 % (±4) at day 60	80 % (±5) DFS at 6 years	Locatelli [73, 74]
	145		91 % (±2) at day 60[a]	91 % (±2) at 4 years[a]	Gluckman [16]
		35	15 of 35 (43 %)	62 % (±9) OS at 2 years; 21 % (±7) DFS at 2 years	Ruggeri [80]
		35	70.7 ± 7.8 %	88 % (±7) OS at 5 years; 74 % (±7) DFS at 5 years	Jaing [78]
		4	100 % by day 42	100 % OS at 1 year; 100 % DFS at 1 year	Parikh [84]
		9	8 of 9	UNK	Shenoy [85]
	Total $n = 236$[a]	Total $n = 86$			Also [70, 75–77, 132]
Sickle Cell Disease	8		75 %; 6 of 8	75 % OS at 1 year	Walters [72]
	30		90 % (±4) at day 60	90 % (±5) DFS at 6 year	Locatelli [17, 73, 74]
		7	43 %; 3 of 7	86 % OS at 2 years; 29 % EFS at 2 years	Adamciewicz [79]
		16	9 of 16 (56 %)	94 % (±6) OS at 2 years; 50 % (±9) DFS at 2 years	Ruggeri [80]
		8	3 of 8	38 % (9–66) EFS at 1 year	Kamani [81]
		8	62.5 % at day 60	62.5 % OS at 2 years; 50 % EFS at 2 years	Radhakrishnan [82]
	48		91 % (±2) at day 60[a]	91 % (±2) at 4-years[a]	Gluckman [16]
	Total $n = 87$[a]	Total $n = 39$			Also [71]

CI cumulative incidence, *95 % CI* 95 % confidence interval, *RCBT* related cord blood transplantation, *UCBT* unrelated donor cord blood transplantation, *UNK* data is unavailable

[a] Overlap of patients may occur

conditioning followed by infusion of primarily HLA-mismatched grafts (2–3 loci: 50 %). High graft failure was seen in this cohort (27 of 51 patients) which was associated with the TNCC cell dose. The 2-year probability of DFS was 45 and 13 % in patients above and below a TNCC threshold of 5×10^7/kg, respectively [80]. While this report reviews retrospective registry data, it raises concerns about the overall feasibility of UCBT as currently practiced by the general transplant community. Standardized approaches for conditioning and supportive care should be developed and validated in specialized centers and then exported for widespread applications.

In an effort to address the transplant-related toxicities, RIC regimens have been used in patients with hemoglobinopathies. However, engraftment is even more challenging in CBT when using RIC regimens. Kamani et al., on behalf of the Blood and Marrow Transplant Clinical Trials Network (Blood and Marrow Transplant Clinical Trials Network 0601, SCURT, trial), reported an unacceptably high graft failure rate in the cohort of SCD patients who received RIC regimen (alemtuzumab/Flu/Melphalen) followed by UCBT. The EFS for these patients was only 37.5 % [81]. Similarly, Radhakrishnan et al., using an alemtuzumab/Flu/Bu RIC regimen, reported a DFS of 50 % [82]. At Duke University, we have successfully treated four thalassemia patients with UCBT using a modified reduced-toxicity regimen by augmenting the alemtuzumab/Flu/Mel backbone [81, 83] with thiotepa and hydroxyurea [84]. All of the patients have had durable engraftment with a median follow-up of 22 months (range, 21–37 months). A multicenter study using a similar conditioning regimen (URTH trial) was recently completed with encouraging results. Nine patients with thalassemia underwent UCBT (HLA-matched = 1, 1-loci mismatch = 8), with graft failure in one patient (median follow-up of 1 year) [85].

Several experimental approaches to increase the available TNCC for transplantation involving *ex-vivo* manipulation to improve engraftment of CBUs in patients with hemoglobinopathies are ongoing. At Duke University, early results are promising using a nicotinamide-based expansion approach (NiCord®) in patients with SCD. Pilot trials are ongoing with other expansion technologies in patients with hematologic malignancies and, if successful, these approaches may ultimately be applied to UCBT for patients with hemoglobinopathies, thus broadening the utility of UCBT in these diseases [86–90].

6 Primary Immunodeficiencies

The PIDs) are a clinically heterogeneous group of disorders associated with frequent life-threatening infections and early mortality. In 1968, the first successful allogeneic transplant was performed in a patient with severe combined immune deficiency (SCID) using bone marrow from an MRD [91]. Over 40 years later, allogeneic HSCT has been successfully used to treat many of the PIDs including SCID, Wiskott-Aldrich syndrome (WAS), CD-40 Ligand deficiency (Hyper-IgM), other combined immune deficiencies, phagocytic function defects such as chronic granulomatous disease (CGD), and defects of immune regulation such as hemophagocytic lymphohistiocytosis (HLH;) [92]. When available, MRD bone marrow remains the preferred graft source for patients with PIDs. Best outcomes are achieved when

HSCT is performed early in life prior to development of serious infections and related comorbidities. For patients without an MRD, CB is an attractive option but is limited by the available TNCC dose. However, patients with PID tend to be younger and adequate to high cell doses are often available. Another immediately available alternative, especially for patients with certain types of SCID, is a haploidentical or mismatched related donor (MMRD) bone marrow or peripheral blood stem cells (PBSCs) from one of the parents or relatives. The relative merits of these two graft sources are a matter of active research.

6.1 Cord Blood Transplantation Experience

The experience using CBT to treat patients with PIDs, as detailed in case reports and smaller series, has generally been successful for both related [40, 93–95] and unrelated donor CBTs (Table 7.3) [96]. Excellent survival (91 %) was noted by Gluckman et al. in a cohort of RCBT recipients with nonmalignant diseases ($n = 301$), 36 of whom had SCID [16]. Comparatively, the 5-year OS for PID patients who received myeloablative conditioning followed by UCBT was approximately 70 % in several retrospective reports [97, 98]. Similar rates of GVHD were seen as compared to other UCBT series. Normal immunologic reconstitution including intravenous immunoglobulin independence was seen in all of the survivors described by Diaz de Heredia. The results of the prospective Cord Blood Transplantation Study (COBLT) performed from 1999–2004, in a cohort of PID patients, were less promising. These patients received myeloablative (Bu/Cy/ATG) conditioning followed by infusion of high TNCC grafts (median 9.3×10^7/kg). The CI of neutrophil engraftment was relatively low in this cohort (58 % at 42 days) and the OS was 62.5 % at 1 year [99]. Higher TNCC and $CD34^+$ dose were associated with better neutrophil engraftment.

7 Experience with Specific Primary Immunodeficiencies

7.1 Severe Combined Immune Deficiency

The use of CBT to treat SCID has been described in many case reports and small series (Table 7.3) [93, 97, 99–102]. In general, the preparative regimen and donor selection for SCID patients is dictated by the immunologic profile of the individual patient. Fernandes et al., on behalf of Eurocord and EBMT, recently described the HSCT outcomes of 249 SCID patients who received either UCBT ($n = 74$) or MMRD HSCT ($n = 175$) [100]. Despite some differences between the cohorts, engraftment rates were similar. However, a higher proportion of UCBT recipients achieved full donor myeloid chimerism, and B-cell engraftment. The UCBT patients were also less likely to need a repeat transplant due to poor graft function. The 5-year OS was similar between the two cohorts (57 and 62 % for UCBT and MMRD HSCT patients, respectively) despite higher incidence of chronic GVHD in the UCBT group.

Improved survival was noted when UCBT patients received HLA-matched CBU grafts (76 %) as compared to mismatched grafts (1-locus: 62 %, 2-loci: 35 %) [100]. Chan et al. described similar findings in two half-siblings with X-linked SCID, one treated with maternal haploidentical BMT and the other with UCBT matched at seven of eight loci, who received the same myeloablative preparative regimen. The sibling with UCBT achieved > 95 % donor T-cell chimerism and B-cell engraftment, whereas the sibling undergoing haploidentical BMT did not achieve B-cell engraftment and had mixed T-cell chimerism (29 % donor) [101]. Therefore, while both donor sources are viable options, immune reconstitution appears to be more robust after UCBT.

7.2 Wiskott-Aldrich Syndrome

Based on a multicenter study of European and American centers, Moratto et al. described the long-term HSCT outcomes of WAS patients ($n = 194$ total), most receiving myeloablative conditioning, with a subset receiving UCBT ($n = 24$, 12.4 %). Excellent 5-year OS (89 %) was seen in patients transplanted more recently (including all UCBT recipients). Compared to other PIDs, higher rates of autoimmunity and neurologic sequelae were observed post-HSCT. Most patients surviving > 1 year achieved full-donor chimerism (72.3 %), although those with mixed chimerism experienced higher rates of posttransplant complications. While MRD BMT recipients experienced the best outcomes, there was no selective benefit to any one unrelated donor source [103]. Similarly, Morio et al. also noted a very good 5-year OS (82 %) in the subset of UCBT recipients with WAS ($n = 23$ of total 88 PID patients), although they had higher rates of GVHD and infection compared to other PID patients [98]. Future studies should include a detailed analysis of immune recovery in these patients to better understand the posttransplant immunological issues seen. In the meantime, UCBT is very reasonable in the setting of myeloablative conditioning.

7.3 Hemophagocytic Lymphohistiocytosis

Due to prior treatment for their underlying disease, many patients with HLH will develop severe organ dysfunction prior to transplant leading to higher rates of TRM with myeloablative transplants [104]. Improved outcomes have been noted with Flu/Mel-based RIC regimens using bone marrow grafts, as reported by the groups from Great Ormond Street Hospital, London, UK and Cincinnati Children's Hospital, USA [105, 106]. To date, the experience with UCBT is limited (Table 7.3). In a recent report from Japan, a 2-year OS and EFS of 65 (± 7 %) and 58 % (± 7 %), respectively, was seen after allogeneic HSCT in patients with HLH ($n = 53$). Of these, 38 patients underwent UCBT after either myeloablative ($n = 25$) or RIC conditioning using Flu/Mel ($n = 13$). Higher graft failure rates were noted after RIC regimens (15 of 22 and 4 of 10 engrafted in the myeloablative and RIC groups, respectively)

Table 7.3 Studies describing the use of cord blood transplantation to treat primary immunodeficiencies (Overlap of patients may occur)

Disease	Cord blood recipients reported in literature		Engraftment CI (95 % CI) or (Engrafted/Total)	Survival CI (95 % CI) or (Alive/Total)	Key citations
	Related	Unrelated			
SCID		3	3 of 3	3 of 3 EFS at 1 year	Knutsen [102]
	1	7	1 of 1 RCBT; 6 of 7 UCBT	1 of 1 RCBT; 5 of 7 UCBT	Bhattacharya [93]
		11	11 of 11 at day 42	7 of 11 OS at 2 years	Diaz de Heredia [97]
		4	2 of 4	1 of 4 at 1 year	Frangoul [99]
		40	74 % at day 100	71 % OS at 5 years	Morio [98]
	36		91 % (± 2) at day 60[a]	91 % (± 2) at 4 years[a]	Gluckman [16]
		74	86 % at day 28	57 % (± 6) at 5 years	Fernandes [100]
	Total $n = 37$	Total $n = 139$			
WAS		5	3 of 5	5 of 5 OS at 1 year; 3 of 5 DFS at 1 year	Frangoul [99]
		23	91 % at day 100	82 % OS at 5 years	Morio [98]
		24	UNK	UNK	Moratto [103]
	Total $n = 0$	Total $n = 53$			Also [97]
CGD	1	7	83 % (55–100) at day 60	100 % OS at 3 years	Tewari [95]
		7	43 % at day 100	43 % OS at 1 year	Morio [98]
	Total $n = 4$	Total $n = 17$			Also [108–110, 133, 134]
HLH		38	UNK	62 % (± 10) OS at 2 years[b]; 59 % (± 10) DFS at 2 years[b]	Sawada [107]
		13		70 %	Duke Unpublished
		5	3 of 5	2 of 5 OS at 2 years	Frangoul [99]
		9	9 of 9	6 of 9	Baker [104]
	Total $n = 0$	Total $n = 65$			

CI cumulative incidence, *95 % CI* 95 % confidence interval, *RCBT* related cord blood transplantation, *UCBT* unrelated donor cord blood transplantation, *SCID* severe combined immunodeficiencies, *WAS* Wiskott-Aldrich syndrome, *CGD* chronic granulomatous disease, *HLH* hemophagocytic lymphohistiocytosis, *UNK* data is unavailable

[a] Refers to overall cohort for engraftment and nonmalignant cohort for survival data

[b] Outcomes for the 25 patients who received myeloablative conditioning. An additional 13 patients received RIC regimens with a 2-year OS of 61.5 % (± 13.5) and EFS of 46.2 %(± 13.8) [107]

[107]. In a CIBMTR retrospective analysis of nine HLH patients undergoing UCBT with myeloablative preparative regimens, Baker et al. reported engraftment in all and survival in six of nine patients [104]. At Duke University, 13 patients with HLH have undergone UCBT with an OS of 70 %. Of these, 10 patients received myeloablative conditioning (Bu/Cy/ATG ± etoposide) with 60 % survival, and three patients received a reduced-toxicity regimen using Campath/Flu/Mel/Hydroxyurea ± thiotepa with 100 % survival. These studies indicate that UCBT using myeloablative approaches can be considered for patients lacking another wise suitable donor. Further studies using reduced-toxicity approaches are warranted to optimize conditioning regimens for these patients.

7.4 Chronic Granulomatous Disease

In a recent multicenter study, Gungor et al. reported on the HSCT outcomes of 58 CGD patients who received an RIC regimen (Flu/rabbit ATG or alemtuzumab/Bu) followed by either MRD ($n = 21$) or MUD ($n = 35$) BMT. They observed a 2-year EFS of 95 % (*95 % CI* 72–99) and 89 % (*95 % CI* 72–96) for MRD or MUD BMT recipients, respectively, with a low TRM (7 %). With these excellent outcomes, this approach should be the preferred approach for patients with a fully matched bone marrow donor. For patients lacking such a donor, the use of CB has been successfully used as an alternative donor. Besides case reports [93, 108–110, 119], the largest pediatric series of CBT for CGD was reported from Duke University, where eight patients underwent UCBT (7 UCBT, 1 RCBT) after myeloablative conditioning using Bu/Cy/ATG ± fludarabine. Two of these patients experienced graft rejection, and were successfully re-transplanted, after additional RIC conditioning, with second UCBTs [111]. All are alive and disease-free at a median of 5.2 years posttransplant [95]. Seven CGD patients were also included in a larger analysis of patients receiving UCBT for PIDs ($n = 88$). Of note, most of these CGD patients (five of seven) had failed 1–2 prior HSCT and were prepared with RIC [98]. The CI of neutrophil engraftment was 43 % with only three of seven patients surviving which most likely reflects the high-risk status of this cohort.

Reports of successful use of UCBT in treating several other immunodeficiency syndromes further emphasize its promise as a therapeutic option [96–99]. Newborn screening for SCID has been initiated in several US states which will likely increase the number of PID patients needing timely access to HSCT. For PID patients, CB provides several advantages as a donor source. Prospective and retrospective multicenter studies are being planned under North American cooperative group (Primary Immune Deficiency Treatment Consortium) to better characterize the outcomes of HSCT in these rare diseases and understand the impact of many variables including graft source and regimen intensity. Interventional studies are also being designed to optimize approaches to transplantation in very young babies.

8 Future Directions

Overall, RCBT is highly successful in treating patients with BMF syndromes, hemoglobinopathies, and PIDs. Strategies to optimize sibling donor CB inventories will be important in families with individuals affected with disorders amenable to transplant. UCBT is more challenging with a higher risk of acute GVHD. Thus, strategies to minimize GVHD are critical in improving outcomes in the future. Late effects of myeloablative chemotherapy conditioning are now well characterized, and therefore, an interest in reducing the chemotherapy intensity has emerged. However, this has been associated with increased graft failure rates. Thus, strategies, such as *ex vivo* expansion, used to overcome engraftment barriers need further study.

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Chapter 8
Umbilical Cord Blood Transplantation for Inherited Metabolic Diseases

Vinod K. Prasad

1 Introduction

Experimental and clinical data supporting the role of hematopoietic stem cell transplantation (HSCT) in general and unrelated donor (URD) umbilical cord blood transplantation (UCBT) in particular for inherited metabolic diseases (IMD), exists only for diseases belonging to the family of lysosomal and peroxisomal storage disorders. All lysosomal storage disorders (LSD) are caused by individual single gene defects, which lead to specific enzyme deficiency and consequent accumulation of toxic substrates and insufficiency of a vital product. The peroxisomal storage disorders (PSD), like adrenoleukodystrophy, stem from a defect in the membrane transporter protein ABCD1, leading to defects in the metabolism of long chain fatty acids and subsequent damage to neuronal and adrenal elements. Both LSD and PSD involve multiple tissues and organs including central and peripheral nervous systems. They are progressive in nature and frequently fatal in childhood.

Currently, allogeneic HSCT is the only available therapy that provides the possibility of lasting amelioration of neurocognitive and functional problems in IMD patients [1]. Enzyme replacement is available for a few diseases and effective in improving somatic features of the disease. However, they are unable to improve the neurological status due to their inability to cross the blood-brain barrier if given by intravenous route. Gene therapies have been developed and hold promise, but the clinical data is early and limited. It is important to note that because of the rarity of these disorders, randomized studies looking at the impact of various types therapies, graft sources, cytoreduction, and other variables do not exist.

The first patient with IMD to undergo HSCT, a child with Mucopolysaccharidosis type I (MPS I), received bone marrow (BM) from a matched-related donor in 1980 [2]. Since then, more than 2000 patients with IMD have been treated with HSCT using related donor BM, URD BM or PBSC, or cord blood (CB) grafts. While these

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studies show favorable impact of HSCT in the short term, their long-term effects on the natural history of these disorders is not as well documented. Except for a few case reports with long follow-up [3–5], the median posttransplant follow-up in published studies is 2–5 years [6–14]. In the subsequent sections, we review the published literature and draw on our center's experience in the use of UCBT for the treatment of IMD.

2 Scientific Basis

To understand how the biochemical and clinical consequences of IMD, a disease group which in most cases does not have any direct or indirect hematopoietic problems, could be corrected by HSCT, one must look at the seminal cellular studies of the late 1960s and early 1970s, subsequent in vivo animal data and the results of human studies. Lysosomal enzymes are synthesized in the endoplasmic reticulum, undergo chemical modification in the Golgi complex, get incorporated into endosomes, and mature in lysosomes. The process is well depicted in the New England Journal of Medicine (NEJM) [15]. To summarize, in the late-Golgi compartments, enzyme modified by mannose-6-phosphate binds to mannose-6-phosphate receptors. The enzyme–receptor complex is packaged into a transport carrier vesicle and delivered to early endosomes in which low pH promotes the dissociation of the enzyme from the receptor. The enzyme is then delivered to the mature lysosome, and the mannose-6-phosphate receptor is recycled to the Golgi apparatus. A small amount of the mannose-6-phosphate-modified enzyme escapes capture by the mannose-6-phosphate receptors and is released into the extracellular space. This enzyme can be recaptured by binding to a mannose-6-phosphate receptor in a clathrin-coated pit on the cell surface. In a patient who has undergone HSCT, enzyme released from a donor-derived stem cell can be taken up by a MPS cell. This intercellular transport enables "cross-correction," by which normal cells can correct the biochemical consequences of enzymatic deficiency within the neighboring cell. This idea was first suggested in very elegant studies by Elizabeth Neufeld's group 40 years ago [16–18]. In the first report, the biochemical defect of cultured skin fibroblasts from Hurler or Hunter patients (faulty degradation of sulfated mucopolysaccharide, resulting in excessive intracellular accumulation) was corrected if cells of these two genotypes were mixed with each other or with normal cells [16]. Following transplantation of BM or CB, donor-derived stem cells and progenitor cells have the ability to differentiate and migrate to affected organs. It is well known that cells derived from the hematopoietic system can specialize to take other roles and become an integral part of nonhematological organs. For example, microglial cells in the brain, alveolar macrophages in the lungs, and Kupffer cells in the liver have hematological origins. Donor-derived cells containing normal levels of lysosomal enzymes migrate to and engraft in nonhematopoietic organs in close proximity to patient's enzyme-deficient cells and provide long term enzyme replacement by "cross-correction". Possibly, there may be other mechanisms by which HSCT in

general and UCBT in particular contribute to improvement in IMD. Tracy et al. isolated and expanded oligodendrocyte-like cells from umbilical cord blood (UCB) and characterized them using multiple oligodendrocyte markers [19] and in *ex vivo* co-culture experiments demonstrated initiation of myelination in mature neuronal cells from the brains of myelin-deficient mice. These or other similar cells could potentially serve as a source of myelin-producing cells for cellular therapy of genetic and acquired degenerative neurologic diseases. In 2004, Kogler et al. isolated "unrestricted somatic stem cells" from UCB and demonstrated their expansion and differentiation into neural cells, liver cells, pancreatic cells, osteoclasts, chondrocytes, and cardiac myocytes in tissue cultures [20]. Multilineage differentiation of UCB-derived cells into cells expressing bone, fat, and neural markers has also been demonstrated [21, 22]. Human UCB cells have also been shown to differentiate into hepatocytes in a mouse liver [23]. In 2009, Canadian investigators demonstrated that lineage negative cells (Lin^{neg}) from UCB can differentiate into neuronal cells, oligodendrocytes, and Schwann cells [24]. Interestingly, in a separate study published in Science in 2002, BM-derived cells failed to give rise to neural cells following transplantation in a mouse model [25]. The above studies support the hypothesis that UCB may be a rich source of nonhematopoietic stem and progenitor cells or cells capable of transdifferentiation and thus may be a better graft source than BM for patients with IMD where engraftment of the hematopoietic system is merely a vehicle for enzyme delivery and other cells can facilitate tissue repair and regeneration. In a recent study, subsets of human CB cells, in particular the aldehyde dehydrogenase positive, lineage negative fraction, ($ALDH^{hi}Lin^{-}$) were shown to home to and engraft in nonobese diabetic/severe combined immunodeficiency/mucopolysaccharidosis type VII (NOD/SCID/MPS VII) mice [26]. Tissue sections showed the presence of donor-derived cells in many organs including liver, retina, brain, pancreas, cartilage, and bone. Additional evidence of cross correction in animal studies include decrease in mannose-rich oligosaccharides in neurons of alpha-mannosidosis cats treated with bone marrow transplantation (BMT; [27]) and MPS VII mice treated with genetically modified fibroblasts [28]. An elegant experiment using "twitcher" (*twi*) mice, a model for Krabbe disease and "shiverer" (*shi*) mice, a model of myelin deficiency, showed that upon transplantation, galactosylceramidase (GALC)-deficient oligodendrocytes from *twi* achieved widespread myelination in the brain and spinal cord of the myelin-deficient *shi* mouse. The positive effect was preserved for the lifespan of the host.

3 Clinical Basis

Most of the evidence for use of HSCT including UCBT for IMD is derived from registry-based data, multicenter questionnaire studies, case reports, and a few large single-center publications. These data sources have many inherent limitations, in particular those of selection bias, investigator preferences, and lack of standardization for treatment approaches and outcomes analyses. To date, more than 2000 patients

with almost 20 different LSD and PSD have been treated with HSCT. Of these, MPS I (Hurler syndrome), adrenoleukodystrophy (ALD), metachromatic leukodystrophy (MLD), and globoid leukodystrophy (Krabbe disease) have accounted for more than 80 % of the cases [29]. Within the field of HSCT, there are many unanswered questions, including the timing of transplantation, criteria for patient selection, impact of different graft sources, and the choice of preparative regimen.

The graft source for most patients in published reports prior to 2003 was BM while unrelated UCB has been the preferred graft source since that time. The results of BMT from matched-related donors in IMD are well reviewed by a number of eminent authors [1, 29–32]. Overall engraftment and survival rates span 63–85 % and 55–90 %, respectively. The outcomes following URD BMT have not been as good. For example, in a study of 40 Hurler syndrome patients undergoing URD BMT in 14 different centers, the probabilities of engraftment and 2-year overall survivals were 62.5 and 49 %, respectively [7]. Approximately 30 % of the survivors experienced primary graft failure. In a European Group for Blood and Marrow Transplantation (EBMT) retrospective study of 146 Hurler patients from 16 centers, 94 underwent transplants from URDs. The "alive and engrafted" rate in URD transplant was 55 % at 3.7-year follow-up [12]. Importantly, full donor chimerism was significantly higher in recipients of CB grafts (odds ratio, OR, 9.31; cumulative incidence, CI, 1.06–82.03; $p = 0.044$). Similarly, the probability of overall survival (OS) following URD BMT for patients with ALD was 53 % in a retrospective questionnaire-based study [10]. Outcomes following haploidentical BMT for Hurler syndrome were very poor with 35 % "engrafted and alive" at a median follow-up of 4.6 years [8].

The use of sibling donors who are carriers of the disease and thus deliver approximately 50 % "dose" of enzyme may yield inferior results as compared to noncarrier donors. Supporting this hypothesis is the fact that urinary clearance of MPS in Hurler syndrome patients was lower in the recipients of heterozygote or carrier (related) donor compared to noncarrier donors ($p = 0.0002$) [33]. Clinical inferiority of a carrier sibling donor compared to a noncarrier donor was shown in a multicenter study of Hurler disease [8, 34]. Children were more likely to maintain normal cognitive development if they were fully engrafted following BMT from a donor with homozygous normal leukocyte enzyme activity ($p = 0.02$). No child whose ultimate enzyme activity level was low (i.e., in the carrier range) due to either a heterozygous carrier donor or partial engraftment from a homozygous noncarrier donor had normal mental functioning at follow-up study. In contrast, three children whose mental development indices were normal (> 80) at follow-up study had received BMT from homozygous normal donors and were fully engrafted and had normal enzyme activity levels.

4 Unrelated Cord Blood Transplantation (UCBT)

UCB is increasingly being utilized as a graft source for HSCT. Almost 30,000 UCBT performed worldwide in the last 20 years points to higher clinical acceptance likely due to lower risk of graft-versus-host disease (GvHD) and rapid availability of 4/6–6/6 matched UCB units even for patients who lack fully matched adult BM donors.

With almost 600,000 publicly banked CB units that have been collected from healthy donors, screened for genetic diseases, tested for infectious diseases markers, HLA typed, and available for transplantation, almost every pediatric patient has a possibility of finding a suitable UCB unit. These units are frozen in many repositories all over the world and can be searched electronically.

Boelens et al., in a retrospective study of 146 children with Hurler syndrome, identified that T-cell depletion and the use of a reduced-intensity conditioning regimen increased the risk of graft failure while use of targeted busulfan protected against graft failure [35]. Most importantly, UCBT recipients showed high rates of full donor chimerism associated with normal enzyme levels after engraftment (Fig. 8.1a–8.1f; [13, 36]). Because full donor chimerism associated with normal enzyme levels are thought to be associated with superior long-term outcomes, including neurocognitive outcome after HCT, CB is the preferred stem cell source for HS patients [37]. An intercontinental study of 258 children with Hurler syndrome treated with HSCT using myeloablative conditioning regimen from 1995 to 2007 was recently published [14]. Median age at transplant was 16.7 months and median follow-up was 57 months. The CI of day-60 neutrophil recovery was 91 %, day-100 acute-GvHD (grade II–IV), 25 %, and 5-year chronic-GvHD, 16 %. OS and event free survival (EFS) at 5 years were 74 and 63 %, respectively. EFS after HLA matched sibling donor and 6/6 matched unrelated CB were similar at 81 %, 66 % after 10/10 HLA matched URD and 68 % after 5/6 matched CB. EFS was lower after transplantation of 4/6 matched unrelated CB (57 %; $p = 0.031$) and HLA-mismatched URD (41 %; $p = 0.007$) as shown in Fig. 8.2. Most importantly, full donor chimerism ($p = 0.039$) and normal enzyme levels ($p = 0.007$) were higher after CB transplantation (92 and 98 %, respectively) compared to the other grafts sources (69 and 59 %, respectively). The authors concluded that the results of allogeneic transplantation for Hurler syndrome are encouraging with similar EFS rates after matched sibling donor (MSD), 6/6 matched unrelated CB, 5/6 unrelated CB, and 10/10 matched unrelated donor (MUD). The outcomes were much better if the patients are transplanted early (Fig. 8.3). The editorial accompanying the paper supported the idea of early transplants and advantage of UCBT in the context of high donor chimerism and its eventual impact on the functional outcomes following transplant [38]. In comparison to previously published BMT experience, UCBT study had demonstrated higher near-total chimerism, better enzyme recovery in the blood, and superior "engrafted and alive" rates [13]. In addition, graft failure and GvHD were lower despite significant donor-recipient HLA mismatching. It took a median of only 35 days to proceed to UCBT after the child was first seen. This is of critical importance because early transplantation in severe forms of IMD significantly improves outcome. Despite gains in the outcomes, transplant-related mortality (TRM) following UCBT was 28.3 % in the whole group ($n = 159$) and 16.1 % in patients with performance status score of 80–100 ($n = 93$) [13]. Thus, it is important to further analyze the impact of conditioning-related toxicity for UCBT.

Busulfan, Cyclophosphamide, ATG Conditioning Outcomes of 159 consecutive IMD patients (median age, 1.5 years; weight, 12 kg) with IMD (Hurler syndrome, $n = 45$, Hunter syndrome, $n = 6$, Sanfilippo syndrome, $n = 19$, Krabbe disease,

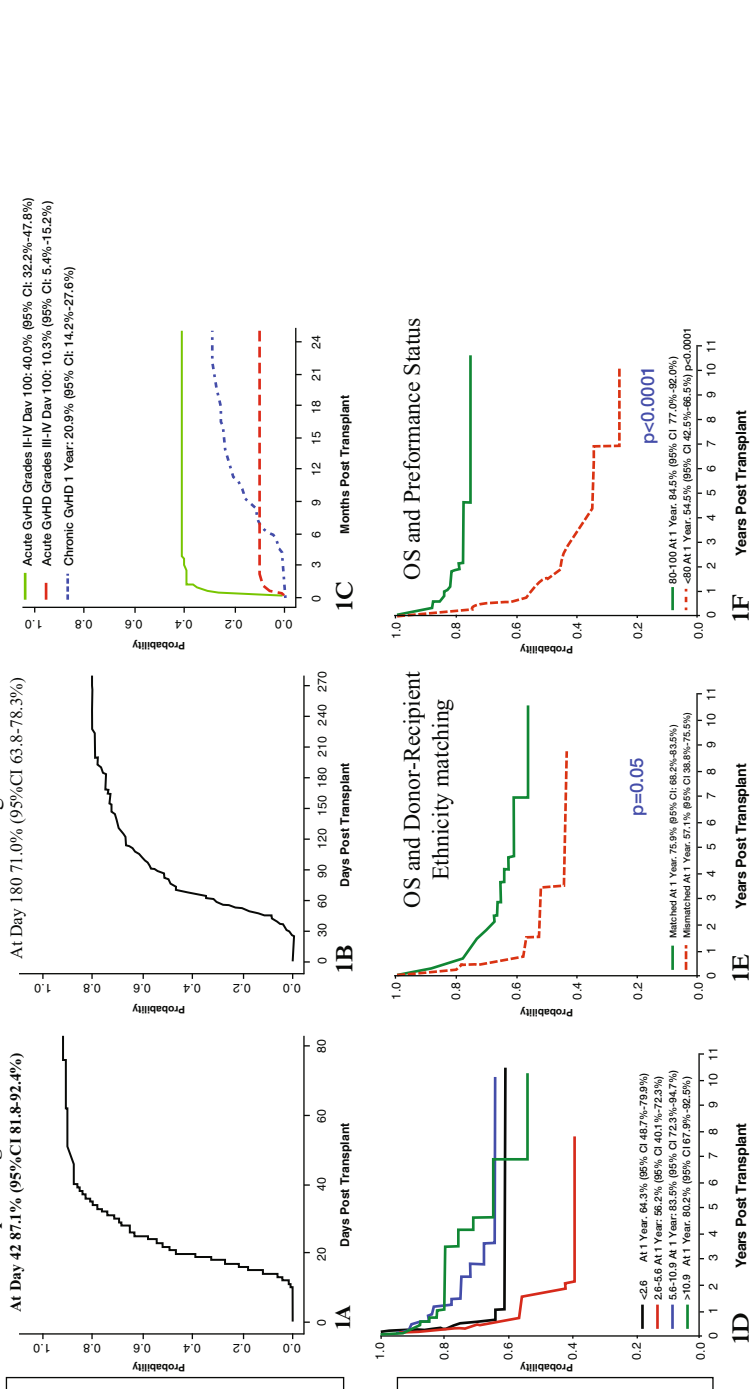

Fig. 8.1 Probability of *engraftment*, *GvHD*, and *OS* and the impact of certain patient characteristics [13]. **a** Probability of *neutrophil engraftment*, **b** Probability of *platelet engraftment* (50K), **c** Probability of *grades II-IV acute GvHD*, *grades III-IV acute GvHD*, and *chronic GvHD*, **d** Impact of CFU infused (x10e4/kg recipient weight) on probability of *OS*, **e** Impact of the donor-patient ethnicity matching on the *OS*, **f** Impact of *performance status* (80–100 vs. < 80) on the *OS*. *GvHD* graft-versus-host disease, *OS* overall survival

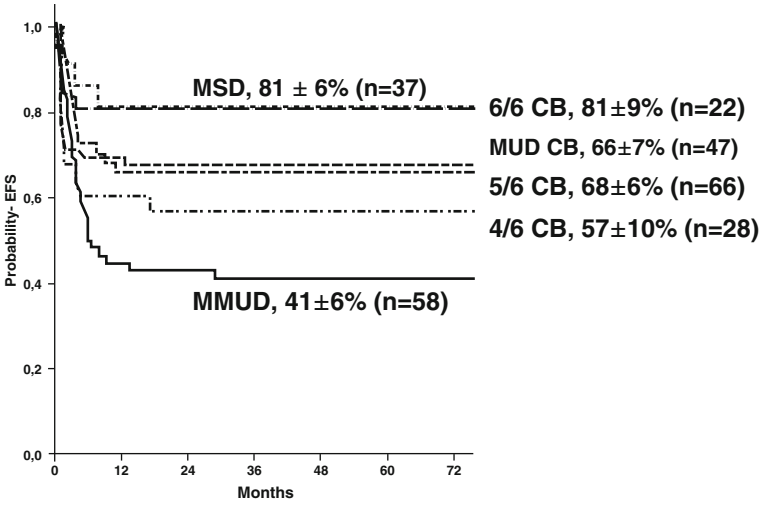

Fig. 8.2 DFS in Hurler syndrome by graft source and HLA match [14]. *CB* cord blood, *EFS* event free survival

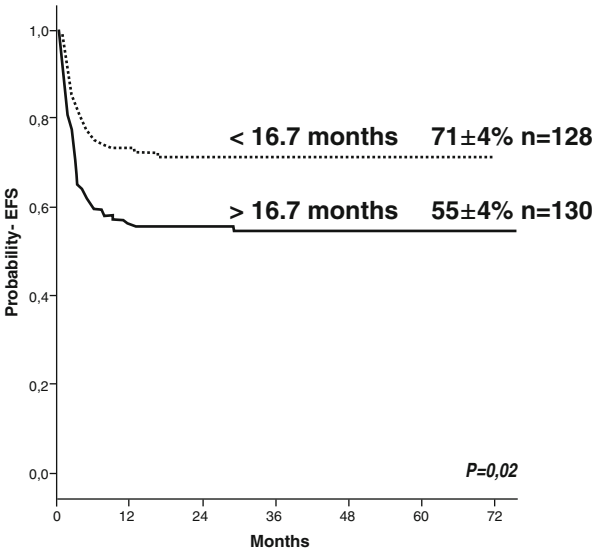

Fig. 8.3 DFS in Hurler syndrome by age at transplant [14]. *EFS* event free survival

$n = 36$, ALD, $n = 13$, MLD, $n = 15$, and others) receiving UCBT from 1995 and 2007 and followed for a median of 4.2 years (range 1–11 years) were published by our group [13]. All patients in this study received myeloablative conditioning with busulfan, cyclophosphamide, and horse antithymocyte globulin (ATG) and GvHD prophylaxis by cyclosporine with either steroids or mycophenolate. Cord blood unit

(CBU) cell doses were high (9.37 × 10e7/kg cryopreserved; 7.57 × 10e7/kg infused). The total graft failure rate was low at 8.2 %. The CI of full donor chimerism (> 90 %) in engrafting patients was very high (97 %). Serum enzyme normalized in 97 % patients with diseases for which testing exist. Risk of GvHD was low (Fig. 8.1c). Grade III/IV acute GvHD occurred in 10.3 % (95 % CI, 5.4–15.2 %). Overall, 1-year risk of any chronic GvHD was 20.9 % (95 % CI, 14.2–27.6 %) and that of extensive chronic GvHD was 10.8 % (95 % CI, 5.7–15.9 %). OS at 1 and 5 years were 71.8 % (95 % CI, 64.7–78.9 %) and 58.2 % (95 % CI, 49.7–66.6 %) in all patients and 84.5 % (95 % CI, 77.0–92.0 %) and 75.7 % (95 % CI, 66.1–85.3 %) in patients with high (Lansky or Karnofsky 80–100) performance score (Fig. 8.1f). In multivariate analysis, OS improved with high (80–100) performance status ($p < 0.0001$), high colony forming unit (CFU) infused $> 5.7 \times 10^4$/kg ($p = 0.02$) and matched patient-donor ethnicity ($p = 0.05$).

Fludarabine, Busulfan, and ATG conditioning In a recent study from the Netherlands, the investigators compared outcomes in 2 cohorts of pediatric HSCT recipients with a variety of diagnoses including those with IMD between 2009 and 2012 where 64 children received fludarabine (160 mg/M2) and busulfan at a target dose of 80–95 mg h/L [39]. CB recipients were additionally given rabbit ATG (10 mg/kg over 4 days). Estimated 2-year survival and event-free survival were 82 and 78 %. Compared with historical Bu-Cy (Mel) arm, less toxicity was noted in the Flu-Bu arm with lower rates of acute (noninfectious) lung injury (16 vs. 36 %; $P = 0.007$), veno-occlusive disease (3 vs. 28 %; $P = 0.003$), chronic GvHD (9 vs. 26 %; $P = 0.047$), adenovirus infection (3 vs. 32 %; $P = 0.001$), and human herpes virus 6 reactivation (21 vs. 44 %; $P = 0.005$). The median duration of neutropenia was shorter in the Flu-Bu arm (11 vs. 22 days; $P < 0.001$), and the patients in this arm required fewer transfusions. Their data indicated that Flu (160 mg/m^2) with targeted myeloablative Bu (90 mg h/L) is less toxic and equally effective as Bu-Cy in patients with similar indications for allo-HCT. Although the follow-up period in Flu-Bu cohort was short to allow optimal assessment of efficacy, the high rate of donor engraftment was promising across a wide range of diagnoses, including metabolic disorders, where donor chimerism can be difficult to achieve. This study shows potential promise of Flu-Cy-ATG for UCBT in IMD. However, longer experience and a larger cohort are required before a more established Bu-Cy-ATG regimen is replaced. A randomized study of these two regimens in IMD may be explored.

In addition to the above study, multi-institutional cord blood transplantation study (COBLT) trial sponsored by National Heart Lung and Blood Institute [11], publication from the European Group for Blood and Marrow Transplantation (EBMT) registry [12], data from a Japanese study [40], and a number of disease specific reports [12, 36, 41–43] support the argument that UCBT is an appropriate and viable option for HSCT for infants and children with IMD. Advantages of UCBT include its ready availability, quick search and procurement process, less stringent HLA matching requirement, higher probability of finding a UCB donor for racial and ethnic minority patients and those with rarer HLA types, potentially less risk of graft-transmitted infections, lower incidences of GvHD, and no risk to the donor. It

is possible that UCB contains a greater dose of nonhematopoietic progenitor cells. The ability of donor-derived cells to distribute and differentiate was demonstrated in the autopsied brain of a Krabbe disease baby who died a year after UCBT [44]. Donor-derived cells were seen in blood vessels, periventricular tissues, white matter, cerebellum, choroid plexus, and forebrain parenchyma. Differentiation of donor cells to microglia and choroid plexus cells was present, but not into neuroectodermal cells (e.g., neurons, astrocytes, or oligodendrocytes).

5 HSCT and Enzyme Replacement Therapy (ERT)

ERT therapy for short periods of time prior to transplantation may be useful in decreasing the respiratory problems as noted in a cohort of seven patients undergoing pretransplant ERT [45]. However, all patients developed serum antibodies against the α-L-iduronidase protein. The clinical significance of these antibodies is not clear, but there is concern that, over time, they may neutralize the effects of ERT. In Hurler syndrome patients, the residual substrate in patients can be measured as the ratio of urinary dermatan sulphate to chondroitin sulphate (DS/CS ratio). Using this marker, it was seen that the recipients of URD CB transplants had significantly lower DS/CS ratios than either recipients of heterozygote (related) donor grafts ($P = 0.0002$) or patients receiving ERT ($P = 0.012$) [33] suggesting poorer substrate clearance with ERT.

6 Functional, Neurological, and Cognitive Outcome

The benefits of HSCT over long term must be measured not only on the basis of survival- or transplant-related toxicity alone but also on the basis of improvement in neurocognitive functioning and all other aspect of the underlying disease. For example, in Hurler syndrome, it must assess joint integrity, motor development, linear growth, hydrocephalus, corneal clouding, cardiac function, hepatosplenomegaly, obstructive airway symptoms, hearing, visual, and auditory processing. There are a number of studies looking at these improvements following BMT and UCBT [9, 12, 34, 36, 46–49]. Improvements observed in the skeletal deformities, particularly after BMT are not as good. Many Hurler patients require corrective hip, back, knee, and carpal tunnel surgeries during later childhood after otherwise "successful" HSCT. All Hurler patients showed either stabilization or improvement of neurocognitive function and continued to gain new skills after UCBT which was performed at a median age of 16 months [36]. The gain in neurocognitive function was slower in some patients than others. There was also an improvement in the somatic features, linear growth, and bone disease in these patients. In most patients, organ dysfunction from accumulation of glycosaminoglycans (GAG) in the heart, liver, and spleen are reduced.

In a study from Duke University and University of North Carolina, 25 patients with early and late onset infantile Krabbe disease [41] who were transplanted before ($n = 11$) or after ($n = 14$) the onset of clinical symptoms showed dramatic efficacy if the patient was transplanted early in the course of the disease. All 11 patients diagnosed prenatally or neonatally because of a family history of an affected sibling and transplanted in the first month of life were alive and well with a median follow-up of 71 months. All had full donor chimerism, normal peripheral blood galactocerebrosidase (GALC) levels, and outlived their affected siblings who had not been transplanted. Detailed analysis of neurodevelopmental testing showed significant and continuing gains in gross motor, fine motor, adaptive behavior, and receptive as well as expressive language domains in most patients who were transplanted as asymptomatic neonates. Adaptive behavior refers to self-care skills (e.g., eating and drinking independently) and self-calming behavior; receptive language refers to the ability to understand communication through gestures, facial expressions, and words; expressive language refers to the ability to express needs with the use of gestures, vocalization, facial expressions, and words. In contrast, survival was only 45 % in the 14 infants who were symptomatic at transplant, and while their disease stabilized, no appreciable gains were seen in neurologic development. MRI images as well as fractional anisotrophy demonstrate normal to near-normal myelination for age following transplantation in these patients [50, 51]. Peripheral nerve conduction studies stabilize and, in some patients, improve. A majority of the patients develop some degree of spasticity in the legs which is partially responsive to physical therapy and muscle relaxants. Some of these children require assisted devices for ambulation between 3 and 5 years of age. In later onset, more slowly progressive forms of Krabbe disease, patients show improvements even if they are transplanted after developing neurological symptoms. BMT has been shown to arrest progression of the disease after stabilization 3–6 months post transplant in the most severe "infantile" form of Krabbe disease (globoid-cell leukodystrophy) as well as in the milder juvenile and adult types [52].

Analyses of the outcomes of UCBT following chemotherapy-based myeloablative conditioning in a cohort of 12 young boys with X-ALD showed the probability of OS to be 71.9 % in a median follow-up of 3.3 years [42]. Symptomatic patients exhibited lower survival and rapid neurologic deterioration. Baseline Loes scores in the MRI correlated with cognitive and motor outcome. The three youngest children (age at transplant, 2.6–3.5 years) transplanted before the onset of clinical symptoms continue to develop at a normal rate 5–7 years post transplant. In a large registry- and questionnaire-based study of BMT in ALD, the patient's with performance IQ < 80 (also referred as nonverbal IQ) at baseline were significantly more impaired post transplant and those with parietal-occipital pattern demonstrate greater mean loss in their performance IQ [10]. In a recent publication, Martin and colleagues reported on the results of a longitudinal study to evaluate long-term outcomes after URD UCBT in 27 pediatric patients with MLD [53]. Twenty-four patients engrafted after the initial transplantation. Seven patients died of infection, regimen-related toxicity, or disease progression. Twenty patients (6 with late-infantile onset and 14 with juvenile onset) were followed for a median of 5.1 years (range, 2.4–14.7).

They found that patients with motor symptoms at the time of transplant did not improve after transplantation. Brainstem auditory evoked responses, visual evoked potentials, electroencephalogram, and/or peripheral nerve conduction velocities stabilized or improved in juvenile patients but continued to worsen in most patients with the late-infantile presentation. Pretransplant modified Loes scores were highly correlated with developmental outcomes and predictive of cognitive and motor function. Children who were asymptomatic at the time of transplantation benefited most from the procedure. Children with juvenile onset and minimal symptoms showed stabilization or deterioration of motor skills but maintained cognitive skills. Overall, children with juvenile onset had better outcomes than those with late-infantile onset. As in other leukodystrophies, early intervention correlated with optimal outcomes and UCB transplantation benefited children with presymptomatic late-infantile or minimally symptomatic juvenile MLD.

7 Current Recommendations

There is no all-encompassing curative option for patients with IMD. HSCT and ERT are currently available therapy for many of these children depending on the diagnosis and stage of the disease and are likely to provide selective benefit. HSCT offers the advantage of benefit in the neurological and cognitive areas in addition to the somatic benefits offered by both ERT and HSCT. HSCT involves administration of high dose chemotherapy which, by itself, is associated with early mortality risks and later morbidity including adverse effects on growth, fertility, and dentition. BMT or UCBT usually cost US$ 300,000–800,000 with minimal additional costs on yearly basis. In contrast, ERT must be given lifelong with an estimated recurring cost of about US$ 250,000–300,000 every year for a 35 kg child needing laronidase for treatment of MPS 1 [54].

There is general consensus that the earlier a patient is diagnosed and treated, the better their prognosis. This is true for ERT, BMT, and UCBT. Thus, newborn screening is attractive and has been implemented in a number of states in the USA. The first such program was started by New York State (NYS) in 2005 [55]. Babies found to have mutations associated with early infantile disease undergo a second blood test and are simultaneously analyzed for confirmation of diagnosis by enzyme levels and HLA typing to allow searching for potential UCB donors. In situations with poor genotype-phenotype correlation, screening would provide longitudinal data to study this and other issues.

The choice of treatment for patients with IMD is dependent on the age of presentation, genotype–phenotype correlations—when known, the degree of organ involvement, the performance status of the patient at diagnosis, donor availability for HSCT, and the availability and feasibility of ERT. Carrier screening and mutation analysis should also be performed for all first-degree relatives and other distant relatives to identify all individuals at risk within that family pedigree [49].

8 General Principles (adapted from [49])

1. Establish diagnosis as early as possible; clinician should have a high degree of suspicion when seeing children with delayed milestone development and dysmorphic facial or other features. In many cases, the family history is negative:
   a. If patient has infantile form of disease, refer immediately for HSCT.
   b. If patient has juvenile or adult forms of disease, consider available treatment options.
2. Perform mutation analysis and establish genotypic and phenotypic correlations, if known:
   a. If patient has severe phenotype disease, refer for HSCT.
   b. If patient has milder or other phenotypes, consider HSCT or ERT depending on the clinical features.
3. Evaluate patient for disease and performance status:
   a. If patient has central nervous system (CNS) involvement, pursue HSCT if disease manifestations are early.
   b. If patient has no CNS involvement or no potential for CNS involvement and ERT is available, consider ERT.
4. For patients considering HSCT:
   a. Refer to a transplant center experienced in HSCT for IMD patients.
   b. Search for and identify donors, screen donors for disease carrier state:
      i. Matched related noncarrier donor should be identified.
      ii. If not suitable or available, UCB donors should be strongly considered:
         1. Cell dose $> 3 \times 10e7/kg$ based on precryopreserved total nucleated cell count.
         2. Use a donor with the highest HLA match and $\geq 4/6$.
         3. Relevant enzyme levels should be checked from 3–5 CB units identified on the basis of HLA and cell dose; use the units with high enzyme level.
      iii. Carrier related or unrelated BM donors should not be used.
   c. Perform formal workup to evaluate disease status and overall organ function.
      i. Performance status should be $\geq 80\,\%$ (Lansky/Karnofsky).
      ii. Disease should not be rapidly progressing (deterioration should not be observed during workup).
      iii. Organ function should not preclude administration of high dose chemotherapy.
      iv. Patient should not have uncontrolled seizures.
      v. Patient should not have uncontrolled aspiration.
      vi. Patient should not have uncontrolled infections.
   d. If patient meets eligibility criteria for the particular IMD and if parents grant informed consent, proceed to HSCT.
5. Refer for ERT:
   a. If patient has mild phenotype, non-CNS involving disease and ERT is available for that disease.
   b. If patient has advanced disease and is not a candidate for HSCT and ERT offers palliative benefit.
   c. As a temporary measure if HSCT cannot be performed for medical or insurance reasons in the next 3–4 weeks.

6. Patients with poorer performance status and/or advanced disease should be considered for reduced intensity HSCT or other experimental therapies as they become available.
7. All families at risk for IMD should receive extensive genetic counseling.

8 Conclusions

Cumulative experience of 30 years for HSCT and 18 years for UCBT in the treatment of IMD points to promising and effective therapy for many but not all patients with IMDs. Use of UCBT offers highest level of donor chimerism and enzyme levels, increases access to transplantation for almost all patients, and allows for quicker donor identification and selection. Delays in diagnosis and referral as well as significant transplantation-related mortality and morbidity continue to pose challenges for the future. Strategies to decrease procedure related risks should further improve short- and long-term outcomes. Patient performance status at the time of transplant is the best predictor of the likelihood of benefit and best clinical outcomes. While novel alternatives like gene therapy, CNS cell therapy, and regenerative medicine continue to be explored, every effort should be made to perform transplantation early in the course of disease before extensive damage to nervous system and other organs. Collaborative studies of functional outcomes will further define the factors impacting the success of transplantation.

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Chapter 9
Myeloablative Single-Unit Cord Blood Transplantation in Adults

Jun Ooi

1 Introduction

Although allogeneic stem cell transplantation from a human leukocyte antigen (HLA)-identical-related donor offers a potential cure for patients with hematological disorder, a suitably matched related donor is unavailable for approximately two-thirds of patients. Recently several reports investigating the clinical results of adult UCBT have been published [1−5]. In this review, we focus on recent results of myeloablative single-unit UCBT in adults.

2 Conditioning Regimen

The optimal myeloablative conditioning regimen for single-unit UCBT in adults has not been established. Recently, the efficacy of a new chemotherapy-based conditioning regimen (TBF regimen) using thiotepa 10 mg/kg, busulfan (Bu) 9.6 mg/kg, fludarabine (Flu) 150 mg/m^2, and rabbit antithymocyte globulin (ATG, total dose 8 mg/kg) was reported in a single center experience [6]. In 2013, Ruggeri et al. reported the outcome of single-unit cord blood transplantation (CBT) after TBF regimen for 88 patients transplanted for acute leukemia in first complete remission (CR1) in the Eurocord registry [7]. All patients received a total nucleated cell (TNC) dose $> 2.5 \times 10^7$/kg. The cumulative incidence of neutrophil recovery at day 60 was 89 %. The cumulative incidence of 2-year nonrelapse mortality (NRM) and 2-year relapse were 33 and 18 %, respectively. The 2-year probability of leukemia-free survival (LFS) was 48 %. These results suggest the choice of TBF conditioning regimen for single-unit UCBT may improve results.

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In the Institute of Medical Science, University of Tokyo (IMSUT), most adult patients received four fractionated 12 Gy total body irradiation (TBI) on days -9, -8 or days -8 and -7, cytosine arabinoside (Ara-C), and cyclophosphamide (Cy) for myeloablative single-unit CBT [8–10]. Ara-C was administered intravenously over 2 h at a dose of 3 g/m^2 every 12 h on days -6 and -5 or days -5 and -4 (total dose 12 g/m^2). For patients with myeloid malignancies, recombinant human granulocyte colony-stimulating factor (G-CSF), thought capable of reducing the post transplant relapse rate because of increasing the susceptibility of myeloid leukemic cells to Ara-C, was combined with Ara-C. G-CSF was administered by continuous infusion at a dose of 5 μg/kg/d. Infusion of G-CSF was started 12 h before the first dose of Ara-C and stopped at the completion of the last dose. Cy was administered intravenously over 2 h at a dose of 60 mg/kg once daily on days -4 and -3 or days -3 and -2 (total dose 120 mg/kg).The cumulative incidence of neutrophil recovery at day 50 was 91–95 %. The 5-year cumulative incidence of treatment related-mortality (TRM) was 4–14 %. The 5-year cumulative incidence of relapse was 16–27 %. The probability of event-free survival (EFS) at 5 years was 57 % for patients with acute lymphoblastic leukemia (ALL) [9], 63 % for patients with acute myeloid leukemia (AML) [8], and 70 % for patients with myelodysplastic syndrome (MDS) [10]. These results suggest that the myeloablative IMSUT regimen may be safely and effectively used for single-unit UCBT in adults.

In 2013, a prospective multicenter study of single-unit UCBT for adult patients with high-risk hematologic malignancies was performed to assess the safety and efficacy of myeloablative IMSUT regimen in Japan [11]. Thirty-three adult patients with hematologic malignancies, such as acute leukemia, chronic myelogenous leukemia (CML), or MDS, either lacking an HLA-identical sibling/HLA-matched unrelated donor or requiring urgent transplantation were enrolled. Conditioning consisted of 12 Gy of TBI, Ara-C, and Cy. Diagnoses were acute leukemia in 26 patients, CML in 4, and MDS in 3; 12 patients were in CR1, and the others were in advanced stages at the time of CBT. For myeloid malignancies, G-CSF was given by continuous infusion, starting 12 h before the first dose of Ara-C and continuing until the last dose of Ara-C. Thirty-one patients achieved engraftment. The 1-year cumulative incidence of NRM was 15 %. The 3-year relapse rate was 42 %. The disease-free survival (DFS) rate was 42 %. These results suggest that the IMSUT regimen can safely provide a high DFS rate in patients with high-risk hematologic malignancies.

3 Comparisons of Cord Blood and Other Stem Cell Sources

3.1 CBT vs. Unrelated Bone Marrow Transplantation (BMT)/Peripheral Blood Stem-Cell Transplants (PBSCT)

In 2004, two registration-based studies comparing both single-unit UCBT and bone marrow transplantation (BMT) from unrelated donors in adult patients with acute leukemia after myeloablative conditioning were published [12, 13].

The study by Laughlin et al. [12] included patients 16 to 60 years of age who had received either an HLA-matched BMT ($n = 367$) or a BMT with single HLA mismatch ($n = 83$) from an unrelated donor or had received a UCBT with one or two HLA mismatches ($n = 150$). Hematopoietic recovery was slower in mismatched BMT and UCBT than matched BMT. The rate of TRM was lowest among patients who received matched BMT. Patients with mismatched BMT and UCBT had similar rates of TRM and overall mortality. There were no differences in the rate of recurrence of leukemia among the groups. LFS at 3 years was 19 % for mismatched BMT, 23 % for UCBT, and 33 % for matched BMT.

The study by Rocha et al. [13] included patients at least 15 years of age who had received a single cord-blood unit ($n = 98$) or HLA-matched bone marrow ($n = 584$). Neutrophil recovery was significantly delayed after UCBT as compared with BMT. The incidence of chronic GVHD, TRM, relapse rate were not significantly different in the two groups. LFS at 2 years was also similar in the two groups (33 % for UCBT and 38 % for BMT).

In 2004, our group also reported on a comparative analysis of UCBT versus BMT from unrelated donors in adults after myeloablative conditioning at IMSUT [14]. The study included data from 113 patients with hematologic malignancies, 16 years or older, who received unrelated BMT ($n = 45$) or unrelated UCBT ($n = 68$). Transplantations were performed between 1996 and 2003. The median number of nucleated cells before freezing in recipients of cord blood was 2.47×10^7/kg. Neutrophil recovery was significantly delayed after UCBT (22 days) as compared with BMT (18 days). However, the overall results for UCBT recipients were better than for BMT recipients in terms of GVHD, TRM, and DFS. DFS at 2 years was 74 % for UCBT and 44 % for BMT. In our assessments, the availability of grafts containing sufficient cell number because of a smaller size, the shorter time from donor search to transplantation, the low requirements of steroid therapy for GVHD, the conditioning regimen including TBI, avoidance of ATG, the GVHD prophylaxis with standard cyclosporine (CyA) and methotrexate (MTX) used in our institution, and a more genetically homogeneous population might have contributed to our favorable results of adult UCBT in Japan.

Recently, Eapen et al. [15] reported a comparative analysis of UCBT from unrelated donor with bone marrow or peripheral blood stem-cell transplants (PBSCT) from unrelated donors in adults after myeloablative conditioning. They used data reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) , the European Group for Blood and Marrow Transplantation (EBMT), the Eurocord-Netcord Registry, and the National Cord Blood Program (NCBP) at the New York Blood Center for adults with acute leukemia. The study included data from 1525 adults aged 16 years or older with acute leukemia (AML, $n = 880$; ALL, $n = 645$) who received BMT ($n = 472$) or PBSCT ($n = 888$) from unrelated donors or unrelated UCBT ($n = 165$). All transplantations were done between 2002 and 2006, and used a myeloablative transplant-conditioning regimen, identified by total Bu dose 8 mg/kg or more, or TBI of 10 Gy or more. All UCBT recipients received a single unit containing a minimum of 2.5×10^7 total nucleated cells/kg bodyweight at cryopreservation. LFS in patients after UCBT was comparable with that after

8/8 and 7/8 allele-matched PBSCT or BMT. However, TRM was higher after UCBT than after 8/8 allele-matched PBSCT or BMT. Grades II-IV acute and chronic GVHD were lower in UCBT recipients compared with allele-matched PBSCT, while the incidence of chronic, but not acute GVHD, was lower after UCBT than after 8/8 allele-matched BMT. These data support the use of cord blood for adults with acute leukemia when there is no HLA-matched unrelated adult donor available, and when a transplant is needed urgently.

In 2012, a Japanese registry-based comparative study of UCBT and HLA-mismatched unrelated BMT was reported [16]. This was an HLA-mismatched locus-specific comparison of the outcomes of 351 single-unit cord blood and 1028 unrelated bone marrow adult recipients 16 years old or older at the time of transplantation who received first stem cell transplantation with myeloablative conditioning for acute leukemia or MDS. All patients in the UCBT cohort received a single-unit cord blood. Transplantation years were between 1996 and 2005 for unrelated BMT and between 2000 and 2005 for UCBT to avoid the first 3 years of a pioneering period (1993–1995 for unrelated BMT and 1997–1999 for UCBT). With adjusted analyses, HLA 0–2 mismatched UCBT showed similar overall mortality compared with that of single-HLA-DRB1-mismatched unrelated BMT. UCBT showed inferior neutrophil recovery, lower risk of acute GVHD, and lower risk of TRM compared with single-HLA-DRB1-mismatched unrelated BMT. No significant difference was observed for risk of relapse. HLA 0–2 antigen-mismatched UCBT is a reasonable second alternative donor/stem cell source with a survival outcome similar to that of single-HLA-DRB1- mismatched or other seven of eight unrelated BMT.

3.2 CBT vs. Related BMT/PBSCT

In 2007, we reported a comparative analysis of UCBT from unrelated donor with BMT or PBSCT from related donors in adults after myeloablative conditioning at IMSUT [17]. The study included data from 171 patients with hematologic malignancies, 16 years or older, who received BMT ($n = 55$) or PBSCT ($n = 16$) from related donors or unrelated UCBT ($n = 100$). Transplantations were performed between 1997 and 2005. The median number of nucleated cells before freezing in recipients of cord blood was 2.43×10^7/kg. Neutrophil recovery was significantly delayed after UCBT (22 days) as compared with related BMT/PBSCT (17 days). However, overall engraftment rates were almost the same for both grafts. The cumulative incidence of grades III–IV acute and extensive-type chronic GVHD among UCBT recipients were significantly lower than those among related BMT/PBSCT recipients. Multivariate analysis demonstrated no apparent differences in TRM, relapse, and DFS between both groups. DFS at 3 years was 70 % for UCBT and 60 % for related BMT/PBSCT. These data suggest that unrelated cord blood could be a safe and effective stem-cell source as related bone marrow or mobilized peripheral blood when patients are treated in experienced centers.

4 Disease-Specific Outcomes

4.1 Acute Myeloid Leukemia

Several reports highlighting the disease-specific outcomes of adult UCBT have been published (Table 9.1) [8–9, 18–24]. In 2008, we reported the results of unrelated UCBT after myeloablative conditioning for 77 adult patients with AML at IMSUT [8]. Between August 1998 and February 2008, 77 adult patients with AML were treated with unrelated UCBT. All patients received four fractionated 12 Gy TBI and chemotherapy as myeloablative conditioning. The median age was 45 years, the median weight was 55 kg, the median number of nucleated cells was 2.44×10^7/kg and the median number of CD34 positive cells was 1.00×10^5/kg. All patients received a single UCBT with an HLA mismatched cord blood unit. The cumulative incidence of neutrophil recovery at day 50 and platelet recovery at day 200 was 94.8 and 91.7 %, respectively. As previously described, a higher CD34 + cell dose was associated with faster hematopoietic recovery. The cumulative incidence of grade III–IV acute GVHD and extensive-type chronic GVHD was 25.1 and 28.6 %, respectively. With a median follow-up of 78 months, the probability of EFS at 5 years was 62.8 %. The 5-year cumulative incidence of TRM and relapse was 9.7 and 25.8 %, respectively. In multivariate analyses, the risk factor identified for EFS was disease status and cytogenetics.

In 2010, a Spanish institute reported the outcome and prognostic factors of 49 adults with high-risk AML receiving single-unit UCBT from unrelated donors after myeloablative conditioning [18]. Conditioning regimens were based on the combination of thiotepa, Bu, Cy or Flu, and ATG. The cumulative incidence of myeloid and platelet engraftment was 96 and 73 % at a median time of 20 and 62 days, respectively. Engraftment was significantly faster for patients receiving higher doses of CD34 + cells. The cumulative incidence of acute GVHD grade II–IV, III–IV, and extensive chronic GVHD were 26, 15, and 30 %, respectively. LFS, NRM, and relapse at 2 years were 42, 39, and 19 %, respectively. Low number of TNC had a negative impact on NRM and LFS. Patients transplanted in CR1 receiving TNC above 2×10^7/kg had a 4-year LFS of 75 %. These results show that UCBT from unrelated donors is a curative treatment for a substantial number of patients with high-risk AML, particularly if transplant is performed with UCB units with $> 2 \times 10^7$/kg and for patients whose AML is in CR1.

In 2009, Atsuta et al.[19] made a disease-specific comparison of unrelated UCBT and HLA allele-matched unrelated BMT among 484 patients with AML (173 UCBT and 311 BMT) who received myeloablative transplantations in Japan. In multivariate analyses, lower overall survival and LFS were observed in CBT. The relapse rate did not differ between the 2 groups, however, the TRM rate showed higher trend in UCBT. For patients with AML, decreasing early posttransplant mortality is required to improve the outcome for cord blood recipients.

Table 9.1 Disease-specific outcomes of adult single-unit UCBT after myeloablative conditioning

Reference	Diagnosis	Patients, *n*	Median age, *y*	TRM	Relapse	DFS	Comment
Ooi et al. 2008 [8]	AML	77	45	10 % at 5 years	26 % at 5 years	63 % at 5 years	Disease status and cytogenetics were associated with DFS
Sanz et al. 2010 [18]	AML	49	34	39 % at 2 years	19 % at 2 years	42 % at 2 years	Patients in CR1 and with high TNC doses had better DFS
Astuta et al. 2009 [19]	AML	173	38	33 % at 2 years	31 % at 2 years	42 % at 2 years	UCBT was inferior to BMT due to higher TRM
Ooi et al. 2009 [9]	ALL	27	36	4 % at 5 years	27 % at 5 years	57 % at 5 years	A larger-sized study is needed
Atsuta et al. 2009 [19]	ALL	114	34	24 % at 2 years	31 % at 2 years	46 % at 2 years	UCBT was similar to BMT
Sanz et al. 2010 [23]	CML	26	33	50 % at 1 year	0	41 % at 8 years	UCBT can be a curative treatment for a substantial number of CML patients
Sato et al. 2011 [10]	MDS	33	42	14 % at 5 years	16 % at 5 years	70 % at 5 years	Better supportive care and G-CSF-combined regimen may be associated with high DFS

AML acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *CML* chronic myeloid leukemia, *MDS* myelodysplastic syndrome, *TRM* transplant-related mortality, *DFS* disease-free survival, *CR1* first complete remission, *TNC* total nucleated cell, *UCBT* umbilical cord blood transplantation, *BMT* bone marrow transplantation, *G-CSF* granulocyte colony-stimulating factor

4.2 Acute Lymphoblastic Leukemia

In 2009, we updated the results of unrelated UCBT after myeloablative conditioning for 27 adult patients with ALL [9]. Between October 2000 and November 2007, 27 adult patients with ALL were treated with unrelated UCBT at IMSUT. All patients received four fractionated 12 Gy TBI and chemotherapy as myeloablative conditioning. The median age was 36 years, the median weight was 57 kg, and the median number of nucleated cells was 2.47×10^7/kg. All patients received a single and HLA mismatched cord blood unit. The cumulative incidence of neutrophil recovery at day 30 and platelet recovery at day 200 was 92.6 and 92.3 %, respectively. With a median follow-up of 47 months, the probability of EFS at 5 years was 57.2 %. The 5-year cumulative incidence of TRM and relapse was 3.7, 27.4 %, respectively.

Atsuta et al. [19] also made a disease-specific comparison of unrelated UCBT and HLA allele-matched unrelated BMT among 336 patients with ALL (114 UCBT and 222 BMT) who received myeloablative transplantations in Japan. In multivariate analyses, there was no significant difference between the groups for relapse and treatment-related mortality, which contributed to similar overall survival (OS) and LFS.

In 2013, Nishiwaki et al. [22] retrospectively analyzed data of 1,726 patients who received myeloablative allogeneic stem cell transplant (SCT) for adult Philadelphia chromosome-negative ALL [Ph(−) ALL] in Japan. The sources of the allo-SCT were related donors (RD; $n = 684$), unrelated donors (URD; $n = 809$), and cord blood ($n = 233$). OS in patients after UCBT in CR1 was comparable with that after RD or URD allo-SCT. UCBT was not a significant risk factor for relapse or NRM as well as for OS in multivariate analyses. Similarly, the donor source was not a significant risk factor for OS in subsequent CR or non-CR. Allo-SCT using cord blood led to OS similar to those of RD or URD in any disease status. Therefore, UCBT should be considered early in the disease courses of patients with Ph(−) ALL who require an allogeneic transplant.

4.3 Chronic Myeloid Leukemia

There are very limited reports of adult CML patients who received UCBT after myeloablative conditioning.

In 2010, Sanz et al. [23] analyzed the outcome of 26 adults with CML receiving single-unit UCBT from unrelated donors after myeloablative conditioning at a single institution. Conditioning regimens were based on combinations of thiotepa, Bu, Cy or Flu, and ATG. At the time of transplantation, 7 patients (27 %) were in first chronic phase (CP), 11 (42 %) were in second CP, 2 (8 %) were in accelerated phase (AP), and 6 (23 %) were in blast crisis (BC). The cumulative incidence of myeloid engraftment was 88 % at a median time of 22 days and was significantly better for patients receiving higher doses of CD34 + cells. The cumulative incidence of acute GVHD grade II–IV was 61 %, that of acute GVHD grade III–IV was 39 %, and

that of chronic extensive GVHD was 60 %. TRM was 41 % for patients undergoing UCBT while in first or second CP and 100 % for patients in AP or BC. After a median follow-up of 8 years, none of the patients relapsed, giving a DFS at 8 years of 41 %. The DFS for patients undergoing CBT while in any CP was 59 %. These results demonstrate that UCBT from unrelated donors can be a curative treatment for a substantial number of patients with CML. Advances in supportive care and better selection of cord blood units and patients are needed to improve TRM.

4.4 Myelodysplastic Syndrome

Again, reports of disease-specific outcomes for adult patients with MDS after CBT are still limited [24].

In 2011, we updated the results of disease-specific outcomes of adult patients with advanced MDS treated with UCBT after myeloablative conditioning [10]. Between August 1998 and June 2009, 33 adult patients with advanced MDS were treated with unrelated UCBT at IMSUT. The diagnoses at transplantation included refractory anemia with excess blasts ($n = 7$) and MDS-related secondary AML (sAML) ($n = 26$). All patients received four fractionated 12 Gy TBI and chemotherapy as myeloablative conditioning. The median age was 42 years, the median weight was 55 kg and the median number of cryopreserved nucleated cells was 2.51×10^7 cells/kg. The cumulative incidence of neutrophil recovery at day 50 was 91 %. Neutrophil recovery was significantly faster in sAML patients. The cumulative incidence of platelet recovery at day 200 was 88 %. Platelet recovery was significantly faster in CMV seronegative patients. The cumulative incidence of grade II–IV acute GVHD and extensive-type chronic GVHD was 67 and 34 %, respectively. Degree of HLA mismatch had a significant impact on the incidence of grade II–IV acute GVHD. TRM and relapse at 5-years was 14 and 16 %, respectively. The probability of EFS at 5 years was 70 %. No factor was associated with TRM, relapse, and EFS. These results suggest that adult advanced MDS patients without suitable related or unrelated BM donors should be considered as candidates for UCBT.

5 Conclusion

Cord blood has emerged as an acceptable alternative source of hematopoietic stem cell for transplantation in adult patients with hematologic malignancies. Several single-institute and registry-based reports have demonstrated encouraging outcomes of a single unit UCBT after myeloablative conditioning in adults. To improve outcomes, the use of optimal conditioning regimen, better unit selection, and optimized supportive care are required.

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Chapter 10
Quantitative and Qualitative Immune Reconstitution Following Umbilical Cord Blood Transplantation

Sarah Nikiforow and Jerome Ritz

1 Introduction

Clinical outcomes using umbilical cord blood cells (UCB) as a source for hematopoietic stem cell transplantation (HSCT) have improved along with more detailed appreciation of cord blood's unique cellular composition and biology. Despite many variations between clinical trials of umbilical cord blood transplantation (UCBT), several distinct aspects of hematopoietic reconstitution in UCB recipients have been consistently demonstrated. First, neutrophil and platelet engraftment are delayed in comparison to recipients of adult stem cells [1, 2]. Second, the presence of multiple human leukocyte antigen (HLA) mismatches in double UCBT (dUCBT) does not have the same negative clinical impact as for other stem cell sources with respect to GvHD [2, 3]. Third, despite aggressive antiviral and antifungal prophylaxis, infection remains a significant cause of death in UCBT recipients. While current 8 % rates of infection-related mortality are lower than initial reports, viral reactivation particularly of cytomegalovirus (CMV), Epstein-Barr virus (EBV), Human herpesvirus 6, and adenovirus remain persistent challenges, particularly in adult recipients [4–7]. The characteristics of UCBT that account for its particular profile of hematopoietic and immune reconstitution and susceptibility to viral infections are described in the following sections.

2 Innate Immunity—Neutrophil Engraftment and Chimerism

The first readily quantifiable step in hematopoietic and immunologic reconstitution is neutrophil engraftment. This occurs with delayed kinetics after dUCBT, at least 1 week longer than neutrophil engraftment after adult stem cell products [2, 3]. Graft

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failure can occur in up to 20 % of dUCBT recipients versus 7 % of adult bone marrow (BM) and an even smaller percentage of peripheral blood stem cell (PBSC) recipients [1]. Neutrophil reconstitution has been shown to correlate with infused doses of total nucleated cells, CD34 + progenitor cells, and myeloid colony-forming units [8–10]. Increasing degree of HLA disparity correlates with slower neutrophil recovery [10–12]. The presence of preexisting recipient anti-HLA antibodies to a mismatched antigen expressed by cord units has recently been found to correlate with increased incidence of graft failure, prolonged time to neutrophil engraftment, excess 100 day transplant-related mortality (TRM), and inferior progression-free survival (PFS) and overall survival (OS) [13].

To provide a higher dose of stem cells, adult patients often receive two UCB products. This approach seems to enhance kinetics of neutrophil engraftment, especially in adults. Following dUCBT, predominance of one unit is usually established early after transplant. One report demonstrated that peripheral blood or marrow chimerism (largely reflecting myeloid chimerism) was derived from a single cord in 76 % of recipients by 21 days [14, 15]. Dominant chimerism of one cord by day 21 after dUCBT has been associated with speed and success of engraftment [16]. Higher total nucleated cell (TNC) numbers, TNC viability, CD34 + numbers, order of infusion, and degree of HLA matching have not consistently predicted dominance by a particular cord. A role for higher CD3 + T cell doses and perhaps higher CD34 + cell viability has been proposed [14, 17]. Recently, immune rejection of the nondominant unit mediated by interferon-gamma (IFN-gamma)-expressing CD8 + effector T cells derived from naive precursors in the dominant cord has been suggested [18–20].

3 Adaptive Immunity—Lymphocyte Reconstitution After HSCT—General Principles

Quantitative and functional reconstitution of lymphoid cell subsets after allogeneic HSCT follows a general pattern regardless of stem cell source (Fig. 10.1). After resolution of the initial phase of myeloid aplasia, the next few months are characterized by quantitative and qualitative lymphocyte deficiencies. Innate immune cells recover early after transplant, but cellular and humoral adaptive immunity are impaired for prolonged periods, rendering recipients particularly susceptible to viral and fungal infections. Natural killer (NK) cells are the first to recover with an initial profound expansion of an IFN-gamma-producing CD56 + CD16− subset; in the first few months NK cells kill KIR ligand-negative targets quite efficiently [21]. Numbers of blood monocytes and tissue macrophages normalize within months of transplant but their functionality in the first year after transplantation is controversial. CD8 + T cell recovery then commences followed by dendritic cells and then slowly by B cells and CD4 + T cells [22, 23].

T Cell Reconstitution Recovery of T cells occurs in two phases. In the first phase, naive and memory T cells preexisting in the stem cell graft or residual T cells from the recipient either (1) expand in the periphery following antigen stimulation, leading to

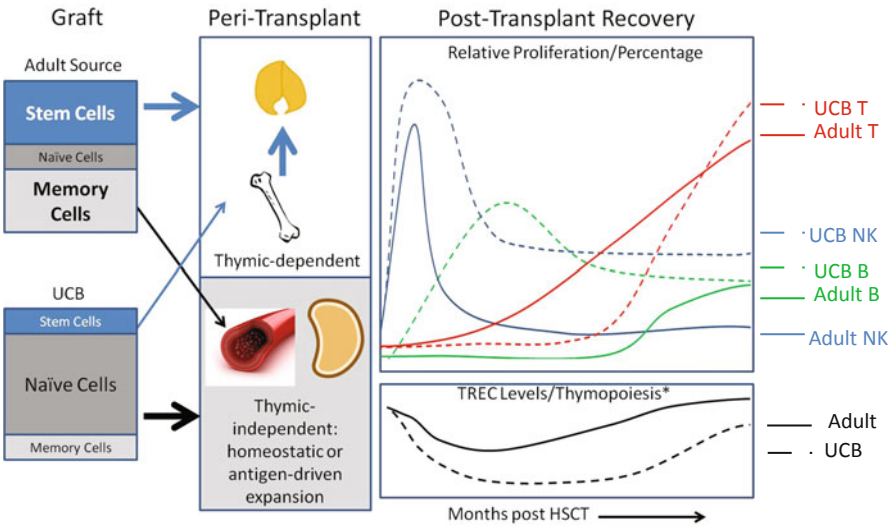

Fig. 10.1 Unique aspects of graft composition and reconstitution after umbilical cord blood transplantation (*UCBT*) versus hematopoietic stem cell transplantation (*HSCT*) with an adult stem cell product. *Left panel*: Adult stem cell grafts are characterized by higher numbers of stem cells and a predominance of memory cells within the T cell compartment. *UCB* grafts contain at least tenfold fewer stem cells and primarily naïve T cells. *Middle panel*: Both grafts are dependent on an initial peripheral homeostatic or antigen-driven expansion of committed precursors and memory and naïve lymphocytes that are independent of the thymus (*black arrows*). This is followed by a thymic-dependent phase of proliferation driven by stem cells that have migrated to the bone marrow, producing T cell precursors that then home to the thymus (*blue arrows*). *Right panel: Upper*—Schematic of robust initial natural killer (*NK*) cell proliferation and reconstitution (*blue*) followed by exuberant *UCB* B cell proliferation (*green*). T cell proliferation and numerical reconstitution (*red*) lags behind. *UCB* T cells and adult B cells are slowest to repopulate. *UCB* reconstitution—*dashed lines*. Adult *HSCT* reconstitution—*solid lines*. *Lower*—Recovery of T cell receptor excision circle (*TREC*) values, an indicator of thymic function and output, is slower in *UCB* recipients (*dashed line*) versus adult *HSCT* recipients (*solid line*). *Asterisk*: Particularly for *UCBT,* variables such as patient age, conditioning regimen intensity, use of in vivo T cell depletion, and occurrence of GvHD may impact the kinetics of thymopoiesis and establishment of T cell diversity

a selective, restricted repertoire or (2) undergo homeostatic expansion through low-affinity interactions with self-peptide / major histocompatibility complexes (MHCs), a process driven by lymphopenia and high levels of cytokines which has no impact on T cell diversity. Antigen recognition during this period of peripheral T cell expansion is limited by the starting repertoire of T cells within the cord units and becomes progressively more oligoclonal or skewed over time [24]. Donor-derived (in the case of adult stem cell products) or residual recipient memory T cells provide much of the protection against infectious pathogens during this time [25]. However, many of these cells do not express homing receptors necessary to enter secondary lymphoid organs and interact efficiently with professional antigen-presenting cells (APCs) and B cells. Graft content of CD4 + T cells, particularly memory or effector cells, influences early CD4 + counts and may affect the incidence of infections [26, 27].

Conditioning regimen, graft composition, HLA mismatches, exposure to infections, immune suppression, and incidence of acute GvHD all impact this expansion phase, making patterns of reconstitution somewhat unique in each individual.

Thymic-dependent T cell neogenesis comprises the second phase of T cell reconstitution. This entails homing of BM-derived progenitors to the thymus, commitment of progenitors to the T cell lineage, T cell receptor (TCR) rearrangement, maturation, export, and migration to peripheral niches where antigen recognition and conversion to an effector or memory phenotype can occur. This process is crucial to maintaining a constant supply of naive T cells and robust repertoire for antigen recognition [28, 24]. This phase of reconstitution takes months to years, during which recipients continue to be highly susceptible to viruses, fungi, and encapsulated bacteria. In contrast to mature T cells in the stem cell product, naive T cells generated from donor stem cells are subjected to positive and negative selection in the recipient thymic environment. T cell recovery in this phase is therefore inextricably linked to age-dependent thymic involution, pretransplantation cytotoxic therapy and damage to marrow and thymic niches, and stimulation of alloreactivity during GvHD. Only about one third of recipients reach age-adjusted normal values of thymic output at a median follow-up of almost 2 years, emphasizing the extended duration of this impairment, particularly in adult recipients [23, 29].

Monitoring Thymic Function Until recently, evidence of thymic recovery and emigration of T cells from the thymus was quantified primarily via naive T cell numbers assessed by flow cytometry and by TCR excision circle (TREC)- containing cells (see chapter by Politikos). Recent thymic emigrants (primarily CD45RA + CD31 +) can be enumerated ex vivo by measuring episomal DNA circles of the TCR locus excised during recombination of the locus (signal-joint TREC, sjTREC), excision circles created during TCR chain recombination (TREC), or a ratio of the two [30, 29]. TREC copy number per CD3 + T cell count or absolute numbers of TREC/mL of blood reflects the number of cells exiting the thymus; the number of TREC-containing T cells decreases with rapid proliferation. Based on TREC analysis, thymic regeneration of CD8 + T cells and resulting diversification seems to occur faster than for CD4 + T cells. By 1 year after transplant, the correlations between thymic activity, T cell repertoire diversity and patient outcomes such as GvHD, severe infections, chimerism, and relapse are clear [31–34]. However, more accurate and prognostic measurements of immune reconstitution in the first 6 months are needed.

B Cells and Dendritic Cells Reconstitution of a diverse B cell population can likewise take up to 2 years. Transitional B cells characterized by CD19 + CD24 + CD38 ++ surface expression increase first, followed by mature B cells. Antibodies early post transplant are primarily of recipient origin and then transition to donor-type antibodies [27]. A prolonged deficit of memory B cells with restricted immunoglobulin gene rearrangement and impaired immunoglobulin class switching and production leads to long-lasting vulnerability to *S. pneumoniae*, *H. influenza*, and other encapsulated organisms [35, 36]. Low levels of epithelial, extrafollicular, follicular, and peripheral blood dendritic cells (DCs) normalize over 6 months to 1 year. Conventional or myeloid DC1 cells reappear prior to plasmacytoid DC2 cells [37, 38]. However, the kinetics and source of DC recovery are not well characterized.

4 Quantitative Comparisons of Lymphocyte Recovery After UCBT and Adult Stem Cell Products

In multiple clinical settings, lymphoid recovery is slower after UCBT than after adult donor transplantation. Single UCBT recipients had lower absolute lymphocyte counts at 30 and 60 days after myeloablative conditioning (MAC) with anti-thymocyte globulin (ATG) than matched unrelated donor (MUD) recipients [39]. Rapid recovery of absolute lymphocyte counts correlated with improved overall survival in UCBT recipients [40]. After reduced-intensity conditioning (RIC) including ATG, a rapid and sustained increase in NK cell levels to above the baseline of healthy donors and an exuberant expansion of B cells to above normal levels occurred starting day 30 post single UCBT. Increases in CD4 and CD8 T cells were not seen until at least 6 months. At 1 year, CD8 + numbers approached normal levels, but CD4 + T cell counts remained approximately half of normal. In this setting, sjTREC quantitation detected thymopoiesis in only 10 % of UCBT subjects, and TREC levels were significantly lower than after autologous or allogeneic adult HSCT [41]. Both CD4 + and CD8 + T compartments contained few naive cells after UCBT; central and effector memory CD4 + T cells and effector memory and late effector memory CD8 + cells predominated. Recipients with CD4 + naive or central memory counts above the median at day 30 had improved survival, linking thymopoiesis to improved clinical outcomes.

Similar data were obtained when lymphocyte reconstitution of dUCBT recipients was compared to recipients of MUD PBSCs after RIC HSCT, which included ATG for the dUCBTs only. The UCBT cohort again demonstrated accelerated recovery of NK and B cells with higher levels maintained from 3 to 24 months. Numerical reconstitution of T cells, specifically, naive and memory CD4 +, CD4 + CD25 + regulatory T cells (Tregs), and CD8 + T cells was significantly lower for the first 6 months after dUCBT, with median values for the two cohorts converging by 12 to 18 months [42]. dUCBT recipients had infection rates of 59 % within 100 days and 69 % overall, significantly higher than the 8 % and 33 % rates of infection seen after MUD PBSCs. Rates of relapse, TRM, PFS, and OS were no different. After MAC without ATG, B cell and NK recovery was also more rapid in the dUCBT cohort than after Matched Related Donor (MRD) or MUD HSCT. Significantly lower numbers of CD4 +/CD8 + T cells and Tregs were seen for 6 and 12 months, respectively, after dUCBT. The cumulative incidence of CMV reactivation was 0.84 in the dUCB and 0.53 in the MRD/MUD recipients, again confirming increased viral susceptibility after dUCBT [43]. In contrast to the prior study which involved RIC dUCBT with ATG in older recipients, this second study reported that thymopoiesis as measured by TRECs was comparable between dUCBT and MRD/MUD recipients by only 6 months after HSCT.

Comparisons between recipients of UCBT (with heterogenous conditioning regimens) and recipients of 9/10 mismatched unrelated adult HSCT showed similar kinetics of naive and memory CD4 + and CD8 + T cells, Tregs, and naive B cell

recovery. Both groups were characterized by very low absolute CD4 + T cells numbers, barely 500/μl at 1 year, and a prevalence of naive B cells. In multivariable analyses, lower CD4 + and higher CD8 + cell counts at 3 months correlated with increased risk of overall and specifically viral infection, but not bacterial infection. Higher numbers of memory CD4 + T cells protected against infections, while, surprisingly, higher numbers of effector and late effector memory CD8 + subsets at 3 months predicted for high rates of viral infections. Cumulative incidence rates for infection over 18 months were 57 % and 72 % for mismatched unrelated adult donor and UCBT recipients, respectively [44]. Thus, when compared to recipients of adult stem cells, UCB recipients exhibit a consistent pattern of robust naive B and NK cell numerical recovery followed by delayed CD4 + and CD8 + T cell recovery and impaired functional T cell immunity. Variations in kinetics of thymic recovery are likely affected by age, intensity of conditioning regimens, and use of ATG for in vivo T cell depletion but are reproducibly delayed by at least 6 months after DUCBT (Fig. 10.1) [45–48].

5 Unique Cellular Composition of UCB

UCB Stem Cell Qualities Delays in quantitative myeloid and lymphoid reconstitution reflect the unique composition and function of cells present in umbilical cord units (Table 10.1). Median numbers of hematopoietic stem cells are a factor of 10 fewer in cord units [39, 42]. UCB units contain about half as many B, CD4 +, and CD8 + T cells than BM and PBSC sources; CD4 + counts early after transplant are influenced by the number of graft CD4 + T cells [27]. UCB stem cells appear more primitive, survive longer in culture, and have greater proliferative capacity. UCB contains greater numbers of immature colony-forming cells [49–52]. There is differential expansion of UCB stem cells in response to cytokines, resulting in variations in cell cycle and homing ability. The higher proportion of CD133 + primitive stem cells in UCB results in higher levels of Notch1 and increased NF-κB signaling [53]. This superior proliferative potential likely enables the use of comparatively few stem cells in UCBT and is being exploited in ex vivo expansion systems.

UCB T Cells UCB T cells are almost exclusively naive in contrast to adult blood in which antigen-experienced T cells predominate [54]. UCB mononuclear cells show reduced capacity to secrete multiple cytokines and lymphokines, particularly interleukin (IL)-2, IFN-gamma, and TNF-beta after allogeneic activation. There is a relative absence of granzyme and perforin expression and aberrant Fas ligand-mediated cytotoxicity [55, 56]. Unlike their adult counterparts, UCB T cells express telomerase. More UCB T cells are actively cycling and entering apoptosis, but these proliferating T cells retain a naive phenotype and may reflect potent homeostatic expansion rather than stimulation by antigen [57]. As a result of decreased expression of the central activation signaling molecule NFAT2c (Nuclear Factor of Activated T cells), UCB T cells may have more stringent activation requirements compared to their adult counterparts [58]. Naive

Table 10.1 Characteristics of umbilical cord blood products

Cell type	Umbilical cord graft compared to adult stem cell source
CD34 + stem cells	
Absolute numbers	Reduced over tenfold
Proportion of primitive stem cells	Increased
Proliferative potential/rate	Increased
Colony-forming capacity	Increased
Engraftment capacity	Increased
Notch levels and NF-κB signaling	Increased
Homing to central lymphoid organs	Increased
T cells	
Naïve/memory distribution	Primarily naïve cells
Cytokine production to mitogens	Decreased
Activation requirements	More stringent
Cytotoxic effector function	Decreased
Proliferative potential	Similar
Susceptibility to apoptosis	Increased
Expression of CD40 L	Decreased
Expression of perforin	Decreased
Telomerase expression	Increased
Impact of IL-15 on survival	Increased
CD4 + T cells	
Bias towards Th2 differentiation	Increased
IL-17 expression after activation	Decreased
CD8 + T cells	
IFN-gamma production	Decreased
Terminal differentiation after activation	Increased
Treg cells	
Level of CD25 and FoxP3 expression	Increased
Proliferative potential	Increased
Suppressive activity after stimulation	Similar to Increased
CTLA-4 expression	Increased
TGF-beta production	Increased
NK cells	
Proportion of CD56 + cells	Decreased
Perforin/granzyme expression	Similar to increased
IFN-gamma production after stimulation	Decreased
Cytotoxicity	Decreased
Adhesion molecule expression	Decreased
Ability to form immunologic synapses	Decreased
B cells	
Proportion of CD5 + B1 cells	Increased
Proportion of CD23- immature B cells	Increased
Ig class switching/IgH rearrangement	Decreased
Susceptibility to apoptosis	Increased
Dendritic cells	
Activation in response to LPS, CpG	Decreased
Expression HLA-DR/costimulatory molecules	Decreased
IL-12 production	Decreased
Expression of Th1-related genes	Decreased

Table 10.1 (continued)

Cell type	Umbilical cord graft compared to adult stem cell source
TLR-4 expression	Decreased
Adhesion molecules	Decreased
Plasmacytoid to myeloid DC ratio	Increased
Ability to stimulate naïve T cells	Decreased
Bias towards Th2 priming	Increased
Induction of Treg cells	Increased

Th T-helper, *DC* dendritic cell, *TLR* toll-like receptor, *IL* interleukin, *HLA* human leukocyte antigen, *LPS* lipopolysaccharides, *IgH* immunoglobulin heavy chain, *IFN* interferon, *NK* natural killer, *CTLA* cytotoxic T-lymphocyte antigen, *Tregs* regulatory T cells, *TGF* transforming growth factor

UCB T cells have a bias towards T-helper type 2 (Th2) functional lineage commitment with robust production of IL-13 and lower IFN-gamma production upon stimulation than naive adult T cells. Expression of T-bet and STAT4, which play critical roles in Th1 differentiation, is reduced in UCB CD4 + T cells [59]. This functional skewing may be influenced by factors at the maternal-fetal interface such as IL-10, IL-4, Prostaglandin E_2, and elimination of activated T cells by Fas-ligand expression. Antigen-specific CD8 + UCB T cells are more likely to reach terminal differentiation following polyclonal stimulation and produce less IFN-gamma in response to cognate peptide than their adult counterparts. These characteristics unique to UCB T cells likely impact both incidence of GvHD and opportunistic infections after UCBT [60].

CD25 + FoxP3 + Treg cells are found at a relatively high frequency in UCB and expand more robustly to anti-CD3/anti-CD28 and IL-2 than do Tregs from adult blood. Resulting changes in cytotoxic T-lymphocyte antigen-4 (CTLA-4) expression and cytokine production differ from those of adult Tregs [61]. UCB Tregs seem to exhibit more potent suppressor function after polyclonal T cell activation, which may be resistant to drugs used in GvHD prophylaxis [62]. However, there are conflicting data on how effectively UCB Tregs function to suppress antigen-specific responses [63–65].

UCB Antigen-Presenting Cells UCB DCs exhibit impaired antigen-presenting function and are only weakly stimulatory to T cells in mixed lymphocyte reactions as compared to adult DCs. They show less HLA-DR, CD40, CD80, CD83, and CD86 up-regulation; less signaling downstream of toll-like receptor-4 (TLR-4); and decreased TNF-α and IL-12 secretion after activation [66]. UCB DCs are characterized by a higher ratio of plasmacytoid to myeloid DCs than adult DCs [67]. Expression of Th1-response-related genes and chemokines is lower, perhaps favoring induction of tolerance [68, 69]. It has been suggested, however, that prolonged stimulation can restore UCB DC activity, possibly contributing to delayed onset of GvHD after UCBT. Thus, UCB T cells and DCs undergo different programming than their adult counterparts [70, 71].

UCB NK and B Cells UCB NK cells are functionally mature and express cytotoxic perforin and granzyme at or above normal levels [72]. They respond similarly to adult NK cells upon stimulation with IL-12 or IL-15. However, the majority of studies seem to demonstrate less potent lytic activity and IFN-gamma production against

target cells than adult BM-derived NK cells, with impaired ability to express adhesion molecules and form immunologic synapses with target cells. Some of these deficiencies can be reversed with exposure to IL-15 or ex vivo expansion using IL-2 [73, 74]. UCB B cells contain higher percentages of CD5 + B1 cells and CD23-immature B cells. It is been postulated that relative lack of terminal deoxynucleotidyl transferase (TdT) expression in UCB pre-B cells and fewer productive rearrangements in the immunoglobulin heavy chain (IgH) gene might delay B cell maturation after UCBT [75].

6 Qualitative Reconstitution of Immunologic Diversity

Standard Assays for Immunologic Competence For the reasons noted above, numbers of T and B cells do not correlate with their functional capacity after UCBT. Flow cytometric monitoring of naive lymphocyte subsets and recent thymic emigrants and quantification of TRECs are used to infer thymic activity and more robust functional immune recovery. These surrogates have correlated with fewer opportunistic infections and improved survival [34, 76, 77]. Analysis of pathogen-specific T cell reconstitution has also been performed as an indicator of global immune function. Reactivity of antigen-specific T cells, e.g., to CMV or EBV, can be tracked quantitatively via cytokine secretion in enzyme-linked immunosorbent spot (ELISPOT) assays, cytokine production by intracellular staining, and by MHC-multimer staining [78]. As an example, such assays have revealed reconstitution of functional cytotoxic CD8 + T cells and CMV-reactive CD4 + T cells by 3 months after adult HSCT [79]. However, each of these assays has limitations and reflects reactivity to only a single or restricted panel of pathogens/antigens.

Repertoire Diversity by TCR Sequencing Historically, spectratyping to assess distribution in lengths of TCR beta-chain complementarity determining region 3 (CDR3) using polymerase chain reaction (PCR) and primers specific for individual Vβ subfamilies served as a readout of T cell diversity or polyclonality. This technique revealed that αβ T cells in UCB grafts are extremely polyclonal [33, 32]. This approach to quantifying the T cell repertoire has recently been superseded by multiplex PCR techniques and high-throughput sequencing. Although methods vary by substrates Vβ (e.g., complementary DNA vs. genomic DNA, mixed vs. sorted T cells), unique V and Jβ primers, different algorithms for bioinformatic analysis, and variable statistical descriptors of T cell population diversity, TCR sequencing is a powerful tool that enables description of the whole T cell population as well as quantification and tracking of individual T cell clones (Fig. 10.2). Techniques allow analysis of both T and B cell diversity through sequencing of TCR αβγδ genes and IgH genes [80–83].

Spectra typing of the TCR repertoire of MRD, MUD, and UCB adult recipients following MAC without T cell depletion showed poor diversification of the T cell

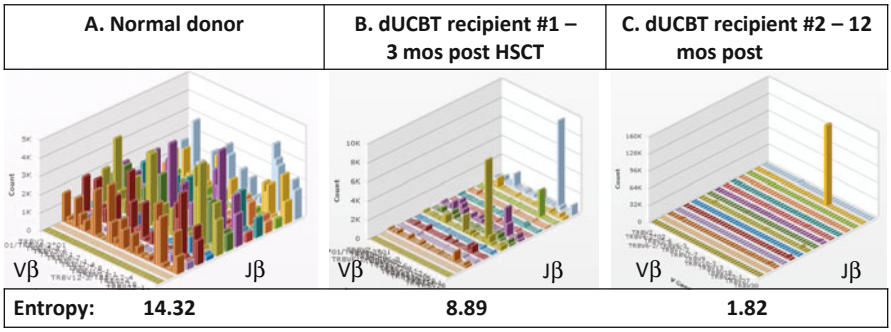

Fig. 10.2 Representative TCRβ sequencing from a healthy donor, dUCBT recipient at 3 months, and double umbilical cord blood transplantation (*dUCBT*) recipient at 12 months after hematopoietic stem cell transplantation (*HSCT*). Absolute numbers of productive CDR3 sequences for each Vβ/Jβ pairing are shown. *Left:* normal donor—diverse T cell repertoire. *Middle: dUCBT* recipient—moderately restricted. *Right: dUCBT* recipient several months prior to succumbing to CMV and *Aspergillus* infections—extremely restricted

repertoire for up to a year in nearly all recipients, without any clear correlation between repertoire diversity and the higher incidence of infections seen in the first 100 days after UCBT. B cell repertoire diversified in most recipients by 90 days after HSCT [84]. TCR sequencing has shown similar T cell diversity indices in MRD/MUD and UCBT recipients who have similar TREC levels, both of which were below diversity values for healthy controls; this supports using TCR sequence diversity as a reflection of thymopoiesis [43]. Van Heijst et al. showed that CD4 + T cell diversity was 50 times that of CD8 + T cell diversity in all recipients of allo-HSCT (PBSC, T cell depleted, PBSC, Tcell depleted PBSC or dUCBT without ATG) and healthy individuals. Perhaps surprisingly, by 6 months after dUCBT CD4 + and CD8 + T cell populations showed higher diversity than in PBSC and TCD PBSC recipients [83]. Interestingly, some recipients had very low TCR diversity but normal T cell counts, indicating lack of concordance between diversity and numerical repopulation. Although the prognostic significance of such detailed repertoire analysis has not yet been demonstrated, recent data suggest that decreased TCR diversity within the first 3 months after UCBT can predict poor survival [85, 86].

A diverse T and B cell repertoire is presumed to be central to effective pathogen-specific immunity, but evidence that TCR diversity is critical to successful control of human infections is limited. Recently, an inverse correlation between CD8 + TCR repertoire diversity and CMV-specific antibody levels, a surrogate for prior viral reactivation, was described in normal donors, strengthening this hypothesis [87]. CD8 + T cell diversity seemed to be more relevant than the number of CMV-specific tetramer-binding cells. Additionally, low TCR diversity in both CD4 + and CD8 + T cells after HSCT from PBSC or UCB has been associated with CMV or EBV infection, although cause and effect have not been established [83]. Thus, TCR repertoire may prove a valuable early readout to quantify functional T cell recovery and evaluate therapeutic strategies for enhancing immune reconstitution.

7 Functional Reconstitution and Antigen-specific Immunity After UCB

To date, the most relevant indicators of functional reconstitution after HSCT come from studies of viral-specific immunity, commonly tracked against the herpes viruses. This is relevant as rates of CMV reactivation and disease after UCBT range between 22–100 % and 6–16 %, respectively; the wide range reflects heterogeneity in study populations and treatment regimens [88]. EBV (particularly in the presence of ATG), Human herpesvirus 6, and Varicella Zoster (VZV) have reactivation rates of up to 21 %, 87–100 %, and 46 %, respectively [89–92]. BK virus reactivation and disease is a significant cause of morbidity for which UCBT is a significant independent risk factor [93]. Antigen-specific immunity to bacterial and fungal pathogens is more difficult to assess. Data regarding whether overall rates of infectious complications are higher after UCBT than after adult stem cell HSCTs is contradictory. However, increased rates of early viral reactivation and infection are consistently observed [88].

Unlike the consistent observation of delayed numerical reconstitution, recovery of antigen-specific immunity after UCBT seems somewhat inconsistent. Only 66 of 153 pediatric recipients of single UCB following ATG developed detectable T cell activity to herpes simplex virus, CMV, and VZV over 4 years following UCBT. In this subset of patients, however, T cells specific for herpes simplex virus, CMV, and VZV were detected as early as 29, 44, and 94 days after UCBT, respectively. The presence of one of these antigen-specific T cell responses decreased the likelihood of infection-related death, demonstrating the favorable prognostic impact of pathogen-specific immunity after UCBT. In contrast, absolute lymphocyte or CD3, CD4, CD8, and CD19 counts did not correlate with the recovery of antigen-specific T cell responses, emphasizing the pitfalls of using surrogate numerical markers [94]. In another study, 44 % of adult UCB recipients were found to have circulating CMV-specific CD4 + and 50 % to have CMV-specific CD8 + T cells, by day 100; the average frequency of CMV-specific CD8 + T cells in those individuals actually approached that expected in healthy CMV-seropositive individuals [41]. A link between thymopoiesis and detection of anti-viral immunity was nicely demonstrated by Brown et al. CD8 + T cells capable of secreting IFN-gamma were seen within 8 weeks of dUCBT, but the dependence on later recovery of naive T cells and TREC levels demonstrated that thymopoiesis was necessary to clear CMV viremia [95] (see chapter by Politikos). Recovery of antigen-specific responses to herpes viruses also correlates with a PFS advantage, primarily due to a decrease in relapse [96].

8 Current Approaches to Improve Immune Reconstitution After UCBT

Neutrophil Engraftment Several strategies for improving kinetics of myeloid/innate immune engraftment to avoid early infections are under development [97, 52]. Approaches to improve homing and engraftment of umbilical cord progenitors include priming ex vivo by fucosylation, fibronectin, hyaluronic acid, Prostaglandin

E2; inhibiting CD26/dipeptidylpeptidase IV to improve SDF-1/CXCR4-mediated chemotaxis; or direct injection into iliac crest sites [98–102]. Expansion of early and late progenitors ex vivo resulting in shorter time to neutrophil engraftment has been achieved using ex vivo co-culture with allogeneic mesenchymal stromal cells; stimulation with Notch ligand to manipulate signaling pathways; and culturing in media with copper chelators, hydrocarbon receptor antagonists, or other agents to inhibit stem cell differentiation and increase numbers of CD34 + cells [103–106]. Co-infusion of UCB stem cells with additional or accessory cells to achieve early neutrophil recovery has also been attempted. Such cell sources under investigation include mobilized hematopoietic stem cells from a third party adult donor, third party cord, or haploidentical donor; haploidentical mesenchymal stromal cells; and (in murine experiments) ex vivo expanded somatic stem cells or stromal cells [107–110] (see chapters by Van Besien, Delima, and Farag). Despite impacts on neutrophil engraftment, clear effects on clinical outcomes using these approaches have not been robustly demonstrated to date.

Enhancing Adaptive Immunity After expansion with anti-CD3/anti-CD28 beads, UCB T cells retain their naive phenotype and polyclonal TCR diversity, suggesting that ex vivo manipulation need not reduce T cell diversity. Several specific strategies to boost lymphocyte diversity and function after UCBT include treatment with cytokines: specifically, low dose IL-2 to enhance NK cell numbers and activity, IL-7 to improve naive T cell expansion and survival, and IL-15 and IL-21 given their roles in proliferation and function of memory CD8 + T cells [111–115, 28]. Adoptive cellular therapy with specific components derived from UCB products is also being pursued. First, functionally active T cells recognizing CMV, EBV, and adenovirus have been expanded from naive UCB T cell populations and used as both prophylactic and therapeutic agents after dUCBT [116]. UCB-derived antigen-specific T cells have been generated against tumor and leukemic antigens such as melanoma-associated antigen recognized by T cells (MART)-1 and CD19 using chimeric antigen receptors [60, 117]. Second, UCB Tregs have been expanded ex vivo with IL-2 with retention of transforming growth factor (TGF)-beta secretion and suppressive potency [118]. Recently infusion of ex vivo expanded UCB Tregs after dUCBT was tolerated without increased risk of relapse, infection, or early mortality. Compared to historical controls, UCB Treg infusions reduced incidence of acute GvHD [119]. Third, use of UCB NK cells to prevent relapse is being evaluated. Thus, while UCBT presents a scenario where further immunomodulation may be beneficial, UCB components themselves are being exploited to fill this need [36, 97, 120].

9 Summary

HSCT using UCB is characterized by unique kinetics of neutrophil and lymphocyte reconstitution as compared to adult stem cell sources. This impacts clinical management and outcomes after UCBT. While the general order of cell subset recovery and paradigm of initial peripheral oligoclonal expansion followed by thymic-dependent

reconstitution still pertains, umbilical cord units contain stem cells, NK cells, T cells, and APCs with different proliferative, intracellular signaling, and functional capacities from their adult counterparts. Quantitative recovery of lymphocyte subsets after UCBT is complicated by variations in recipient age and conditioning regimens but is increasingly well characterized. However, the most relevant way to assess and impact functional reconstitution of immunologic diversity remains unclear. Recovery of naive cell populations, TRECs, polyclonality/diversity of TCR sequences, and activity against individual pathogens are all reasonable methods which generally correlate with each other. Data is emerging to link each of these measures with infectious and other clinical outcomes. The current challenge facing the UCBT field is to expand the umbilical cord stem cell population, to improve thymic function and homing/engraftment of T cell progenitors, and to augment lymphocyte subsets that mitigate GvHD and infectious complications while preserving graft-versus-tumor effects. Many new enhancements to UCBT are in clinical development. Long-term follow-up and comprehensive assessment of immunologic recovery will be necessary to demonstrate progress. UCBT should be regarded as an immunologic platform significantly different from HSCT using adult stem cell sources.

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Chapter 11
Thymic Regeneration after Umbilical Cord Blood Transplantation: Mechanisms, Measurements and Implications on Anti-Viral Immunity

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1 Introduction

Umbilical cord blood (UCB) represents a useful alternative source of hematopoietic stem cells (HSCs) for patients lacking sibling or unrelated human leukocyte antigen (HLA)-matched adult donors. UCB grafts are increasingly used for transplantation in the treatment of both malignant and nonmalignant diseases in children and adults [1–3]. While offering some advantages over peripherally mobilized or bone marrow HSC units, including noninvasive procurement, timely availability, and allowance for greater HLA mismatch [4], UCB grafts also exhibit distinct biological characteristics which may contribute to a higher risk of infection. Specifically, UCB units contain a lower number of hematopoietic progenitor cells and fewer T lymphocytes, which are uniformly naïve, resulting in prolonged time to engraftment and delayed and incomplete immune reconstitution [5]. Consequently, infectious complications and Epstein-Barr virus (EBV)-driven posttransplant lymphoproliferative disorder (PTLD) remain a major cause of mortality and morbidity after umbilical cord blood transplantation (UCBT). In this chapter, we will review the imperative role of the thymus in the reconstitution of adaptive immunity and in clinical outcomes after UCBT.

2 Review of Normal Thymopoiesis

The thymus is the primary site of T lymphopoiesis and is essential for the durable maintenance of a broad T cell receptor (TCR) repertoire, capable of responding to foreign antigens while maintaining self-tolerance. The thymic stroma, consisting of thymic epithelial cells (TECs) and other supportive cells, plays a pivotal role in

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this function by providing the microenvironment that supports T cell proliferation, differentiation, and selection via the production of cytokines (interleukin (IL)-7, Stem Cell Factor (SCF), keratinocyte growth factor (KGF), chemokine ligand 25 (CCL25)) and surface proteins (chemokine receptors, peptide, or major histocompatibility complex (MHC)) that facilitate trafficking and cell-cell interactions [6]. Thymopoiesis in humans is most active in the fetal and perinatal period. Following the first year of life, the size of the thymus gradually decreases, a process known as thymic involution [7, 8]. However, de novo thymic production of naïve T cells has been detected throughout life [9, 10], whereas the involution process can be potentially reversed (thymic rebound), as seen in the lymphopenic setting following autologous stem cell transplantation [11] or in HIV patients initiated on highly active antiretroviral therapy (HAART) [12, 13].

T cell development begins with thymic seeding with early lymphoid progenitors (ELPs) that originate from multipotent HSCs in the bone marrow and reach the thymus through the circulation. T cell progenitors initially settle in the cortex, where they undergo T cell lineage commitment and expansion under the influence of Notch signaling and interactions with the cortical TECs (cTECs) [14, 15], and they subsequently begin the process of differentiation from the $CD4^{-}CD8^{-}$ double negative (DN) to the $CD4^{+}$ $CD8^{+}$ double positive (DP) stage. DN thymocytes rearrange the T cell receptor (TCR) beta (TCRB) locus to create a TCR-β chain, which leads to the formation of the pre-TCR. Cells that produce a functional pre-TCR are selected to survive the beta-selection checkpoint and develop into DP thymocytes, which undergo rearrangement of the alpha (α) chain, resulting in the assembly of the αβ-TCR. DP cells expressing surface αβ-TCR are then subject to positive selection. Only thymocytes with a TCR capable of binding self-peptide/self-MHC complex expressed by cTECs are allowed to survive, whereas the rest will undergo death by neglect. Finally, positively selected DP thymocytes differentiate into CD4 and CD8 single positive (SP) thymocytes, according to the restriction of their TCR to recognize either MHC class I or II molecules. SP cells then migrate to the medulla, via up regulation of the chemokine receptor CCR7, and undergo negative selection, a process that assures the elimination of self-reactive lymphocytes. Negative selection is mediated by the medullary TECs (mTECs), which have the unique capacity of promiscuous expression of tissue restricted antigens (TRAs), under the control of the transcription factor Aire [16, 17]. Thymocytes with high affinity TCRs for self-peptides presented on MHC class I or II molecules are eliminated by programmed cell death. Only the small percentage of thymocytes that have successfully undergone positive and negative selection exit the thymus and are termed recent thymic emigrants (RTEs).

3 Assessment of Thymopoiesis in Humans

The homeostatic maintenance of the peripheral T cell compartment depends on the constant export of functional RTEs from the thymus throughout adult life. Immunophenotyping of peripheral blood lymphocytes and determination of the naïve

T cell subsets is a valuable tool for the in vivo assessment of thymic RTE export. The expression of CD45 isotopes has been most widely used for the approximation of the thymus-derived naïve ($CD45RA^+$) T cells, although not always reliably [18]. A panel of adjunctive surface markers, including CD62 L, CCR7, CD31, CD27, αEβ7 integrin (CD103), has improved the differentiation between naïve and memory T cells but, similarly to CD45RA, their cell surface expression is not specific for RTEs [19–21].

A more precise quantification of thymic RTE export is provided by the *ex vivo* measurement of TCR excision circles (TRECs) [13, 22]. These are episomal molecules of circular DNA and represent byproducts of the sequential TCR recombination events that take place during the intrathymic T cell development and result in the formation of diverse αβ-TCRs [23]. The rearrangement of the TCRB locus (encoding segments of the TCR-β chain) occurs first at the DN thymocyte stage, which generates a great variety of βTREC products. The TCR alpha (TCRA) locus is rearranged next in DP thymocytes and is similarly characterized by enormous diversity. However, a common requirement for the productive TCRA variable joining (TCRAVJ) recombination is deletion of the TCR delta (TCRD) locus which it encompasses, a two-step process that gives rise to a signal-joint TREC (sjTREC) and a coding-joint TREC (cjTREC). Both DNA families of TRECs are stable [24] and do not replicate during mitosis. These sequences are also unique to naïve αβ T cells and they have not been detected in memory T cells or the small subset of γδ T cells [13], therefore they serve as a valuable marker of RTEs. Measurement of sjTRECs with quantitative competitive polymerase chain reaction (QC-PCR) has been extensively used to assess thymopoiesis and RTE output after HSC transplantation (HSCT). This is a very sensitive method and requires a small amount of DNA isolated from peripheral blood samples or T cell subpopulations of interest. While useful in the monitoring of thymopoietic recovery after HSCT, the sjTREC assay may be influenced by the proliferation history of the peripheral T cell pool, since TRECs do not replicate with mitosis. This is a limiting factor for interpatient comparisons, especially in the posttransplant setting that is characterized by significant variability in the kinetics of naïve T cell expansion and recovery. Therefore, the determination of the sj/β TREC ratio (thymic factor, TF) has recently been introduced as a more accurate method for the assessment of thymopoiesis [25, 26]. Since both sj and β TRECs are equally diluted with subsequent cell divisions, their ratio is independent of peripheral cell proliferation and it is considered as a "RTE signature" of the peripheral T cell compartment.

4 Mechanisms of T Cell Recovery After Allogeneic Stem Cell Transplantation

Regeneration of the T cell compartment after HSCT proceeds along two different pathways that act in parallel but follow distinct kinetics. These involve a thymic-dependent and thymic-independent mechanism. The thymic-independent pathway

predominates in the early posttransplant period and is mediated by adoptively transferred lymphocytes contained in the graft or recipient T cells that survive conditioning. These T cell populations undergo homeostatic expansion in response to lymphopenia and high cytokine levels or oligoclonic proliferation upon interaction with cognate antigens. However, the spectrum of immunologic responses provided by the thymic-independent pathway is limited by the starting repertoire of donor T cells and conditioning-resistant host lymphocytes. An additional challenge in UCBT recipients is related to the uniformly naïve nature of the graft-derived T cells. UCB T cells are characterized by less robust proliferation and effector cytokine production in response to pathogens compared to adult donor, non-T cell depleted, allografts [27]. As a result, this first phase of T cell reconstitution after UCBT results in impaired and incomplete adaptive immunity and contributes to the higher rates of infections in the early posttransplant period [28].

Regeneration of a functionally competent T cell compartment after HSCT and diversification of the TCR repertoire involves the thymic-dependent production of naïve T cells [29], a process that requires several weeks to months. The Thymus is also a sensitive target to conditioning chemotherapy and allogeneic graft-versus-host disease (GvHD), which can further delay RTE thymic output. An additional challenge in allogeneic transplantation is posed by the HLA discordance between the recipient and the graft. The intrathymic process of positive and negative selection normally occurs in a MHC restricted way. In the transplant setting, hematopoiesis is reconstituted with donor-derived cells expressing a different MHC haplotype, whereas the epithelial stromal cells remain of host origin. Therefore, MHC mismatches can adversely impact immune reconstitution after HSCT, by compromising thymic selection and naïve T cell homeostasis. This is of particular importance in UCBT, since the lower rate of GvHD observed with UCB grafts allows for a greater degree of HLA disparity (4/6 or $>$) and UCB recipients may receive, one or more, HLA-mismatched units. This greater degree of HLA mismatch might represent an additional factor contributing to delayed thymic reconstitution after UCBT.

5 TREC Levels in the Context of UCBT

Following allogeneic transplantation, all patients experience a period of profound immunodeficiency and absent thymic function, regardless of the graft source. Quantification of TRECs has been extensively used to monitor thymic recovery after HSCT. Although direct comparisons between different patient cohorts can be limited by the unbalanced population characteristics that may have a variable effect on TREC levels, TREC PCR assays have provided valuable insight into the distinct kinetics of thymic reconstitution in different transplant settings and the factors affecting thymic recovery.

TREC levels universally drop and remain extremely low or undetectable in the early posttransplant period. However, TRECs have been detected as early as 2 months in children and younger adults after HSCT and gradually rise towards pretransplant

levels by 12–24 months [30–32]. In pediatric patients receiving UCBT, TREC values reached a nadir at 3 months but recovered to near baseline levels at 6 months after transplant. Furthermore, no significant differences in the timeline of immune recovery were observed between UCB and haploidentical [33] or matched sibling [34] HSCT in pediatric recipients. In contrast, adult patients undergoing UCBT displayed delayed recovery of TRECs, starting 18 months after transplant [31]. Additionally, TREC numbers remained below the age-adjusted normal controls even at 36 months after transplant unlike pediatric patients, all of whom attained normal levels by 1 year. Although one might consider that factors such as conditioning, GvHD prophylaxis, and nucleated cell dose could contribute to these distinct outcomes in pediatric versus adult patient groups, a multivariate analysis showed that age was an independent factor influencing recovery of thymopoiesis after transplantation [35]. The profound thymic deficiency after single-unit UCBT in adult patients has been confirmed by a more recent study that reported a near complete absence of detectable sjTREC levels during the first year after transplantation [36].

The sequential administration of two UCB units in adults has improved the time to neutrophil engraftment [37]. Importantly, this approach has also resulted in earlier thymic reconstitution [38–40]. Adult recipients of double-unit UCBT (dUCBT) have detectable sjTREC levels as early as 100 days, with normalization of TREC values by 1 year after transplant [38]. This striking difference may be related to a cell dose effect in the context of two UCB units, whereas insufficient numbers of lymphoid progenitors might arise from a single UCB unit. Differences in conditioning regimens and GvHD prophylaxis in single versus double UCB recipients might contribute to the distinct outcomes on TREC recovery. However, early dominance of hematopoiesis from one of the two UCB units, which occurs prior to the recovery of thymic function, suggests that yet unidentified mechanisms related to the presence of two UCB units, other than cell dose, might be involved.

Several factors have been shown to influence thymic reconstitution and TREC levels after HSCT. As mentioned above, age is a major determinant of TREC recovery [35]. This is particularly important considering that the development of reduced-intensity conditioning (RIC) protocols with lower toxicity has made allogeneic transplantation feasible in older adults. At the cellular level, the process of thymic involution with aging is characterized by morphological and functional changes that lead to a decline in thymic T cell output and sjTREC levels [41]. However, a more important observation is that the age-related thymic senescence is accompanied by a decrease in the sj/β TREC ratio, which signifies a decreased capacity for intrathymic proliferation of thymocytes between the β- and α-chain recombination stages [26]. Advanced age correlates with a delay in the recovery of naïve T cells, higher risk for opportunistic infections and inferior overall survival (OS) after transplantation [42]. In contrast, donor age does not appear to affect thymic recovery [31], consistent with the hypothesis that thymus intrinsic host-related mechanisms regulate thymic activity. Thus, decreased thymic function with aging may pose a rate-limiting step in the immune reconstitution after HSCT.

Thymus is also a target organ of allogeneic GvHD, affecting both its lymphoid and epithelial compartments. Features of "thymic GvHD" include thymocyte depletion, changes in the number and composition of TEC subpopulations, disappearance of the corticomedullary demarcation, and absence of Hassall's bodies [43]. In turn, the distortion of normal architecture is accompanied by defective thymopoiesis. The severity of GvHD has been inversely correlated with T cell reconstitution both in adult HSC and UCB transplantation. Patients that develop acute GvHD (aGvHD) have reduced TREC numbers, delayed emergence of RTEs and oligoclonal T cell repertoire compared to patients without GvHD [25]. In contrast to the effect of aging, TREC assays have shown that both βTREC and sjTREC levels decrease in the course of aGvHD, whereas the sj/β ratio remains relatively constant [42, 44]. This pattern suggests that the aGvHD-induced delay in thymic recovery is not primarily due to impaired intrathymic proliferation but, instead, it underlines a block of T cell neogenesis at an early stage of development, before TCRB rearrangement. Extensive chronic GvHD (cGvHD) has also been associated with very low and often undetectable thymic function [32]. Furthermore, the use of immunosuppressive agents may negatively impact immune reconstitution in these patients. Consequently, the presence of GvHD and its effect on thymopoiesis is also associated with increased risk of infections and inferior overall outcome. Additional factors that may influence thymic recovery after HSCT include intensity of the conditioning regimen [45], use of ATG [31], radiation dose, T cell graft depletion, and pretransplant TREC levels [46], which in turn depend on the underlying disease, patient age, and treatment history. However, conclusions regarding the impact of these factors on thymic activity are not consistent among the various studies.

6 Prognostic Value of TREC Levels After UCBT

Thymopoietic recovery is a key component of immune reconstitution. TREC PCR assays are a valuable tool for the monitoring of thymic activity and TREC levels correlate with clinical outcomes after HSCT. Cytomegalovirus (CMV) is a common pathogen thought to contribute significantly to HSCT morbidity and mortality [47]. The frequency of CMV reactivation after UCBT varies from 21 to 100 % [48, 49]. In the largest published series of 332 UCBT recipients from the University of Minnesota [50], CMV reactivation was observed in 51 % of seropositive subjects, which is comparable to the rates observed in recipients of adult HSC grafts. This study showed that CMV seropositivity or reactivation did not impact disease-free survival (DFS), OS, or incidence of GvHD, with a trend toward worse 100-day TRM in seropositive patients. However, 27.1 % of patients experiencing reactivation developed clinical CMV disease, resulting in higher TRM and worse OS. Moreover, a different study has reported a positive correlation between aGvHD and CMV disease after UCBT [51]. Despite the seemingly similar rates of CMV reactivation, there is evidence that patients undergoing UCBT display a high incidence of late (post day 100) CMV disease and persistent CMV viremia, and they are more likely to require repeated courses

of preemptive gancyclovir therapy compared to other HSC graft recipients [52]. These findings suggest a delayed recovery of CMV- specific immunity after UCBT.

In contrast to peripherally mobilized or bone marrow HSCT, where CMV reactivation can originate from either a seropositive donor or recipient, the CMV source after UCBT is almost exclusively of host origin, since CMV infections in the prenatal period are rare and the majority of UCB units are seronegative [53]. Similarly to other herpes viruses, CMV enters a latent state after the primary infection and CMV- specific $CD4^+$ and $CD8^+$ T cell populations are essential to prevent reactivation [54, 55]. Therefore, an important concern in UCBT is related to the absence of CMV- specific memory cells in the UCB graft that would confer adoptive immunoprotection against reactivation after transplantation.

Detection of CMV specific effectors in dUCBT adult recipients, as determined by interferon (IFN)-γ enzyme linked immunoabsorbent spot (ELISpot) assay, has been reported as early as 8 weeks after transplantation, before the recovery of thymopoiesis [38]. This observation has been confirmed in another study [56] reporting detection of IFN-γ^+ CMV- specific $CD4^+$ and $CD8^+$ T cells by cytokine flow cytometry after *in vitro* stimulation in the majority of seropositive patients in the first 56 days after transplant. Of note, almost all patients had 100 % lymphoid reconstitution by one CB unit, as determined by chimerism analysis, confirming that the CMV- specific T cells were of UCB origin. These findings suggest that both $CD8^+$ and $CD4^+$ UCB-derived naïve T cells are primed to CMV early after UCBT and can give rise to functional CMV effectors, independent of thymic recovery. However, these $CD8^+$ CMV- specific T cells fail to control CMV reactivation. This functional deficiency of CMV- specific $CD8^+$ T cells may be explained by the profound paucity and slower reconstitution of $CD4^+$ T helper cells after UCBT [38], which are imperative for the development of a functional $CD8^+$ T cell response [57]. This conclusion is further supported by the fact that clearance of CMV viremia was increasingly observed after 6 months and correlated with the recovery of naïve $CD4^+$ $CD45RA^+$ T cells. Furthermore, clearance of CMV viremia was associated with the reemergence of TRECs, and UCBT recipients who attained normal TREC levels were more likely to display absence of CMV viremia, suggesting a critical contribution of the recovering thymopoiesis to the clinical control of the virus in vivo.

The prognostic value of TREC levels extends beyond its use as a marker of reconstitution of pathogen-specific immunity. It is a long-standing observation that successful pathogen-specific immune reconstitution after UCBT in pediatric patients with leukemia is associated with improved RFS and OS [58]. Furthermore, recovery of thymic function was shown to correlate with decreased risk for leukemia relapse in pediatric patients [33]. Specifically, subjects who relapsed had significantly lower levels of sjTREC or βTRECs before transplantation and during follow-up, at 3 and 6 months. Findings in adult dUCBT recipients are consistent with these observations in pediatric patients. As mentioned above, thymic regeneration and increased TREC levels displayed a strong correlation with attainment of CMV- specific immunity and absence of CMV viremia in adult dUCBT recipients [38]. Because CMV immunity was used as a paradigm for immune reconstitution, this study also investigated whether reconstitution of CMV- specific immunity and parameters of cellular T

cell immunity may be directly linked to distinct outcomes of OS and relapse-free survival (RFS). Univariable and multivariable analysis demonstrated that improved OS and RFS were significantly associated with the ability of patients to develop CMV- specific responses as determined by ELISpot assay. Given that reconstitution of functional CMV- specific immunity and absence of viremia were significantly associated with TREC recovery, these investigators hypothesized that the restoration of thymopoiesis might also imply a successful immune reconstitution and an improved capacity for generation of immune responses against other pathogens or tumor antigens. Consistent with this hypothesis, the main causes of death in this patient cohort were relapse, PTLD, and sepsis. Furthermore, assessment of OS showed that patients whose TREC levels were 2,000 copies/μg DNA (the lowest limit of values range in healthy individuals) or more by 1 year after transplantation had significantly improved OS compared with patients whose TREC values remained less than 2,000 copies/μg. Taken together, these observations suggest that thymic differentiation of T cells with pathogen-specific and leukemia-specific functions occurs in parallel, both in pediatric and in adult UCBT recipients. This event is marked by reconstitution of TREC levels, and has clinically important implications in the outcome of UCBT.

7 Approaches to Improve Thymic Regeneration and Function After Allogeneic Stem Cell Transplantation

The use of two UCB units, with or without ex vivo manipulation, is associated with improved recovery of the innate immunity, with earlier engraftment of neutrophils and reduced risk of graft failure [36, 59, 60]. Currently, it is unclear whether this approach may also have a direct effect on thymopoiesis. However studies suggest that dUCBT recipients may display earlier recovery of adaptive immunity, with de novo production of naïve T cells by the thymus [38–40]. This striking difference might be related to a cell dose effect, with higher numbers of lymphoid precursors in dUCBT recipients leading to more efficient thymic seeding. However, alternative yet unidentified mechanisms may also be involved, considering that the majority of patients demonstrate single unit chimerism by 3 months [37, 38], before the emergence of RTEs.

Besides enhancing cell dose, other experimental approaches have been employed to improve thymopoietic recovery after HSCT and are under investigation in preclinical models or human clinical trials. Administration of IL-7 in mice has been shown to enhance thymic reconstitution, but it also has a beneficial effect on the thymic-independent pathway of T cell regeneration and B cell reconstitution [61, 62]. Similar results have been observed in primates and, more recently, in human trials [63]. Patients with advanced malignancies [64] or HIV [65] treated with exogenous recombinant human IL-7 (rhIL-7) displayed a sustained expansion of T cells, higher levels of TRECs and circulating RTEs, and a marked increase in the TCR repertoire diversity. Preclinical data also suggest a synergistic effect of SCF with IL-7 [66].

In a murine model of HSCT, treatment with Fms-related tyrosine kinase 3 (FLT3) resulted in a significant increase in thymic cellularity, thymic output, and TREC levels [67]. Sex steroid ablation with luteinizing hormone-releasing hormone analogue (LHRH-A) before HSCT in mice is associated with increased numbers of lymphoid-myeloid progenitors (LMPs) in the bone marrow and improved T cell recovery [68]. In humans, LHRH-A administration prior to HSCT has been shown to lead to faster recovery of total and naïve $TREC^+$ $CD4^+$ T cells and diversification of T cell repertoire, suggesting an effect of sex hormone blockade on thymic recovery [69]. Finally, improved understanding and exploitation of the signaling events determining lymphoid commitment of HSCs (e.g., ex vivo expansion in Notch ligand-based systems) may prove clinically useful in T cell immune recovery after UCBT [70].

8 Summary and Future Directions

UCB is a viable HSC source for patients requiring allogeneic transplantation who lack suitable sibling or unrelated adult donors. Recovery of thymopoiesis is an imperative component of immune reconstitution after UCBT and plays an essential role in the restoration of the peripheral T cell compartment, by the de novo production of naïve lymphocytes with diverse TCR repertoire. TREC PCR assays have proven to be a valuable tool for the approximation of thymic RTE output and TREC levels are a major determinant of clinical outcomes for UCBT recipients. The use of dUCBT has lead to earlier thymic regeneration in adult patients. Further strategies to improve the thymic seeding, intrathymic proliferation, and differentiation of LMPs, or to protect the thymic microenvironment from the detrimental effects of conditioning and GvHD may further improve the outcomes of UCB transplantation.

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Chapter 12
Cord Blood Transplantation in the East Mediterranean Region

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1 Introduction

There are now multiple centers performing allogeneic hematopoietic stem cell transplantation (HCT) in the Eastern Mediterranean region (EM), and the results are comparable to those published in the international literature [1]. The HCT practice in this region is, however, governed by multiple logistical and cultural issues that are different from those in the western hemisphere. The Eastern Mediterranean Blood and Marrow Transplantation (EMBMT) group was thus established relatively recently to cover the HCT activity in the WHO-defined Eastern Mediterranean Region (EMRO; Fig. 12.1).

2 Potential Factors that Influence the Performance and Outcome of Cord Blood Transplantation (CBT) in the EM

Potential factors that influence the performance and outcome of CBT in the EM are listed in Table 12.1.

3 Availability of HCT Centers and Cord Blood (CB) Banks

One main difference about HCT in the EMRO is that the availability of transplant centers is still limited; the EMRO has a total of 21 countries with a total population of more than 540 million. These 21 countries include 1 country with low-income

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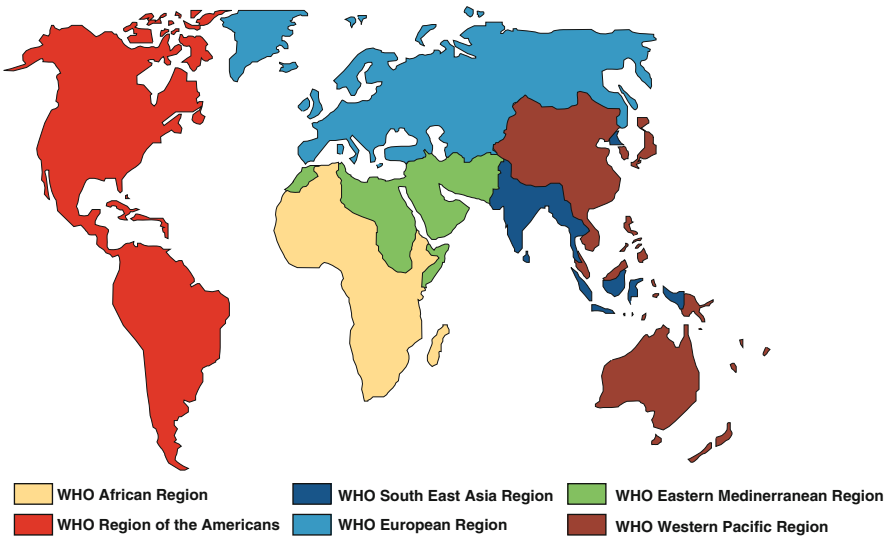

Fig. 12.1 World Health Organization (WHO) defined regional map of the EMBMT countries

Table 12.1 Potential factors that may influence the performance and outcome of CBT in the EMRO

Availability of HCT centers
Availability of cord blood banks
Increase rate of consanguinity
Large average family size
Young median age of the population
High prevalence of inherited diseases
Infectious complications
HLA haplotype frequency

CBT cord blood transplantation, *EMRO* WHO-defined Eastern Mediterranean Region, *HCT* hematopoietic stem cell transplantation

category, 5 with lower-middle-income category, 8 with upper middle-income category, and 7 with high-income category countries according to WHO income groups based on the Gross National Income per capita. Only 9 of the 21 countries (encompassing 70.8 % of the total EMRO population) have at least one active HCT programs available for their population [2]. Consequently, the potential for alternate donor programs is limited with only two countries, Saudi Arabia and Iran, currently offering CBT on a large scale and banking umbilical cord blood (UCB) units for transplantation [3].

4 Consanguinity and Family Size

In EM countries, the social culture promotes interrelated marriage, leading consequently to more consanguinity; this, in addition to the relatively larger family size than the western hemisphere, leads to a relatively higher probability of finding matched related donors within the family, and a higher possibility of a parent or sometimes a cousin being a fully human leukocyte antigen (HLA)-matched donor, all contributing to a reduction in the need for an UCB [4]. In one study from Saudi Arabia, the possibility of finding a fully matched related donor was 60 % [5]. In another regional study from Jordan, the overall probability of finding an HLA-matched related donor was 74 % in adults and 61 % in pediatric patients, presumably because adult patients have a completed family [6]. Many of the potential donors are nonsibling donors identified upon extended family search. On the other hand, the EM has one of the fastest population growth declines and average family size is declining every generation which ultimately will lead to more demand on alternate donor stem cell sources in the future [7].

5 High Prevalence of Inherited Diseases

Consanguinity can, however, be a double-edged sword, and although it may play a role in increasing the chances of finding a matched related donor, it also accounts for the relatively higher incidence of some hereditary diseases in this part of the world. Patients with hereditary diseases, such as primary immune deficiency disorders; osteopetrosis; hemoglobinopathies; and hereditary bone marrow failure syndromes such as Fanconi anemia, dyskeratosis congenital, and others, constitute a relatively large portion of matched related and unrelated CBT performed in this part of the world [2, 8–12].

6 Infectious Complications

Certain bacterial and viral diseases are endemic in the EMRO. This has an important influence of CBT outcome in certain cases; for example, primary immunodeficiency disorders are another highly prevalent disease entity in the region due to consanguineous marriages. The presentation of such patients to the transplant centers in the EM is somewhat different and their management represents a significant challenge; one health measure required by health departments in some of the EM countries is for all neonates to have routine administration of the Bacille Calmette-Guerin vaccine after birth, and hence some patients with a primary immune deficiency disorder who lack a family history for such a disease are referred for transplantation in a critical state with disseminated Tuberculosis after receiving this vaccine.

In addition to the above differences, other differences exist such as high cytomegalovirus (CMV) seropositivity which was reported in the EM to be as high 100 % among the recipients and 96 % among donors in Saudi Arabia and 100 % in donors and recipients in Pakistan [2, 13, 14]; this incidence may affect the incidence of CMV infection/disease post unrelated CBT. Additionally, many EMRO countries suffer from high prevalence of hepatitis B and C infections that may adversely the outcome of HCT and more so in unrelated CB [2].

7 HLA Haplotype Frequencies

Despite the higher probability of finding matched related donors in the EMRO, there are still a considerable number of stem cell candidates in need for an alternative donor. Thus, unrelated UCB transplantation in some of the EMRO countries is now an established viable option for many patients who require allogeneic HCT but lack a suitable family donor.

Because it is clear now that even in UCB transplants, the degree of HLA matching is closely associated with long-term survival, it is now a major goal of public CB banking to provide a large and diverse inventory of HLA typing to extend transplant access to racial and ethnic minorities [15, 16].

Middle-Easterners have a different genetic background from western Caucasians; ethnically most patients have Arabic and Persian background and their populations are relatively homogeneous with little influence from the immigration movements; there is therefore lower probability of finding suitable unrelated CB units for EM patients in western CB banks; data from Memorial Sloan-Kettering Cancer Center on the ancestry of 52 CB recipients between 2005 and 2008 showed only 2 % of the patients to be of Middle Eastern ancestry [15], and hence it is likely that the middle eastern ethnicity is underrepresented in the western CB banks. Consequently, many western banks now aim at recruiting non-Caucasian individuals for CB units. In one study, the authors reported that some DRB1 alleles occurred at higher frequency in CB banks than unrelated donor registries, likely because of increased recruitment of CB donation from ethnic minorities [17].

Recognizing this potential problem, some EMRO countries are now supporting the establishment of national CB banks to enhance the probability of finding suitable units for their nationals who need stem cell transplantation (SCT), but have no suitable matched family donor; Saudi Arabia and Iran both now have active national CB banks with about 5000 and 3000 units, respectively ready for use.

8 CBT Experience in EMRO

The two main programs that perform CBT on a large scale in the EMRO are The King Faisal Specialist Hospital and Research Center (KFSHRC) in Riyadh, Saudi Arabia and The Hematology-Oncology & Stem Cell Transplantation Research Center, Shariati Hospital in Teheran, Iran. Few sporadic cases have also been done in other countries over the last few years.

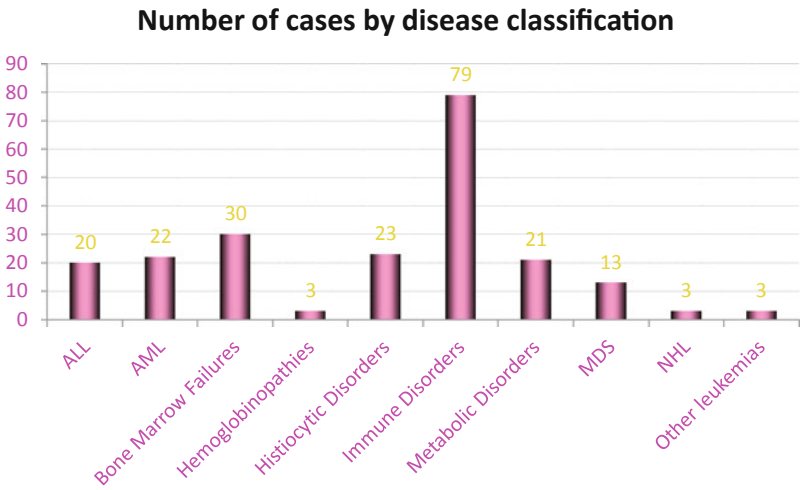

Fig. 12.2 Number of CBT cases performed at King Faisal Specialist Hospital and Research Center by disease. *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome, *NHL* non-Hodgkin lymphoma

The program at KFSHRC in Riyadh, Saudi Arabia was launched in January 2003; the national Cord Blood Program was established in 2008 and the first case from the national bank was done in September 2008.The data presented here are an update of the previous report published in 2009 [18]. Up until 2012, a total of 217 pediatric and 16 adult patients have undergone UCB. Due to the fact that the majority of the CBT were in pediatric patients, the following discussion will focus on the pediatric age group transplants. Out of the 217 patients, 9 had to undergo a second CBT (thus, the total number of transplants was 226); 96 units were obtained from the national bank: 6 were fully matched units (HLA-A, B, and DR-B1), 36 were one antigen-mismatched, and 51 were two antigen-mismatched units; 130 units were procured from foreign banks: 59 were one antigen-mismatched, and 71 were two antigen-mismatched units. The CBT was performed for malignant disorders in 69 cases and for nonmalignant disorders in 157 cases (Fig. 12.2). Conditioning regimens were tailored according to disease, with the addition of anti-thymocyte globulin (ATG) to all patients. Graft-versus-host disease (GvHD) prophylaxis was with cyclosporine and methylprednisolone.

Two hundred and two patients were evaluable for engraftment, and the median time to engraftment (absolute neutrophil count $\geq 500 \times 10^6$/L; ANC) was 30 days; 40 patients failed to engraft (19.8 %). The median time to engraftment in units obtained from the local national bank seemed significantly less than that with units from foreign banks, 24 days versus 32 days ($P = 0.001$). Acute GvHD (aGvHD) grade ≥ 2 developed in 48 patients (22.1 %): 29 grade 2, 10 grade 3, and 9 grade 4, and chronic GvHD (cGvHD) developed in 21 of 156 (13.5 %) patients at risk (14 extensive and 7 limited). No statistical differences were noted in the incidence of aGvHD or cGvHD

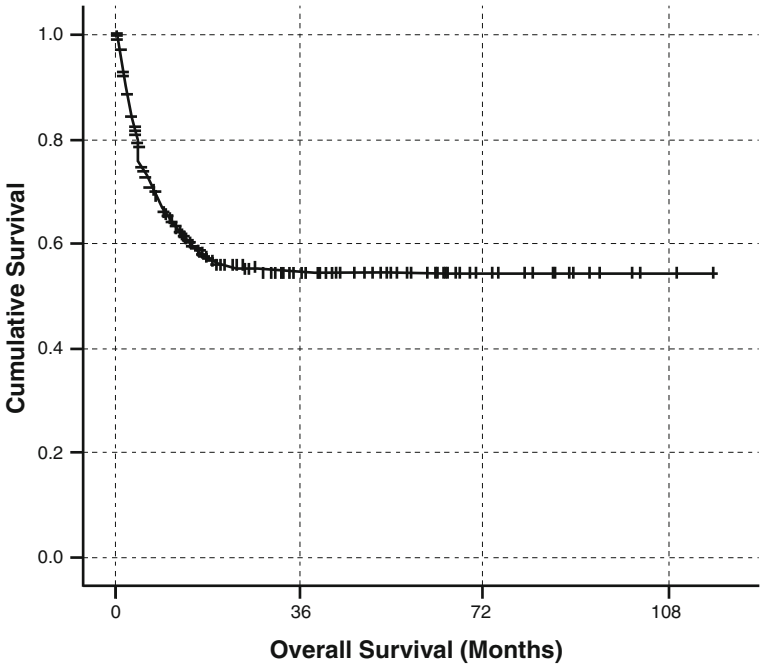

Fig. 12.3 OS of all UCB patients at King Faisal Specialist Hospital and Research Center

according to the source of the transplanted unit (national or local), or according to the degree of HLA mismatch.

The overall survival (OS) of all CBT patients was 55 % (Fig. 12.3); OS was not different between patients who received units from the local bank versus those who received units from foreign banks, (KFSHRC CBT bank: 62 % vs. other CBT banks: 51 %, $P = 0.29$), nor in nonmalignant vs. malignant disorders (49 % vs. 57 %; $P = 0.97$) (Fig. 12.4). Figure 12.5 outlines the survival by indication for transplant. These results are encouraging and similar to other reported results. The Saudi experience also underscores the points noted above about consanguinity, with more than two-thirds of the transplants in patients with nonmalignant disorders; in the above study, ten patients were transplanted for FA, with only three long-term survivors, in keeping with an OS of 40 % in 93 FA patients reported by the EMBMT group. Also, in the above study, 79 patients had primary immune deficiency, and because of the co-morbid conditions (status post BCG vaccine; see above), many of them underwent HCT after reduced intensity conditioning [18].

The Hematology-Oncology & Stem Cell Transplantation Research Center, Shariati Hospital in Tehran has a very active allogeneic HCT program. Presently close to 300 allogeneic HCT procedures are performed annually. The program is supported by an active national alternate donor registry and a national CB bank.

A significant proportion of the transplants are done for young patients with non-neoplastic hematologic disorders. Shariati Hospital has one of the world's largest series of HCT in thalassemia.

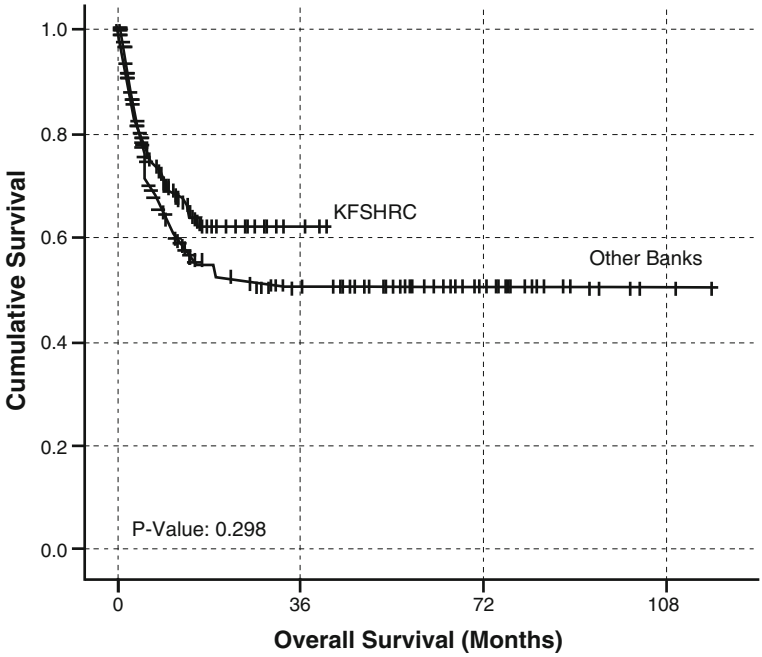

Fig. 12.4 OS of all UCB patients at King Faisal Specialist Hospital and Research Center according to source of cord blood unit

To date, 51 CBT have been performed. Similar to the center in Saudi Arabia, congenital and hereditary disorders constitute 50 % of the indications for CBT; 96 % of the transplants are performed with units obtained from the local CB bank. ATG is used routinely in the conditioning regimens used, with cyclosporine as GvHD prophylaxis. Most of the HCT (88 %) were done using a single unit with satisfactory outcome. Similar to other centers worldwide, infection, disease recurrence, and graft failure are the main contributors to the CBT procedure failure in 40 % of the cases at 100 days.

Most, if not all, EM countries have a national health service and organ transplantation is covered through national health insurance. National health insurance system certainly facilitates patient access to at least primary and secondary health care. It is also expected to facilitate delivery of tertiary care including organ transplantation. The major difficulty that is faced using national health insurance is inability to estimate the cost of medical procedure in an accurate way. This has a great relevance for CBT where patients stay in the hospital for extended period and occasionally several months, added to the purchasing cost of the CB units and supportive care. Implementation of cost-coding methods will be a necessity for proper planning and matching the market needs and potential availability of CBT and other organ transplantation procedures.

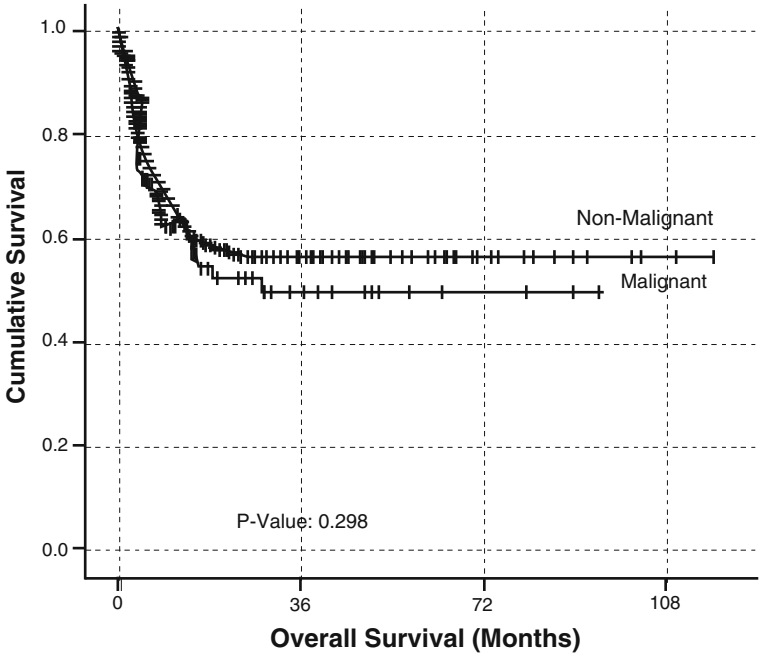

Fig. 12.5 OS of all UCB patients at King Faisal Specialist Hospital and Research Center according to indication for transplant

9 Conclusion

This year, the international community celebrates 25 years of CBT since the first case was performed for a Fanconi anemia patient in France [19]; in the EMRO, CBT is being increasingly recognized as a valid alternative stem cell source, and consequently, the field of CBT as well as CB banking has grown considerably in many EMRO countries. About 8000 CB units have been stored for transplantation in EMRO countries, with almost 350 CBT performed; the continuously growing inventories of the EMRO CB banks have resulted in fewer numbers of units are procured from the western hemisphere circumventing the issue of different ethnicity of the EMRO nations. The available CBT outcome data are encouraging and are in keeping with those reported in other international countries and registries.

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Chapter 13
Targeting Homing to Enhance Engraftment Following Umbilical Cord Blood Stem Cell Transplantation

Tyler Davis and Sherif S. Farag

1 Introduction

Umbilical cord blood (UCB) is a viable source of hematopoietic progenitor and stem cells (HP/SC) for patients who otherwise would not have an available donor for allogeneic hematopoietic stem cell (HSC) transplant (HSCT). For many patients with hematologic malignancies, HSCT represents the only therapy offering the option for cure. However, many patients do not have a human leukocyte antigen (HLA)-matched related or unrelated adult donor. The probability of a patient having a matched-related sibling donor is only 25–30 %. Furthermore, the chance of finding a suitable HLA-matched volunteer unrelated donor in the registry ranges from 10–60 % depending on racial background [1]. UCB, therefore, is an important alternative unrelated donor source for HSC for this large patient population without HLA-matched volunteer donors.

UCB is associated with certain advantages and disadvantages different from those encountered with HSC from bone marrow or mobilized peripheral blood. Advantages of UCB include rapid availability and less stringent HLA-matching requirements. UCB contains a unique naïve immune cell phenotype that results in a lower rate of graft-versus-host disease (GVHD) [2, 3], which allows for less stringent HLA-matching requirements with one or two HLA locus mismatches being acceptable. Recent studies have shown that outcomes of such mismatched UCB transplants are similar to HSCT from HLA-matched unrelated donors [2, 4, 5].

Despite the flexibility that UCB brings to transplant, the major disadvantage remains delayed engraftment. Multiple studies have shown a direct correlation between low nucleated cell (NC) dose and delayed engraftment leading to poorer outcomes and increased transplant-related mortality [6, 7]. Given the typically low NC doses seen in single UCB units, multiple strategies have been investigated to overcome

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delayed engraftment in adults receiving UCB transplants. Strategies have included the use of double UCB units [8, 9], intraosseous infusion of UCB cells [10], *ex vivo* expansion of UCB cells [11, 12], and as described in this chapter, strategies to enhance homing of HP/SC to the bone marrow niche.

In this chapter, we discuss the mechanisms of HSC homing and how targeting this process might lead to increased stem cell engraftment and potentially improved outcomes in patients receiving a UCB transplant. Four major approaches currently being investigated to improve homing are discussed, including *ex vivo* fucosylation, *ex vivo* treatment of UCB cells with prostaglandin E_2, *ex vivo* priming of UCB cells with complement fragment C3a, and the systemic use of CD26/DPP-IV inhibitors. Finally, we discuss potential future areas of investigation that may translate this exciting laboratory work into improved clinical outcomes.

2 Mechanisms of Stem Cell Homing

Homing is the initial process in which infused HSC actively interact with marrow sinusoidal endothelial cells to transmigrate and lodge in the bone marrow compartment. Infused HSC flow through marrow sinusoidal blood vessels until selectins and integrins begin the process of cell rolling and adhesion. Endothelial cells within the bone marrow sinusoids express E- and P-selectin, which are membrane bound C-type lectins that bind to cell surface glycosylated ligands expressed on HSC [13]. One such ligand, P-selectin glycoprotein ligand 1 (PSGL-1) on endothelial cells, interacts with P-selectin on HSC to induce HSC rolling and adhesion [14, 15]. HSCT studies in mice treated using monoclonal antibodies against P- and E-selectin, and in mice genetically lacking these two selectins, have demonstrated the importance of the interaction between P-selectin and PGSL-1 to HSC homing [14, 16, 17].

Independently of selectins, integrins increase adhesion of CD34+ cells to bone marrow endothelial cells [18]. The interaction of α4β1 integrin (very-late antigen, VLA-4) and with vascular cell adhesion molecule-1 (VCAM-1) has been suggested to play a dominant role in HSC adhesion. In murine studies, blocking antibodies to VLA-4 or VCAM-1 significantly reduced the homing of transplanted bone marrow cells to the marrow of lethally irradiated recipients [15, 19, 20]. Although not as efficient as the tethering of VLA-4 to VCAM-1 [21, 22], the tethering of another integrin, leukocyte function-associated antigen-1 (LFA-1) to its receptor, intracellular adhesion molecule-1 (ICAM-1), was also shown to be important in stem cell homing under physiologic conditions [23].

After rolling, tethering, and adhesion, the HP/SC must transmigrate through the marrow sinusoidal endothelium to the extravascular marrow space. Transmigration is accomplished by the interaction of the chemokine, stromal-cell-derived factor 1α (SDF-1α), and its receptor, CXC chemokine receptor 4 (CXCR4) [24]. The SDF-1α:CXCR4 axis has been shown to play a key role in the homing of HSCs. High-dose chemotherapy and/or radiation therapy used in conditioning regimens for HSCT caused an increase in SDF-1α expression by bone marrow stromal cells

in vivo [25]. The mechanism of this increase is likely due to the tissue damage induced by conditioning that then leads to a dramatic increase in the levels of secreted chemokines, cytokines, and proteolytic enzymes, which have profound impacts on stem cell migration and repopulation [25]. Additionally, the successful homing and engraftment of human HP/SC in immune-deficient mice is significantly reduced if the SDF-1α:CXCR4 axis is impeded [26].

SDF-1α has been shown to increase the cell surface expression of VLA-4 and LFA-1. Single administration of a high dose of SDF-1α was associated with increased human HSC repopulation in immune-deficient mice [27]. Pretreatment of human HP cells with SDF-1α in vitro also increased engraftment of these cells in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice [28]. Further supporting the key role of the SDF-1α:CXCR4 axis, overexpression of CXCR4 by human cord blood cells was associated with increased engraftment in NOD/SCID mice [29]. Also, cord blood $CXCR4^-$ cells treated prior to transplant with a neutralizing antibody to CXCR4 also engraft in NOD/SCID mice at low levels [30], suggesting the possible up-regulation of surface CXCR4 expression rapidly following transplantation in vivo.

Finally, after transmigration, HP/SC homing is complete when these cells migrate and lodge in the bone marrow endosteal niche via the calcium-sensing receptor, which is highly expressed on HP/SC [31], and responds to the high calcium ion concentration in the endosteal niche [31]. This process surprisingly occurs within hours after transplantation and takes no longer than 1 or 2 days [25]. Thus, the interactions of HP/SC with endothelial cells and the bone marrow stromal cells through integrins, selectins, and SDF-1, the proper homing of transplanted stem cells to the bone marrow niche occurs. Armed with this knowledge, investigators have endeavored to develop ways to enhance HP/SC homing to improve engraftment of UCB transplants.

3 Methods Targeting HP/SC Homing to Enhance Engraftment of UCB

3.1 Ex vivo Fucosylation

As noted above, selectins and their ligands are necessary to initiate HP/SC homing. PSGL-1 is the best-characterized selectin ligand [32–34]. In vivo, PSGL-1 mediates leukocyte tethering to P-selectin and rolling [13]. In order for PSGL-1 to bind to P-selectin, a small N-terminal region of PSGL-1 must be modified with tyrosine sulfates and a core two O-glycan capped with sialyl Lewis x (sLe^x) moieties [35]. This modification is accomplished through a process called α1–3 fucosylation to form terminal glycan components such as sLe^x. With the observation that human UCB CD34+ cells do not home as well to the bone marrow of irradiated NOD/SCID mice as do CD34+ cells from human adult bone marrow or mobilized peripheral blood [36], Xia and colleagues investigated *ex vivo* fucosylation of UCB cells as a

means to enhance the homing and engraftment of HP/SC from UCB via the improved binding of fucosylated PSGL-1 to P-selectin and E-selectin [35].

It was hypothesized that transient fucosylation of UCB cells with guanosine diphosphate (GDP) fucose and exogenous α1–3 fucosyltransferase (FT) would result in increased binding of these cells to both selectins, which would lead to enhanced engraftment in irradiated NOD/SCID mice [35]. Flow cytometric analysis demonstrated that approximately 25 % of UCB CD34+ cells did not express sLe^x, and that the majority of cells without sLe^x did not bind to P-selectin or E-selectin [35]. Through transient fucosylation of UCB CD34+ cells via *ex vivo* treatment with FTVI, an exogenous α1–3 FV, and GDP fucose, tethering to selectins was enhanced, and more importantly, engraftment of human HPs was significantly improved as compared with control saline or sham-treated UCB mononuclear cells [35].

The role of *ex vivo* fucosylation using FTVI in increasing homing and engraftment of UCB CD34+ cells was confirmed in another murine system by Robinson and colleagues [37]. Further preclinical studies of fucosylation have shown that the major endogenous α1–3 FV in CD34+ cells is FTVII rather than FTVI [38, 39]. Moreover, in vitro FTVII was found to be more effective than FTVI in modifying functional selectin ligands on UCB CD34+ cells [40].

Based on the preclinical data in murine xenograft models showing that human UCB progenitor cells treated *ex vivo* with a recombinant human FTVI (ASC-101; American Stem Cell, Inc., Floresville, TX, USA) resulted in more rapid and higher levels of human engraftment as compared to untreated UCB progenitors [37], a multicenter clinical trial of *ex vivo* fucosylation was initiated (ClinicalTrials.gov identifier: NTC01471067). Patients aged 1–80 years with a broad range of hematological malignancies are eligible, and may be enrolled in one of two arms defined by either myeloablative or reduced-intensity conditioning depending on age and disease type. Double UCB units (each with a minimum NC dose of 1.5×10^7/kg) are transplanted, with the unit containing the higher number of NC unmanipulated and infused first, while the smaller unit is thawed, washed, and treated for 30 min at room temperature with ASC-101 and substrate GDP-fucose. The primary objectives of the trial are the number of patients engrafting within 42 days and the mean time to engraftment. The preliminary results of this trial were recently reported in abstract form [41]. *Ex vivo* fucosylation appeared safe and feasible, and increased the proportion of fucosylated CD34+ cells from a median of 33–99 %. For the first seven patients who received reduced-intensity conditioning and are evaluable for engraftment, the median time to absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L was 14 (range 12–28) days, and the median time to platelet count $\geq 20 \times 10^9$/L was 33 (range 18–100) days. One patient had secondary graft failure. Surprisingly, only four of the six engrafting patients engrafted with the fucosylated unit, with the remaining two engrafting with the unfucosylated unit indicating that other factors beyond enhanced homing are associated with long-term engraftment. If the results of this trial end up being less robust, other methods of *ex vivo* fucosylation, such as with FTVII, may need to be investigated. Also, of greater relevance would be the testing the effect of *ex vivo* fucosylation on transplantation using only single-treated UCB units.

3.2 *Ex vivo* Treatment with Prostaglandin E_2

Prostaglandin E_2 (PGE_2) is a regulatory eicosanoid that plays an important role in many physiologic processes in the human body [42]. One such role is in hematopoiesis. PGE_2 dose-dependently inhibits myelopoiesis in vivo [43], but stimulates erythroid and multilineage progenitor cells [44, 45]. In zebra fish, agents that enhanced PGE_2 synthesis increased hematopoietic stem cell numbers, and those that blocked prostaglandin synthesis decreased stem cell numbers [46]. In the same study, *ex vivo* exposure to stabilized PGE_2 increased the frequency of long-term repopulating HP/SC present in murine bone marrow. Investigators at Indiana University showed that both mouse and human HSC express PGE_2 receptors, and that short-term *ex vivo* exposure of HP/SC to PGE_2 enhanced their homing and led to a fourfold increase in HSCs up to 20 weeks following transplantation [47].

With this knowledge, another study explored whether PGE_2-treated human UCB cells transplanted into NOD/SCID mice would display enhanced homing [48]. These studies demonstrated enhanced homing of UCB cells to the marrow, which was at least partly explained by increased expression of CXCR4 on HP/SC [25]. This increase in CXCR4 has been reported with human CD34+ cells [49] and endothelial cell [50] following exposure to PGE_2. Additionally, chemotaxis of UCB CD34+ cells to SDF-1 was also significantly enhanced by pulse exposure to the stable PGE_2 derivative 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$) and migration was blocked by the selective CXCR4 antagonist AMD3100 [51], indicating a specific effect mediated through the CXCR4 receptor. Further studies with $dmPGE_2$ demonstrated safety in long-term primate transplantation studies [52].

A phase I trial was initiated to evaluate the safety and efficacy of an *ex vivo* treatment with $dmPGE_2$ (FT1050, Fate Therapeutics, San Diego, CA, USA) to improve engraftment following reduced-intensity double UCB transplantation in humans [53]. Double UCB units were used for transplantation, with only a single unit treated with $dmPGE_2$. This study showed that *ex vivo* incubation with $dmPGE_2$ for 30 min did not result in significant cell loss, with a mean viable CD34+ cell recovery of 90 %. Adverse events were manageable, and no patient experienced primary graft failure. The median time to neutrophil engraftment was 17 (range 14–31) days, which was significantly shorter than a historical controls transplanted with unmanipulated UCB units in similar patients at the same institution (median time to neutrophil recovery of 21 days; $P = 0.04$). The median time to platelet engraftment was 43 (range 26–60) days, and 11 of 12 patients had engrafted platelets by day 60. Most importantly, chimerism assessment demonstrated that 10 of 12 patients had early and sustained engraftment of the $dmPGE_2$-treated UCB unit as opposed to the unmanipulated unit, and that the treated unit contributed 100 % to hematopoiesis. Based on these positive findings, the investigators are expanding the investigation of $dmPGE_2$ in a randomized phase II trial, as well as testing *ex vivo* treatment with $dmPGE_2$ of cord blood cells in single-units UCB transplantation (ClinicalTrials.gov identifier: NCT01527838).

3.3 Ex vivo Priming with Complement Fragment 3a

Recent studies have highlighted an important role of the complement fragment C3a in hematopoiesis. Normal human CD34+ cells, as well as lineage-expanded hematopoietic precursors, were shown to express the complement C3a anaphylatoxin receptor C3aR [54]. Furthermore, bone marrow stromal cells secrete C3, and activation of C3aR by C3a sensitizes the response of HP/SC to SDF-1 [54–56]. Specifically, C3aR-mediated signaling increased the chemotactic response of HP/SC to SDF-1 and enhanced migration across subendothelial basement membranes [54]. In mouse transplantation experiment, C3a primed murine Sca-1+ cells engrafted faster than untreated cells in lethally irradiated mice [54]. Also, it was shown that C3a priming of human CD34+ cells resulted in the incorporation of the CXCR4 receptor into membrane lipid rafts [57], the form of CXCR4 that allows hematopoietic cells to be most responsive to SDF-1 [58].

Based on preclinical studies that indicated that C3a priming of CD34+ cells can potentially enhance homing and engraftment of UCB grafts, a pilot clinical trial was initiated to assess the safety and potential efficacy of this approach in adult patients with hematological malignancies undergoing double UCB unit transplantation following nonmyeloablative conditioning [59]. The two UCB units were required to have a minimum combined NC dose $\geq 3 \times 10^7$/kg, with each unit having a minimum of NC dose of 1.5×10^7/kg. One unit was unmanipulated and the second unit was primed after thawing with C3a using a fixed concentration of 1 μg/ml at room temperature for 15 min, as had been previously used in murine studies [54]. As a nonmyeloablative regimen was used, early neutrophil recovery could not be used as an endpoint of efficacy because of autologous reconstitution. Therefore, skewing of long-term chimerism predominance toward the C3a primed unit was used a surrogate for potential enhancement of engraftment. While this trial demonstrated the safety and tolerability of C3a priming, with minimal infusional toxicity except for grades 1–3 hypertension in 9 of 29 patients treated, increased skewing of chimerism toward the treated unit was not demonstrated [59]. The 27 of the 29 patients who achieved neutrophil recovery by day 42 after transplantation, only 9 patients had hematopoiesis from the C3a-primed unit and 18 from the unmanipulated unit, indicating that this approach was not effective in promoting homing of stem cells and engraftment [59]. The investigators, however, have suggested that optimization of the conditions of priming, such as higher C3a concentration or incubation at 37 °C, may be required to improve efficacy. Further studies are required to demonstrate the potential usefulness of this approach.

3.4 Systemic Inhibition of CD26/DPP-IV

The SDF-1α:CXCR4 axis plays a key role in the homing of stem cells, and is modulated by the enzyme CD26/DPP-IV [60]. CD26 is a membrane-associated protein with dipeptidyl peptidase activity that cleaves and inactivates SDF-1α [61]. CD26 cleaves the N-terminus dipeptide of SDF-1α, resulting in a truncated form of SDF-1

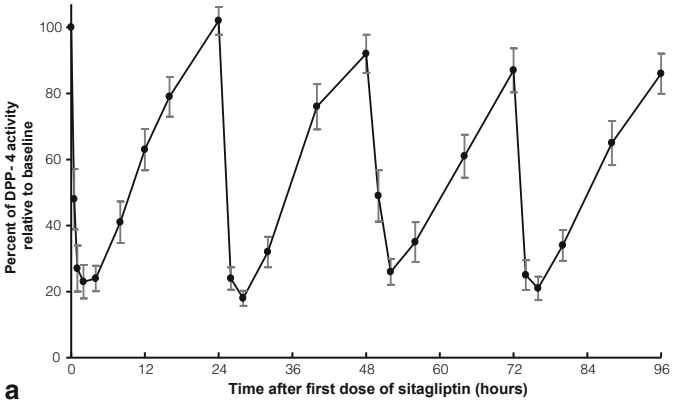
that is unable to activate CXCR4 but can still bind to CXCR4 [62]. CD26 is expressed on the surface of normal CD34+ cells [63], and a subfraction of UCB CD34+ cells that are mainly CXCR4 expressing cells [64]. Also, CD26 circulates in plasma in a catalytically active soluble form [65]. CD26/DPP-IV inhibition was shown to enhance the migration of CD34+ cells along a SDF-1 gradient [66]. Also, inhibition of CD26/DPP-IV or homozygous deletion of CD26 in mouse HP/SC was associated with increased in vivo stem cell repopulating capacity [66].

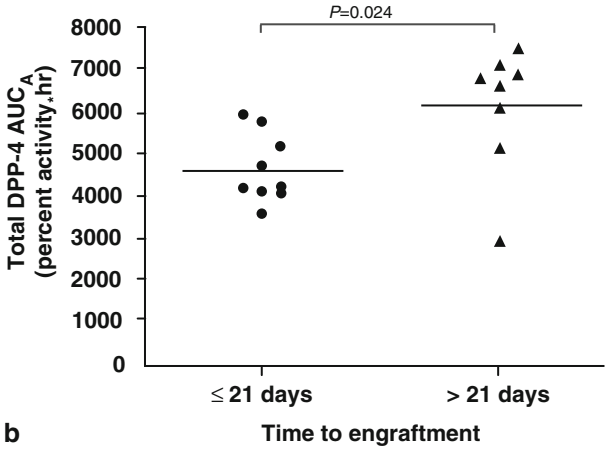
Phenotyping of human UCB hematopoietic progenitor cell (HPC) has shown that only a subpopulation of human UCB CD34+ cells ($8.6\,\% \pm 2.1\,\%$) express catalytically active CD26/DPP-IV [64]. An inhibitor of DPP-IV, diprotin A, further illustrated the importance of CD26/DPP-IV by showing that treatment of CD34+ CD26+ UCB cells with diprotin A enhanced the migratory response to SDF-1α of these cells, which was roughly equivalent to the migratory response to observed in CD26− CD34+ cells [64]. This study went on to show that diprotin A treatment blocked the majority of DPP-IV activity expressed in the total CD34 + population of UCB cells, but treatment did not affect the migration of the CD34+ CD26− cells, suggesting that the action of the inhibitor is specific.

The potential of CD26/DPP-IV inhibition was further provided in a murine model that showed *ex vivo* pretreatment of donor mouse HP/SC with diprotin A for 15–30 min before transplantation dramatically increased short-term homing and long-term engraftment [66]. This effect was also seen with the pretreatment of human CD34+ UCB cells with inhibitor leading to increased in vivo engraftment in sublethally irradiated NOD/SCID mice [66]. Moreover, the enhancing effect of inhibition of CD26/DPP-IV on engraftment was apparent when the number of CD34+ cells transplanted was relatively low, which is of particular interest in UCB transplantation where the number of CD34+ cells is limiting [66].

Systemic inhibition of CD26/DPP-IV also led to enhanced engraftment of human and murine bone marrow HP/SC in preclinical models [67, 68]. Although it is not known whether systemic inhibition of DPP-IV is equivalent to *ex vivo* treatment of cells prior to transplantation, systemic CD26/DPP-IV inhibition offers important logistic advantages over *ex vivo* inhibition. As *ex vivo* manipulation of a graft is more expensive, requires a good manufacturing practice laboratory, and negotiating regulatory hurdles, pharmacological in vivo inhibition using clinically approved inhibitors of DPP-IV lends itself to easier and more immediate clinical translation.

We have investigated in vivo pharmacological inhibition of DPP-IV as a means of enhancing engraftment of single-unit UCB transplantation in adult patients with hematological malignancies. Sitagliptin is an oral DPP-IV inhibitor approved by the Food and Drug Administration (FDA) for the treatment of type II diabetes mellitus. It is a highly selective inhibitor of DPP-IV and has been shown to be safe in human trials even in high doses [69, 70]. We recently reported the results of a pilot trial evaluating the safety and feasibility of high-dose sitagliptin in patients with advanced hematological malignancies undergoing single-unit UCB transplants [71]. Twenty-four patients of median age 39 (range, 21–58) years received myeloablative conditioning, followed by sitagliptin 600 mg/day orally on days − 1 to + 2, and single UCB grafts day 0. The dose of sitagliptin was based on published pharmacodynamic





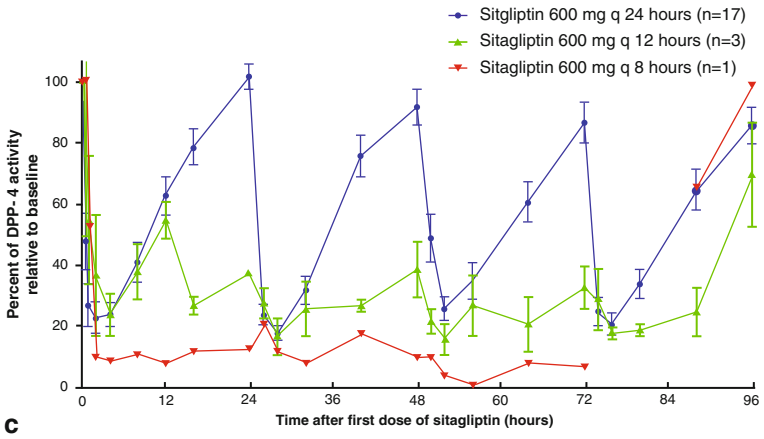


Fig. 13.1 Plasma DPP-IV inhibition by sitagliptin in UCB transplant patients. **a** Plasma DPP-4 activity following sitagliptin dosing. DPP-4 activity is shown as a percentage of baseline activity. Data represent mean values ± standard error for 17 patients who received single unit red

studies in healthy volunteers demonstrating > 90 % plasma DPP-IV inhibition that was sustained for more than 24 h following single 600 mg dosing [69]. UCB units were required to have a minimum NC dose of 2.5 × 10^7/kg, and be at least four of six HLA locus matched. Seventeen patients who received red cell-depleted UCB grafts, matched at four ($n = 10$) or five ($n = 7$) of six HLA loci and with median NC dose 3.6 (2.5–5.2) × 10^7/kg, engrafted at a median of 21 (range, 13–50) days with cumulative incidence of 94 % (95 % CI, 84–100 %) at 50 days. The results compare favorably with major published series where predominantly adult patients received either single or double UCB grafts [4, 72–76], and where the reported median times to engraftment are 22 days or more. While sitagliptin was well tolerated, inhibition of plasma DPP-IV activity was not sustained at the dose used. Plasma DPP-IV activity was reduced to mean of 23 % at 2 h after dosing, but inhibition was rapidly lost by 12–18 h (Fig. 13.1a). Importantly, the area under the plasma DPP-IV activity-time curve significantly correlated with engraftment (Fig. 13.1b), suggesting that plasma DPP-IV activity may be a good surrogate measure of DPP-IV activity in the bone marrow niche and that more sustained inhibition of DPP-IV may have further improved engraftment. Pharmacokinetic-pharmacodynamic modeling indicated that improved inhibition of DPP-IV could be better achieved using multiple daily dosing [77]. Based on this data, a dose-escalation study was subsequently performed testing sitagliptin doses of 600 mg every 12 h, and every 8 h. While sitagliptin dosing of 600 mg every 12 h was well tolerated, grade 5 dose-limiting toxicity (capillary leak syndrome and multiorgan failure) was observed at 600 mg every 8 h. More sustained inhibition of plasma DPP-IV activity was observed with twice-daily dosing of sitagliptin (Fig. 13.1c). Based on these studies, we have initiated a multicenter phase II clinical trial of in vivo DPP-IV inhibition using sitagliptin at 600 mg every 12 h in adult patients with hematological malignancies, with the engraftment as the primary endpoint (ClinicalTrials.gov identifier: NCT01720264).

4 Conclusions and Future Directions

While UCB is an important alternative source of hematopoietic stem cells for transplantation for patients who do not have HLA-matched volunteer donors, delayed engraftment remains a significant limitation of UCB transplantation. While a number of strategies to enhance engraftment and early blood count recovery have been developed, it is important for those in the field to recognize that such strategies need to be relatively simple and affordable to allow exportability of the technology to the greatest number of patients who may benefit from UCB transplants. Economic

cell-depleted UCB grafts. **b** Relationship between area under the plasma DPP-4 AUC_A and engraftment. Patients engrafting within 21 days had significantly lower AUC_A compared with those engrafting beyond 21 days ($P = 0.024$; two-sided Mann-Whitney test). **c** Plasma DPP-IV activity, shown as percentage of baseline, with multiple dosing of sitagliptin compared to single dosing in a dose escalation study. *DPP-4* dipeptidyl peptidase IV, AUC_A activity versus time curve. (**a** and **b** adapted from [71])

concerns remain high for methodologies involving extensive in vitro manipulation, including cord blood expansion, and use of third-party haploidentical cells or of multiple cord blood units, where there is a real danger for the field to price itself out of competition as a source of HSCs for HSCT [78]. Methods targeting the processes involved in HP/SC homing to enhance engraftment, including *ex vivo* fucosylation, priming with $dmPGE_2$, and in vivo pharmacologic inhibition of CD26/DPP-IV are much simpler, and offer the advantages of greater exportability with significantly less expense, particularly if they can be applied successfully to single UCB unit transplants. Further investigations of these technologies are ongoing, and defining their relative efficacies will be important to advance the field. It is important to appreciate, however, that the methods targeting stem cell homing discussed in this chapter are not necessarily competing strategies. It is likely that combinations of procedures targeting different aspect of homing may ultimately result in greater improvement in engraftment capacity than any one procedure itself [79]. For example, *ex vivo* priming with $dmPGE_2$ combined with in vivo DPP-IV inhibition using sitagliptin is a simple approach that should be investigated. It is highly probable that in the next few years, the promise of therapeutic targeting of stem cell homing to the bone marrow niche will be realized, and the optimum method (or combination of methods) to achieve more rapid engraftment of limiting numbers of stem cells will be defined. In this way, it is hoped that overcoming the barrier to engraftment will allow for the full merit of UCB transplantation to be revealed.

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Chapter 14
Cord Blood *Ex Vivo* Expansion

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1 Introduction

Over the last three decades, umbilical cord blood (UCB) has become a frequently used source of hematopoietic stem cells (HSCs) for transplantation. The rapid growth in the rates of UCB transplantation (UCBT) is a reflection of the advantages this modality has over bone marrow (BM)- or peripheral blood (PB)- sourced grafts including rapid availability of stored UCB units, decreased rates of acute and chronic graft-versus-host disease (GVHD), and less stringent histocompatibility requirements. These characteristics have established UCBT as an alternative for patients who lack a suitable matched related donor (MRD) [1].

The main disadvantage of UCB is the presence of delayed engraftment kinetics and a higher rate of engraftment failure than BM transplants (BMT) or PB transplants (PBT). Initial reports of pediatric matched sibling UCBT showed primary graft failure occurred in 15 % of patients [2]. In a matched-pair analysis of pediatric unrelated UCBT and human leukocyte antigen (HLA)-matched unrelated BMT, Barker and colleagues [3] observed neutrophil engraftment was significantly delayed after UCBT although the rates of primary graft failure were not statistically different. Subsequent pediatric studies confirmed that UCBT was associated with delayed engraftment and increased early transplant-related mortality (TRM; [4, 5]). The studies that followed clarified the relevance of cell dose and HLA matching on the outcomes of pediatric UCBT. In an observational study including 102 patients undergoing UCBT, grafts with an HLA match of at least 4/6 and a minimum dose of 1.7×10^5 CD34+ cells/kg of recipient body weight had significantly improved rates of survival [6]. Several reports from the Eurocord registry indicated pediatric patients receiving a total nucleated cell (TNC) dose above 3.7×10^7 cells/kg had a higher probability of engraftment and survival [7, 8].

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Early experiences in adult UCBT confirmed the problem of delayed engraftment. In the initial report of UCBT in 68 high-risk hematologic malignancy patients by Laughlin and colleagues [9], primary graft failure among patients surviving more than 28 days was 10 %, but mortality 100 days after transplant was 51 %. A higher TNC dose ($\geq 2.4 \times 10^7$ cells/kg) was associated with improved event-free survival (EFS). Recognition of cell dose as a critical barrier to engraftment and improvements in supportive care have resulted in major improvements in adult UCBT outcomes. A study performed by the Eurocord and the Center for International Blood & Marrow Transplant Research (CIBMTR) [10] compared outcomes of recipients of unrelated BMT or PBT (7–8/8 HLA matched donors) and UCBT (4–6/6 HLA matched units with a minimum TNC dose of 2.5×10^7 cells/kg). UCBT had slower neutrophil recovery (29 vs. 14, PB, and 19, BM, days, $p = 0.01$), and lower neutrophil engraftment rate by day 42 (80 vs. 96, PB, and 93 %, BM, $p < 0.0001$). TRM was higher with UCBT, while GVHD and relapse rates were lower, leading to comparable leukemia-free survival rates. This report and other studies would suggest that shortening time to hematopoietic engraftment could lead to reduced early TRM and improved overall survival after UCBT.

Adding a second UCB unit (double UCBT, dUCBT), has been investigated as a means to increase cell dose and improve engraftment. Initial studies from the University of Minnesota indicated possibly expedited engraftment (median 23 days, range 15–41) [11]. However, dUCBT appears to lead to a higher incidence of GVHD [12]. In a recent retrospective study, Brunstein and colleagues compared outcomes of dUCBT with those of MRD, matched unrelated donor (MUD), and mismatched unrelated donors [13]. While leukemia-free survival was similar in all four groups, dUCBT was still associated with higher rates of nonrelapse mortality (NRM), slower hematopoietic recovery, and higher incidence of engraftment failure.

UCB is remarkable in that the hematopoietic progenitor cells (HPCs) have a higher proliferative potential than those in the BM [14]. As a result, UCBT leads to engraftment despite CD34 + cell doses that are only 10 % of BM grafts and 5 % of PB grafts [15]. Therefore, the central hypothesis of *ex vivo* expansion strategies is to exploit the high proliferative potential of UCB to increase the cell dose before transplantation. As graft engineering methods continue to advance [15, 16], UCB expansion has become a reality that has reached clinical applications, many of which are currently being tested in clinical trials.

2 Initial *Ex Vivo* Expansion Studies

2.1 Hematopoietic Growth Factors

Shpall and colleagues reported in 2002 the first clinical study of *ex vivo* cord blood expansion [17]. In this trial, a CD34-selected portion of UCB units that were originally frozen in two aliquots was exposed for 10 days to liquid culture conditions with stem cell factor (SCF), granulocyte-stimulating factor (G-CSF) and megakaryocyte

growth and differentiation factor (MGDF). These conditions had been previously reported to lead to 100-fold expansion of myeloid and erythroid colony-forming cells and a 500-fold expansion of megakaryocyte progenitors [18]. A prior study exposing autologous stem cell grafts to these culture conditions had resulted in accelerated neutrophil engraftment [19]. CD34 selection was used due to poor expansion in these conditions when unselected UCB cells were used. The expanded portion was either co-infused with unmanipulated UCB, or given on day 10 after transplantation. Thirty-seven patients (25 adults and 12 children) with hematologic malignancies and breast cancer were treated. The median weight was 61 kg (range 9–116 kg). The median CD34 + cell dose was 10.4×10^4 cells/kg (range $0.97–311 \times 10^4$ CD34 + cells/kg). There was no acute toxicity associated with the infusion of expanded or unmanipulated UCB. Four patients died of disseminated fungal infections before day 28, whereas three additional patients died with extensive BM relapse and pancytopenia on days 41, 51, and 78. None of the remaining patients ($n = 30$) had engraftment failure. The median time to neutrophil engraftment was 28 days. Subjects receiving a cell dose higher than 5×10^4 CD34 + cells/kg presented a more rapid time to neutrophil engraftment, but the difference was not statistically different.

A major concern with expansion approaches is that of stem cell exhaustion. It is believed that most current techniques lead to stem cell differentiation and lineage commitment, thereby decreasing "stemness." Shpall and collaborators then hypothesized that using a dUCBT platform in which one unit was 100 % *ex vivo* expanded, combined to an unmanipulated UCB unit, would allow for early engraftment of the expanded unit, while a safety net would be provided by the unmanipulated unit, which was expected to provide long-term engraftment. The authors then performed a randomized study at the MD Anderson Cancer Center comparing infusion of two unmanipulated UCB units (standard dUCBT) versus the combination of one unmanipulated unit and an *ex vivo* expanded unit [20]. The liquid culture system was similar to that employed by Shpall and collaborators in their preliminary work [17]. In this dUCBT platform, CD133 selection was used instead of CD34, due to the possibility of selection of earlier HPCs. In addition, the unselected cell fraction obtained after CD133 selection was frozen after separation and reinfused at the time of transplant. The authors hypothesized that this cell population could contain engraftment-facilitating cells. The liquid culture was performed in two stages, with culture flasks transferred to larger culture bags after 7 days. Forty-eight hematologic malignancy patients were enrolled. The median TNC dose was 0.36×10^8 cells/kg in both cohorts, whereas CD34 + cell dose was 0.16×10^6 and 0.13×10^6 cells/kg in the expanded and unmanipulated cohorts, respectively. There were no statistically significant differences in hematopoietic engraftment or survival between both cohorts. One UCB unit dominated engraftment in all patients; among those receiving expanded units, the unmanipulated unit predominated in all but three subjects. These data suggested that cytokine-based liquid culture systems push progenitor cells towards a more differentiated stage of maturation, depleting the stem cell potential.

2.2 Coculture with Mesenchymal Stem Cells

The proliferation and differentiation of HPCs is regulated by microenvironmental signals and interaction with cells from the BM stroma [21–23]. Mesenchymal stromal cells (MSCs) are precursors of mesodermal tissues and give rise to the BM stromal compartment [23]. Preclinical studies demonstrated coculture of UCB with BM-derived MSCs [24] required less graft manipulation (i.e., no CD34 + or CD133 + selection), while at the same time, producing higher TNC and HPC numbers than *ex vivo* liquid culture methods previously investigated. The first clinical trial of infusion of UCB expanded by MSC coculture was recently reported [25]; 31 high-risk hematologic malignancies received two UCB units, one of which had undergone *ex vivo* expansion through MSC coculture. Expansion increased TNC numbers by a median factor of 12.2 and CD34 + cells by a median factor of 30.1, leading to grafts with median TNC dose of 5.8×10^7 cells/kg. The median time to neutrophil and platelet engraftment was 15 and 42 days, respectively. Chimerism studies indicated that the expanded unit provided early engraftment, while the unmanipulated unit was responsible for long-term engraftment in the majority of patients. Only a minority of patients had evidence of long-term expanded-unit hemopoiesis, with unmanipulated unit predominance. These encouraging initial clinical results provided the rationale for an ongoing international randomized multicenter study comparing unmanipulated dUCBT versus dUCBT containing one *ex vivo* expanded MSC cocultured unit and an unmanipulated unit.

2.3 Notch-Mediated Expansion

The Notch gene family encode for transmembrane proteins that participate in cell-cell interactions and have a pivotal role in cell-fate decisions of progenitor cells in several organisms and organ systems. Milner and colleagues demonstrated HPCs express Notch1 (TAN1) [26] and subsequent studies showed that constitutive activation of this gene was capable of establishing pluripotent cytokine-dependent hematopoietic progenitor cell lines [27]. Incubation of murine HPCs with engineered Notch ligand and hematopoietic growth factors resulted in inhibition of differentiation and a several log increase in number of HPCs [28]. Delaney and colleagues demonstrated that incubation of human UCB HPCs with Notch ligand resulted in *ex vivo* expansion by approximately 100-fold, with resultant-improved hematopoietic engraftment when transplanted to immunodeficient mice [29]. In a phase I clinical trial conducted at the Fred Hutchinson Cancer Center, 10 hematologic malignancy patients had myeloablative conditioning (fludarabine, cyclophosphamide, and total body irradiation) followed by infusion of one unmanipulated UCB unit and one *ex vivo*-expanded UCB unit. Notch ligand-mediated expansion resulted in a 164-fold expansion of CD34 + cells and 562-fold expansion of TNC. The median time to neutrophil engraftment was 16 days (range 7–34). One subject failed to engraft. GVHD rates were acceptable, and there were no infusional toxicities. One-year actuarial survival was 70 %.

3 Expansion with Preservation of Long-Term Engraftment Potential

An important limitation to expansion of hematopoietic cell grafts with the methods discussed above is the generation of committed HPCs at the expense of less-differentiated progenitor cells, responsible for long-term hematopoietic recovery. It should be noted that the use of dUCBT platforms preclude a direct evaluation of engraftment potential of expanded cells, but the fact that in most cases the expanded UCB lost the "competition" to the unmanipulated unit suggests poorer long-term engraftment potential or reflect loss of engraftment facilitating cells. Additional developments in graft manipulation have identified methods that could potentially allow HPC expansion without loss of long-term engraftment potential.

3.1 Copper Chelation

Intracellular copper concentration regulates differentiation of HPCs. The copper chelator tetraethylenepentamine (TEPA) reduces intracellular copper concentration and prevents HPC differentiation *in vitro* [30]. Culture of UCB cells with TEPA in conjunction with cytokines increases the numbers of early progenitors without differentiation to less-pluripotent cells. Grafts cultured with TEPA led to improved engraftment in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice [31]. The feasibility of *ex vivo* expansion using tetraethylenepentamine (TEPA) in a liquid *ex vivo* expansion system was investigated in a phase I/II study that included ten advanced hematologic malignancy patients [32]. UCB units that were frozen in two fractions were used, and the smaller fraction was cultured for 21 days in a cytokine-based liquid culture system. The median CD34 + cell increase was sixfold, and the mean times to neutrophil and platelet engraftment were 30 and 48 days, respectively. One patient failed to engraft. Grade II–IV acute GVHD occurred in 44 % of cases, while 100-day survival was 90 %. These results led to the design of a multicenter trial investigating the use of a single UCB unit partially expanded with the copper chelation-based system (Stem Ex ®). Expanded unit transplant outcomes were compared with historical controls receiving double UCBT [33]. One hundred and one patients received expanded UCBT. Median TNC and CD34 + expansion were 400-fold and 77-fold, respectively. The median TNC and CD34 + doses were 2.2×10^7/kg and 9.7×10^5/kg, respectively. One-hundred-day survival after transplantation was significantly higher in patients receiving a single expanded unit (84.2 % vs. 74.6 %, $p = 0.035$), while neutrophil and platelet engraftment were faster in patients who received expanded units: 21 days vs. 28 days ($p < 0.0001$) and 54 days vs. 105 days ($p = 0.008$), respectively.

3.2 Nicotinamide

Nicotinamide is the precursor for nicotinamide adenine dinucleotide (NAD^+), and is a potent inhibitor of enzymes that require NAD^+, including sirtuins, protein/histone deacetylases. SIRT1 is a sirtuin whose deficiency was associated with increase *in vitro* proliferative activity of HPCs [34]. Exposure to nicotinamide has been shown to delay differentiation, while increasing the expansion, migration, and engraftment of *ex vivo* expanded UCB CD34 + cells [35]. The authors reported TNC increase of 400-fold, while CD34 + expanded 80-fold. A phase I/II study of infusion of one UCB unit expanded on a nicotinamide-based system combined with one unmanipulated UCB unit was recently completed [36]. Eleven hematologic malignancy patients received the planned UCB infusion after myeloablative conditioning. Eight of the patients engrafted with the nicotinamide-expanded unit, two with the unmanipulated unit, whereas one patient experienced primary engraftment failure. Median neutrophil engraftment time was 12.5 days (range 7–26), and 10.5 days (range 7–18) for the whole cohort and for patients who had engraftment of the expanded unit, respectively. One-hundred-day mortality was 10 %. Three patients had grade I/II acute GVHD, while none had more severe GVHD. These results suggested that nicotinamide-based *ex vivo* expansion could achieve shortened hematopoietic recovery time with preserved long-term engraftment. A recently opened phase I trial (ClinicalTrials.gov identifier NCT01816230) is investigating the infusion of a single unit, 100 % expanded with this nicotinamide-based system.

3.3 Aryl Hidrocarbon Receptor Antagonist

The purine derivative StemRegenin 1 (SR1) can increase the number of CD34 +, CD133 + and CD90 + stem and progenitor cells when added to cytokines in culture media. SR1 does not stimulate proliferation, and importantly, causes a reversible arrest in differentiation [37]. Expansion with SR1 resulted in higher engraftment rates of human hematopoietic cells in a NOD/SCID mouse model. Boitano and colleagues demonstrated that SR1 binds and inhibits aryl hydrocarbon receptor (AHR), a nuclear receptor implicated in the induction of drug-metabolizing enzymes as well as in the regulation of several pathways involved in hematopoiesis, including c-MYC, CCAAT-enhancer-binding proteins (C-EBP), C-X-C chemokine receptor type 4 (CXCR4), among others [37]. Khan and colleagues have recently reported that hypoxic culture conditions can increase the expansion rates in the presence of SR1 and cytokines [38]. Investigators at the University of Minnesota are conducting a phase I study consisting of infusion of one unmanipulated UCB unit and one CD34- selected, SR1-expanded UCB unit. The median CD34 + expansion was 248-fold (range 66–446). Five of nine patients that engrafted with the expanded unit had faster neutrophil engraftment (16 days vs. 24 days).

3.4 *Dimethyl Prostaglandin E_2*

Screening studies showed that agents that increased prostaglandin E_2 (PGE_2) levels in zebra fish resulted in higher numbers of HSCs [39]. Dimethyl prostaglandin E_2 ($dmPGE_2$), a stable derivative of PGE_2, acts through G-protein-coupled receptors and the second messenger cyclic adenosine monophosphate (AMP) to increase expression of genes involved in homing, proliferation, survival, and self-renewal of HSC. It has been reported that this agent induces expansion and long-term maintenance of the HSC population [40]. Based on these data, the Dana Farber Cancer Institute and Massachusetts General Hospital conducted a phase I clinical trial evaluating the safety of a $dmPGE_2$-treated UCB unit co-transplanted with an unmanipulated unit. In contrast with the trials summarized above, $dmPGE_2$ treatment did not consist of *ex vivo* expansion, but of exposure to this agent for a short period of time after UCB units were thawed. Two cohorts of hematologic malignancy patients were treated. In the first cohort (nine patients), UCB units were incubated with 10 μM of $dmPGE_2$ for 60 min at 4 °C; only two patients presented engraftment of the $dmPGE_2$-treated units and neutrophil engraftment times were not improved (median = 24 days). Based on the lack of improvement in engraftment parameters, the investigators sought to optimize the conditions for *ex vivo* treatment with $dmPGE_2$, by increasing the incubation time to 120 min and the temperature to 37 °C. The second cohort included 12 patients who received one of two units treated under the optimized conditions. Median time to neutrophil engraftment was then 17.5 days (range 14–31), with ten patients engrafting with the $dmPGE_2$-treated UCB unit. Grade I–II infusion reactions were observed in four patients, and one patient presented with grade IV ST segment elevation and myocardial ischemia [41].

4 Limitations and Future Directions of UCB Expansion

UCB graft manipulation is coming of age. It is postulated, but not yet proven in a randomized fashion, that improved engraftment time will lead to decreased TRM, less infections, and shorter hospitalizations. However, graft manipulation could lead to the loss of some of the attributes of UCB that permit its application to patients who do not have a matched family donor, in particular the easy and rapid availability of procurement. The use of the dUCBT platform adds to the overall cost of the transplant, and it remains to be proven that expansion will actually decrease transplant costs by decreasing transfusion rates, or by shortening hospitalization, for example. On the other hand, it is likely that once cell dose and engraftment kinetics barriers are overcome, the main limiting characteristic of UCBT will be slower immune and lymphoid reconstitution and long-term infection risk [42, 43].

Alternative approaches to expedite engraftment are now under active investigation. Improving stem cell homing is an alternate approach being studied in a phase I/II clinical trial. Investigators at the MD Anderson Cancer Center have postulated that fucosylation of UCB cells would lead to better homing and to faster engraftment. Preliminary results are promising [44].

The scientific advances in our understanding of hematopoietic cell proliferation and differentiation have led to the early stages of clinical implementation of multiple UCB graft engineering technologies. However, the majority of the reported clinical trials have been nonrandomized. A large, randomized controlled study comparing unmanipulated dUCBT versus the combination of one MSC-expanded UCB unit and one unmanipulated unit (ClinicalTrials.gov identifier NCT01854567) is ongoing. Therefore, the efficacy of UCB expansion methodologies and their applicability in a large number of patients is yet to be formally determined. Moreover, combinations of multiple expansion and homing methods may yield even better results, but the feasibility and efficacy of such an approach will need to be clarified by rigorous research.

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Chapter 15
Intra-bone Marrow Transplant (IBMT) of Cord Blood (CB) Cells: A Transplant Approach that Tries to Optimize Seeding Efficiency and Trafficking of Hematopoietic Stem Cells (HSCs)

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1 The Problem of Engraftment and Speed of Hematopoietic Recovery

In the history of hematopoietic cell transplantation (HCT), the infusion of hematopoietic cells has been used in almost all cases intravenously. Physicians have utilized this route of administration relying on the fact that the cells infused are capable of finding their niches. Although it was published that in rodents less that 10 % of the injected cells were actually capable to seed in hematopoietic organs [1, 2, 3], little attention in the clinical practice was addressed to tackle this problem. While the engraftment has been a problem in the past and, to some extent, still it is, "how many cells actually occupy the niches after transplant" has not attracted major concern. It was also documented that even many years after transplant, the hematopoietic reservoir never reverts to normal values [4]. Thus, the "exhaustion" of hematopoietic stem cell (HSC) remains an interesting isssue that is linked to seeding efficiency but also to the capacity of HSC to "expand" after transplant.

In the clinical practice, the concept that the cell dose was relevant for transplant outcome was brought about only recently; higher cell dose was associated with lower nonrelapse mortality (NRM) [5, 6]. However, the utilization of mobilized peripheral blood has rendered the cell dose effect a very complex isssue.

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2 Trafficking of HSCs After Transplant

Migratory capacity is a key feature of HSCs that persists throughout the whole life of mammalians. This characteristic implies a profound communication as well as an intense exchange of signals and cells between different bone marrow (BM) segments. Similarly, the migration and seeding capacity represent the basis for the intravenous injection of HSCs in the course of BM transplantation (BMT). Without this crosstalk between HSC and microenvironment [7, 8], the transplantation of hematopoietic cells would have never took place. After the systemic intravenous (IV) injection, HSCs must cross the lung microvascular network prior to being transported to the body tissues by the blood stream. Accordingly, lungs represent a first order filter able to reduce the availability of cells for blood-BM exchange. Once they have reached the left atrium, HSCs are diluted in the cardiac output and their first-pass delivery to the different structures follows the mathematical rules predicted by the fractionation law [9, 10]. This principle has been described for all substances characterized by a high tissue-extraction rate and predicts that the fraction of administered molecules (or cells) reaching a specific organ is equal to the fraction of cardiac output delivered to them. This simple theoretical concept implies that a large number of HSCs will first take contact with highly perfused compartments such as brain, kidneys, and spleen, with a very low number reaching the BM. Obviously, the relevance of this "first appointment" is related to the specific extraction rate of HSCs in these organs, i.e., the rate at which these cells escape from blood in each specific tissue. A number of studies have documented that murine progenitors and human $CD34^+$ cells escape from the blood pool of recipient animal within minutes. These considerations imply that the accurate evaluation of HSCs trafficking after administration is a crucial point for understanding their homing features and thus to verify the validity of methods proposed to improve the effectiveness of BMT.

This issue has been extremely difficult to approach for a number of reasons. The sensitivity of conventional methods is too low for this task; moreover, the rapid nature of HSCs clearance, distribution, and homing asks for a high temporal resolution in order to identify in vivo the kinetics of these cells in the whole body as well as in different organs and in blood.

According to these considerations, nuclear medicine now could represent a gold standard in protocols asking for local administration of cells whose persistence in the target tissue can be accurately evaluated by monitoring the local hot spot radioactivity. Yet, the analysis of tracer (cell) accumulation in the time domain can be extremely helpful to this purpose. Temporal trend of tissue uptake associated with the evidence of tracer (cell) clearing from the blood can provide quantitative estimations of cell homing in the different tissues.

A quantitative analysis of early engraftment revealed an advantage in recognition of the BM by more primitive hematopoietic progenitors, manifested by differential homing and seeding efficiency of lineage-negative versus lineage-positive BM cells [11]. Assuming that homing is mediated by preexisting adhesion molecules expressed by the transplanted HSC, the affinity for the marrow stroma may be an

intrinsic property of BM cells at various level of differentiation. In general, 2–10 % of the infused lineage-negative cells home and seed in the marrow stroma in vivo more efficiently by a factor of 20–30 than lineage-positive cells. This process occurs physiologically, with loss of stromal adhesion during differentiation and releasing of matured cells into the peripheral circulation. However, selection of HSC by their repertoire of adhesion molecules failed to accurately predict homing and capacity to engraft.

The early events that occur during the engraftment process may indicate that:

1. There is a functional redundancy in the molecular pathways that mediate the cell-stroma interaction such that blockage of a single pathway has only a minor effect on homing and seeding.
2. Seeding and engraftment of injected HSC may be improved by localized transplant in a relatively small marrow space bypassing the peripheral circulation, increasing seeding efficiency by avoiding a massive passage through the lung and other organs in which HSC may be trapped and lost. In addition HSC may reach remote BM sites "according to a more physiological situation."

3 Intra-bone Transplant (IBT)

The story of clinical IB transplant of hematopoietic cells began in 1999 when a talented neuroscientist came to our group and proposed a collaboration. At that time the slogan "from brain to blood" was very popular and Dr Magrassi, who was working with fetal neural cells, was interested to expand the possibilities of turning fetal neural cells into blood cells [12].

In addition, we had just observed that children grafted with cord blood (CB) cells had more long-term colony-initiating cells (LTC-IC) 1 year after transplantation than comparable children grafted with BM in spite of the fact that they had undergone slower neutrophil and platelet recovery [13]. We considered this finding very important because there was a big discrepancy in the data available in the literature: (1) the percentage of human CD34 cells recovered after injection of equal numbers of CD34 in conditioned nonobese diabetic/severe combined immunodeficiency (NOD/SCID) was higher if they were injected with CB cells versus BM, or mobilized peripheral blood (PB cells); (2) CB progenitors were considered insufficient in numbers (approximately 10 time less that a standard BM harvest) to sustain a rapid engraftment. Then, we hypothesized that CB progenitors cells are more prone to self-renewal respect to differentiation and maturation than adult BM or peripheral blood stem cells. These results were a major advance as we had previously documented that patients allografted with BM never recovered the LTC-IC reservoir [4].

We irradiated C57BL mice and injected fetal neural cells. The control animals (injected with syngeneic BM) reconstituted, but we did not observe donor hematopoiesis in mice injected with fetal neural cells (FNC). Therefore, we considered to inject FNC into the tibia for overcoming the problem of BM seeding. No hematopoiesis was observed locally or in other hematopoietic sited after FNC injection; surprisingly

in the control mice transplanted with hematopoietic cells, we found 10 time higher engraftment in the mice transplanted directly IB as compared to those transplanted intravenously [14]. Briefly, by serendipity, it was observed that IB transplantation was associated with better engraftment. In the same period, the group of Osaka had also observed and published (before our own data) a better engraftment when cells were delivered directly into the bone [15].

Therefore, we need to understand the pathophysiological mechanisms underlying these improvements. Briefly, Lewis rats underwent IB or IV administration of HSCs harvested from syngeneic animals and purified according to CD90 expression. These cells were labeled with 37 MBq, mTc-exametazime and injected either IV or IB. Cell trafficking and distribution in heart, lung, spleen, liver, and forelimb was evaluated by dynamic radionuclide imaging [11]; graphical approach was used to estimate tissue recruitment of HSCs. More than 90 % of cells escaped from the injected bone to the bloodstream in a few minutes. However, this short contact profoundly modified HSCs kinetics, reducing their lung sequestration and shortening their blood persistence with respect to IV. More importantly, IB passage resulted in reduced lung uptake and in a fourfold increase in homing of remote BM sites. From these experiments, we hypothesized that the first-entry contact with the hematopoietic microenvironment immediately readdresses the fate of transplanted HSCs, providing them with "the final destination stamp" to define their BM homing. This study confirmed that the capability to cross the pulmonary microcirculation represents an important step in defining the therapeutic potential of transplanted HSCs. This striking effect produced by IB on cell kinetics strongly supports the concept that specific signaling pathways selectively regulate the interaction between HSCs and the surfaces of BM sinusoid endothelium. On the other hand, this finding indirectly suggests that, once harvested and kept for few hours (or much more time in the case of frozen CB cells) out from their native habitat, HSCs undergo relevant modifications of their natural characteristics.

However, this direct analysis is hampered by major limitations in evaluating cell recruitment in organs downstream from this filter. In fact, the radioactivity content of the latter reflects the contribution of two different HSCs pools: those actually recruited by the organ and those still circulating in the blood volume included in it. The simple HSCs kinetics varies according to the injection protocol. The seeding efficiency of transplanted HSCs remains controversial.

While previous studies documented an extremely low BM homing of transplanted HSCs, more recent experiments documented that the low frequency of permanent BM reconstitutions is not only caused by a low BM homing but also by the failure of initially engrafted cells to sustain self-renewal.

On one side, our kinetics data indicate the relevance of injected bone microenvironment in addressing the fate of HSCs after IB. On the other hand, they also confirm the up-regulation of adhesion molecules, such as vascular cell adhesion molecule and intercellular adhesion molecule in irradiated lung microcirculation. Obviously, the relatively decreased BM homing observed after total body irradiation (TBI) does not conflict with the well-established clinical protocols requiring host preconditioning before allogeneic transplantation. In fact, durable engraftment with high levels of

repopulation by transplanted stem cells is largely dependent on this procedure both in experimental models and in patients. However, our study aimed to identify the immediate trafficking of donor HSCs as a function of site of injection and BM integrity rather than to verify the ultimate effectiveness of this procedure [11]. In this line, cell homing represents only the first step of the process of BM repopulation, with later ones being more dependent upon the relationship and competition between donor and recipient cells.

Finally, although our study did not evaluate the lymphocyte trafficking, the large difference in lymph nodes recruitment of HSCs after IB indicates that further studies might indicate a possible role for the first interaction with recipient bone as a factor able to modify the cross talk between donor and recipient hematopoietic systems.

4 Technical Aspects

In the IB study, CB units were obtained from several CB banks. Human leukocyte antigen (HLA)-A, HLA-B, and HLA-DRB1 typing was done to select the most closely matched donor unit–recipient pair, with preference given to HLA-DRB1-matched units. HLA-DRB1 alleles were determined by high resolution DNA typing, by use of polymerase chain reaction (PCR) sequence-specific primers and sequence-based typing. Class I antigen- or allele-level minimum requirements were a 4/6 HLA match, and a total number of nucleated cells greater than 1×10^7/kg of recipient body weight, as determined before freezing.

On the basis of patient characteristics and pretransplant therapy, patients were prepared for transplantation with different conditioning regimens. The majority of patients received fractionated 10–12 Gy total body irradiation on days 6, 5, and 4 before transplantation (subdivided into 1 fraction/day or fractions of 2 Gy twice a day) and cyclophosphamide (60 mg/kg/day) on days -2 and -1. Some patients received thiotepa (8 mg/kg) on day 8 before transplantation, treosulfan on day -7 to -5, and fludarabine on days -7 to -3.

Some patients received a reduced-intensity conditioning, which consisted of fludarabine from day -6 to -2 plus cyclophosphamide (50 mg/kg) on day 6 before transplantation, and a single 2 Gy fraction of total body irradiation on day 1 before transplantation.

Cyclosporin was started intravenously from day -7 before transplantation at a daily dose of 1 mg/kg and was tapered to discontinuation from day $+90$ to $+180$ after transplantation in the absence of graft-versus-host disease (GVHD). The dose of cyclosporin was adjusted to maintain serum trough concentration between 150–300 μg/L. Mycophenolate mofetil was given at a dose of 15 mg/kg orally twice a day from days $+1$ to $+28$ after transplantation. Antithymocyte globulin was given at a dose of 3 mg/kg per day on days -3 and -2 before the procedure. No patients received steroids for GVHD prophylaxis. Patients received granulocyte colony-stimulating factor.

4.1 CB Unit processing and IB transplant

CB units were thawed in a 37 °C water bath as described by Rubinstein and coworkers [16]. We decided to remove dimethylsulphoxide before IB injection of the cells by washing the cells with a saline solution plus dextran and human albumin. CB cells were resuspended in 20 mL of saline solution plus dextran and albumin, and aliquoted in four 5-mL syringes. The first procedure was done under local anesthesia, which is usually used for BM aspiration. However, because the first patient described unbearable pain, for all subsequent procedures, anesthesia consisted of short propofol sedation. Propofol is a short-acting IV sedative drug used for the induction of general anesthesia in adults and children, maintenance of general anesthesia, and sedation in medical contexts.

The entire IB-injection procedure lasts 8–15 min from the beginning of anesthesia. The patient was positioned in the flank posture because there was no intubation. Once sedation is established, a standard needle for BM aspiration (14 gauge) was inserted a few centimeters into the superior-posterior iliac crest; an aspiration of about 1 mL was then done to assess that the needle was securely inserted into the BM cavity. Subsequently, we insert the syringe containing 4–5 mL of CB-cell suspension, which is then gently infused.

This procedure is then repeated for all the remaining aliquots at a distance of about 3–5 cm from the previous injection site.

We report here two series of patients transplanted IB with CB. In the initial 90 patients [17–18], HLA matching was 4/6 and 5/6 for 85 and 15 % of patients respectively; 54 % were in advanced stage of disease and in poor clinical conditions. Median infused total nucleated cells (TNC) and CD34 + cells were 2.5×10^7/kg and 1.4×10^5/kg (0.6–4.3) respectively; at day + 30, cumulative incidence (CI) of neutrophil (PMN) and platelet (PLT) recovery was 82 and 75 % with a median time to engraft of 23 (PMN) and 36 days (PLT). TNC dose did not impact on PMN and PLT recovery, while the number of CD34 + cells showed a borderline impact.

In another series of 30 patients (unpublished), the TNC and CD34 + cells infused were 2.06 (0.3–3.92) x10^7/kg and 0.54×10^5/kg (0.23–2.9). In this series, the number of CD34 + cells infused correlated with PMN and PLT recovery as well as with the NRM. The difference of impact of CD34 + cells on PMN and PLT recovery between the two groups may lie in the fact that in the second group the CD34 + cells infused were much lower and therefore below the threshold dose. In both series (120 pts), 60 % of patients did not have acute GVHD (aGVHD) and only two experienced Grade III aGVHD, no patient had Grade IV aGVHD. Conclusion: IB-UCBT is associated with faster myeloid and platelet recovery and very low incidence/severity of aGVHD.

An important finding was the low incidence of severe GVHD. This finding compares favorably with the incidence of GVHD in adult recipients of an IV CB transplant, which is reported to be between 35 and 45 %. Two combined factors might contribute to the low incidence of aGVHD in our study. First, lymphocyte trafficking is known to be a crucial factor in immunity. The possibility exists that only a proportion of transplanted T cells will reach the lymphatic organs, where they

would be immediately confronted with host antigen-presenting cells, as probably occurs after IV injection. Second, injected T cells immediately come into contact with mesenchymal stem cells and osteoblasts in the marrow spaces (niches), which are known to be potent immunosuppressants. A decreased incidence of GVHD has also been reported in animal models in which the IB technique has been used compared with IV injection [19].

A decreased incidence of GVHD is usually associated with an increase in relapse, due to its effect on leukemic cells, referred to as graft-versus-leukemic disease. CB transplants (CBT) do not follow this pattern completely because a decreased incidence of chronic GVHD does not seem to increase relapse from leukemia. Our study seems to be in keeping with these findings [20].

Therefore, we can conclude that this approach may extend the possibility to offer a transplant to more patients.

5 The Potentialities of CB HSC Do Not Emerge in the Setting of Allogeneic Transplants

CB HSC have superior proliferative potentialities respect to BM HSC. This has been documented *in vitro* and in vivo. *In vitro* progenitor cells have the capacity of re-cloning, whereas BM progenitors do not. In vivo CD34 + cells from CB repopulate better the NOD/SCID mice and, in clinical CBT, the hematopoietic reservoir, as measured by LTC-IC, is approximately 1 log higher that after BMT. Thus, the slow recovery of hematopoietic parameters after CBT remains a paradox. We have also documented that there is not cell dose effect on the recovery of neutrophils or platelets suggesting that, when hematopoietic cells are transplanted via IB, the cell dose threshold is indeed much lower than when cells are administered via IV. Nevertheless, the immune recovery remains slow and the proliferative potentiality of CB HSC does not translate in superior outcomes, at least in these transplant settings. It might be that patients who have successfully undergone CBT might have a younger hematolymphopoietic system long-term. Whether this will translate in some survival advantage is unknown.

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Chapter 16
Haplo-cord Transplantation: Overcoming the Limitations of Umbilical Cord Blood (UCB) Transplantation (UCBT)

Koen van Besien

1 Introduction

Originally proposed by Fernandez et al., the infusion of umbilical cord blood (UCB) cells with CD34-selected donor stem cells from an adult typically results in rapid hematopoietic recovery from adult donor cells that are over time replaced by definitive hemato- and lymphopoiesis from the cord blood graft. Fernandez et al. reported in 2001 three patients with advanced hematologic malignancies who underwent UCB transplant (UCBT) after myeloablative conditioning supported by coinfusion of adult haploidentical CD34 cells [1]. The same group has since performed 87 such transplants and detailed results were reported in 2009 on the 55 initial patients [2, 3]. Six other groups have reported outcomes of cord blood transplant supported by coinfusion of adult, usually haploidentical cells [4–8]. The group at the University of Chicago pioneered this technology in the USA.

2 Initial Data on Haplo Cord—University of Chicago

Our original protocol initiated at the University of Chicago in 2005 included two cohorts with different intensity conditioning. In the intensive cohort, patients received myeloablative conditioning, consisting of thiotepa, fludarabine, and total body irradiation (TBI 200 cGy BID for 3 days). Only 13 patients were accrued, most of them with advanced hematologic malignancies. Though all engrafted rapidly, there was excessive toxicity mainly due to interstitial pneumonitis and this regimen has been abandoned. It is likely that this toxicity was related to excessive toxicity of the conditioning regimen, because similar problems were observed in recipients of related and unrelated donor transplantation [9]. The large majority of patients since then have received a widely utilized reduced intensity conditioning consisting of

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fludarabine (25 mg/m2 × 5), melphalan, (140 mg/m2 × 1 or 70 mg/m2 × 2) and thymoglobulin® (4 mg/kg × 4 doses, and more recently × 3 doses for patients over the age of 50). Posttransplant graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus (target level 5–15 ng/ml) and mycophenolate mofetil. Supportive care followed usual transplant procedure with prophylactic quinolone until engraftment, antifungal, pneumocystis, and antiviral prophylaxis. Cytomegalovirus (CMV) prophylaxis was modeled on our initial studies of CMV prophylaxis with high-dose valacyclovir in T-depleted transplant recipients; a variant of this regimen was also recently used by the Seattle group. Monitoring for Epstein–Barr virus (EBV) was not routinely performed in the initial patients.

We initially reported on 45 patients; their median age was 50, weight 80 kg, and 58 % had active disease. Neutrophil engraftment occurred at 11 days (interquartile range, IQR, 9–15) and platelet engraftment at 19 days (IQR, 15–33). In the majority of patients, early haploidentical engraftment was replaced by durable engraftment of UCB by 100 days, with regular persistence of minor host and/or haplo-hematopoiesis. Percentage of haplo chimerism at day 100 correlated with haplo CD34 dose ($p = 0.003$). Cumulative incidence of acute GVHD (aGVHD) was 25 % and chronic GVHD was 5 %. Actuarial survival at 1 year was 55 %, progression-free survival (PFS) 42 %, nonrelapse mortality (NRM) 28 %, and relapse 30 % [7]. Average duration of admission and transfusion requirements compared favorably with those reported from other institutions with double UCBT.

3 Modifications to the Initial Protocol

While the general treatment strategy has remained unchanged since the report on our initial patients, important modifications have been introduced as these studies were continued at the University of Chicago and at Weill Cornell Medical College in New York (WCMC). The most important ones relate to donor selection and cell dose infused.

3.1 Donor Selection

Many groups have reported on the role of donor-specific human leukocyte antigen (HLA) antibodies (DSA) in graft rejection, and anecdotally we too have observed cases of graft failure associated with DSA [10–12]. We currently check for the presence of DSA in all recipients, attempt to avoid donors targeted by DSA, and, if unavoidable, attempt to reduce the burden of DSA by plasma exchange, intravenous immunoglobulin (IVIG), rituximab, and/or proteasome inhibitor [13]. UCB selection now also takes into account cord blood viability, an important determinant of cord blood engraftment [14].

3.2 Cell Dose Haplo Donor

In the initial trial, the graft cell dose was calculated based on T-cell dose. Some of the recipients received extremely high doses of CD34- selected cells, which was associated with failure of the cord blood graft. Currently we limit the dose of the haplo graft to a maximum of 5×10^6 CD34/kg recipient weight.

Since the introduction of these modifications in graft selection and cell doses, failure of the UCB unit has become rare, with only three instances among 40 patients. In two of the three cases, the failure of the cord blood graft could be attributed to technical issues.

3.3 Cell Dose Cord Blood Unit (CBU)

While emphasizing cord blood quality, we have deemphasized cord blood cell dose. In an ongoing study, we are systematically studying the lowest threshold for cord blood units that are associated with durable CBU engraftment. Doses as low as 0.5×10^6 nucleated cells/kg have been followed by durable CBU engraftment. Definitive conclusions are not possible yet, but at present, no adverse impact of lower cell dose on long-term engraftment has been observed. We hypothesize that the acceptance of lower UCB cell doses may ultimately result in improvement in outcomes, because often, and particularly in minorities, smaller CBU's are better matched CBU's and there is increasing evidence that better matching is associated with improved outcome in UCB transplant [15, 16].

3.4 Supportive Care

Because of the low incidence of chronic GVHD, GVHD prophylaxis has been tapered and mycophenolate is discontinued on day 28. Close monitoring for EBV reactivation and aggressive intervention with rituximab upon reactivation are now standard. For patients more than the age of 50, with a high incidence of EBV reactivation, the dose of antithymocyte globulin (ATG) has been reduced [17]. During 2013, pretransplant trimethoprim-sulfamethoxazole, long standard, was omitted at WCMC, and three cases of toxoplasmosis (two disseminated and one central nervous system, CNS) were observed. Polymerase chain reaction (PCR) monitoring for toxoplasma is now routinely performed for patients at WCMC [18]. Other aspects of supportive care, particularly broad spectrum azoles, posttransplant neupogen, and intensive CMV prophylaxis remain standard [19].

4 Comparison Between Haplo-Cord and Double Cord Transplantation

As of October 2013, 99 patients had been treated at University of Chicago and WCMC, using the same conditioning regimen. We compared our outcomes with those of 737 adults undergoing double UCBT as reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) [20]. Patients undergoing haplo-cord transplant were significantly older (median age 54 vs. 48, $P = 0.0096$), more frequently were of minority ethnicity (60 % white vs. 76 % white, $p = 0.0013$) and had higher risk disease (45 % American Society for Blood and Marrow Transplantation, ASBMT, high risk vs. 23 % ASBMT high risk). In order to adjust for such imbalances, a case cohort study was performed and controls were selected based on patient age, gender, race, disease type, disease stage pretransplant, Karnofsky Performance Status (KPS), and year of transplant. One to four matched controls were identified for each patient and the final control group had 344 patients. Patient characteristics are shown in Table 16.1. The median age of the patients was 54. More than half had acute leukemia or myelodysplastic syndromes (MDS) and one-third had lymphoid disorder. A considerably higher percentage of patients in the haplo-cord group had high-risk disease, (44 % vs. 34 %, $p = 0.06$).

In multivariate analysis, engraftment of neutrophils and platelets was considerably faster after haplo-cord transplantation than after double UCBT. By day 30, 91 % of haplo-cord transplant recipients had achieved neutrophil recovery and 53 % platelet recovery vs. 72 % and 6 % of controls respectively ($P < 0.0001$ for both platelet and neutrophil recovery (Fig. 16.1 and Table 16.2). Survival was also superior at all time points ($p = 0.0069$) and the survival advantage became more apparent over time. At 4 years, 43 % of haplo-cord recipients remained alive vs. 21 % of double-cord recipients ($p = 0.0053$). These data strongly suggest a considerable advantage of haplo-cord transplant over double-cord-blood transplantation. This advantage extends from more rapid hematologic recovery to shorter duration of admission and improvement in overall survival.

5 Experience in Other Groups

Two groups in Spain have also extensively studied cord blood transplantation supplemented by third party donor cells as has a group from the Netherlands, investigators from the National Institute of Health (NIH) and recently from Memorial Sloan Kettering Cancer Center in New York [4–7]. All of them confirmed rapid hematologic recovery, long-term dominance of the UCB graft, and most also described a low incidence of acute and chronic GVHD. The Spanish group has the largest experience using myeloablative conditioning. Their GVHD prophylaxis consisted of calcineurin inhibitors combined with steroids. In their series, there were six cases of grade III–IV acute GVHD out of 55 recipients. There were also three cases of extensive chronic GVHD. Despite this low incidence of severe GVHD, the relapse rate was only 17 % at 1 year. The incidence of opportunistic infections particularly CMV disease was

Table 16.1 Characteristics of haplo-cord recipients and CIBMTR matched double cord transplant recipients

Variable	Control ($N = 344$)	Case ($N = 99$)	P
Age, med (range) Y	51 (19–80)	54 (19–73)	0.6483
Male	202 (58 %)	61 (62 %)	0.6053
Race			
White	225 (65 %)	59 (60 %)	0.5192
Black	70 (20 %)	25 (25 %)	
Others	49 (14 %)	15 (15 %)	
Disease type			
AML	191 (56 %)	54 (55 %)	0.9839
ALL	45 (13 %)	12 (12 %)	
CLL/Other	15 (4 %)	3 (3 %)	
CML	11 (3 %)	4 (4 %)	
MDS/MPS	41 (12 %)	12 (12 %)	
Other AL	6 (2 %)	1 (1 %)	
NHL	24 (7 %)	9 (9 %)	
HL	11 (3 %)	4 (4 %)	
Disease stage			
Early	147 (43 %)	34 (34 %)	0.1520
Intermediate	80 (23 %)	21 (21 %)	
Advanced	117 (34 %)	44 (45 %)	
KPS			
60–80 %	71 (21 %)	21 (21 %)	0.9015
90–100 %	273 (79 %)	78 (78 %)	
Year of TX			
2007–2009	79 (23 %)	19 (19 %)	0.4254
2010–2013	265 (77 %)	80 (81 %)	

AML acute myeloid leukemia, *ALL* acute lymphocytic leukemia, *CLL* chronic lymphoid leukemia, *CML* chronic myelogenous leukemia, *MDS* myelodysplastic syndromes, *KPS* Karnofsky Performance Status, *TX* treatment, *CIBMTR* Center for International Blood and Marrow Transplant Research, *MPS* Myeloproliferative syndrome, *AL* acute leukemia, *NHL* non-Hodgkin's lymphoma, *HL* Hodgkin Lymphoma

considerable in their studies and seemingly higher than we identified in our studies. This may reflect differences in infection prophylaxis, and in GVHD prophylaxis and treatment. The routine use of steroids for GVHD prophylaxis as utilized in Spain may result in an increased propensity for infectious complications. Incidence of EBV reactivation and EBV posttransplant lymphoproliferative disorder (PTLD) was similar to what we observed in our series.

In our own studies, the source of adult donor cells has been restricted to haploidentical related donors. The Spanish group used mismatched unrelated donors in a substantial proportion of their patients; this allows additional opportunity for occasional patients who lack access to family donors. It is also possible that characteristics of the adult donor graft such as HLA type and killer immunoglobulin-like receptors (KIR) type further modulate gamma-Valerolactone (GVL) and Graft versus Host (GVH) occurrence. The ability to select from a large pool of unrelated donors based provides further opportunities to prospectively study the impact of such parameters on transplant outcome.

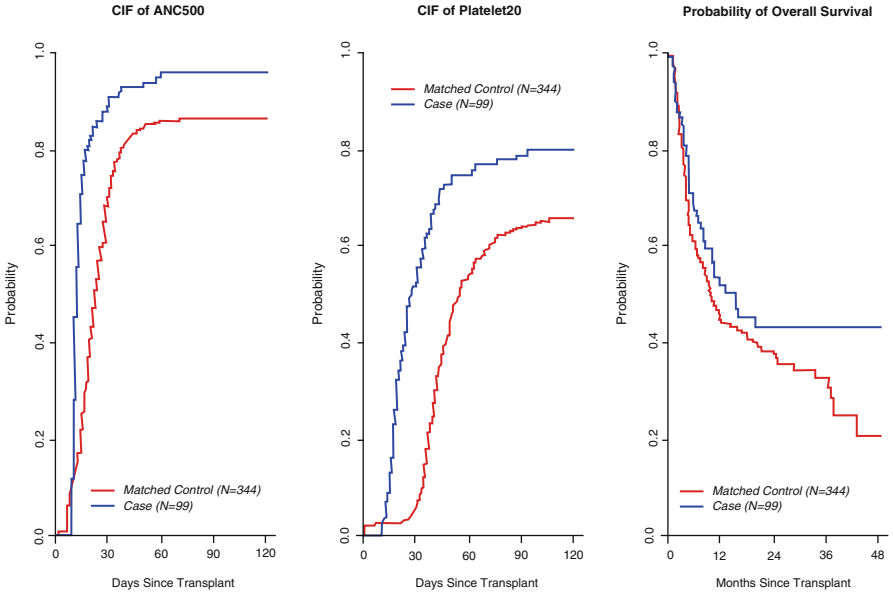

Fig. 16.1 Haplo-cord transplant vs. matched CIBMTR DCBT controls. **a** Neutrophil engraftment ($p < 0.0001$). **b** Platelet engraftment ($p < 0.0001$). **c** Overall survival, $p = 0.07$. *CIBMTR* Center for International Blood and Marrow Transplant Research, *DCBT* double umbilical cord blood transplant, *ANC* absolute neutrophil count, *CIF* Cumulative Incidence function

Table 16.2 Multivariate analysis comparing haplo-cord transplant vs. CIBMTR double UCBT (dUCBT)-matched controls

	Double cord ($N = 344$)	Haplo-cord ($N = 99$)	P
ANC recovery			
30 Day	72 (67–76)%	91 (84–95)%	< 0.0001
60 Day	86 (82–89)%	96 (90–98)%	0.0001
90 Day	87 (83–90)%	96 (90–98)%	0.0001
120 Day	87 (83–90)%	96 (90–98)%	0.0001
Platelet recovery			
30 day	6 (4–9)%	53 (43–61)%	< 0.0001
60 day	54 (46–59)%	75 (65–82)%	< 0.0001
90 day	64 (59–69)%	79 (70–85)%	0.0014
120 day	66 (61–70)%	80 (71–86)%	0.0019
Overall survival			
1 year	44 (39–50)%	52 (40–60)%	0.2277
2 year	38 (32–43)%	43 (32–54)%	0.3846
3 year	33 (26–40)%	43 (32–54)%	0.1219
4 year	21 (11–33)%	43 (32–54)%	0.0053

ANC absolute neutrophil count, *CIBMTR* Center for International Blood and Marrow Transplant Research, *dUCBT* double umbilicical cord blood transplant

The group from Madrid addressed occasional graft failures of the UCB unit by infusion of a second UCB unit, which tended to engraft and outcompete the residing adult graft. More recently they have analyzed the patterns of early chimerism, and found that the day 14 or day 21 detection of UCB T-cell chimerism was predictive of long-term CBU engraftment [21]. Absence of early CBU-derived T-cell chimerism could thus be used to guide treatment decisions.

The groups from the Netherlands and Spain have utilized haplo-cord transplant in pediatric patients and also as a way to expedite engraftment after transplant of a low cell dose CCR5−graft in HIV patients [22].

The group from NIH has focused on patients with aplastic anemia, a disease in which outcomes of cord blood transplantation were previously poor. In their series, 11 of 12 heavily transfused patients engrafted and had durable responses [5]. In a recent abstract, they provide preliminary evidence that natural killer (NK)-cell-mediated reactions between haplo and cord blood grafts due to KIR incompatibility may provide yet another mechanism of graft failure/rejection [23]. If confirmed, KIR incompatibility between different donors may need to be avoided.

Lastly, the group from Memorial Sloan Kettering Cancer Center (MSKCC) has recently presented preliminary data introducing yet another variant of the procedure [8]. They combined double UCB Stem Cell Transplant (SCT) with a haploidentical graft. The rationale for using two UCB units was threefold: (1) allowing comparison with their own historical data in double UCB SCT, (2) avoiding graft failure due to poor quality of occasional UCB units, and (3) exploiting potential GVL effects from infusion of two UCB units. As opposed to the other groups, they do not utilize ATG, for concern over excessive immunosuppression. They again confirm rapid hematopoietic reconstitution with a time to discharge that is much shorter than in their previous experience with double UCBT. The long-term benefit of a triple graft remains to be demonstrated.

6 Long-term Immune Reconstitution

The Spanish group as well as the Chicago group studied long-term immune reconstitution [24]. The Chicago group assessed lymphocyte subsets, T-cell diversity, Cylex Immuknow assay (a measure of T-cell responsiveness), and serological response to pneumococcal vaccination [25]. NK-cell and B-cell reconstitution were rapid at 1 month and 3 months, respectively. T-cell recovery was delayed with gradual increase in the number of T cells, starting around 6 months posttransplantation, and was characterized by a diverse polyclonal T-cell repertoire. Recovery of immunoglobulins and responsiveness to pneumococcal vaccination was observed. T-cell spectratype was often remarkably diverse. They concluded that immune reconstitution after haplo-cord transplantation was similar to that seen after cord blood transplantation, despite infusion of much lower cord blood cell doses.

The Spanish group previously reported similar observations in their patients [26]. NK and B cells recovered to normal values by the 6th and 9th months respectively. This was somewhat slower than in the Chicago series, possibly because of routine use

of posttransplant steroids for GvHD prophylaxis. Recovery of T cells was slower, naive cells lagging behind those of memory and effector cells. Serial analyses of signal joint T cell receptor (TCR) excision circles showed a general pattern of very low levels by the 3rd month after CBT, followed by recovery to levels persistently similar or higher than those observed before transplantation and in normal controls. In both the Chicago and Madrid series, early T-cell recovery derives from the adult donor followed by gradual replacement by cells of UCB origin. It is likely that most of the early B cells after haplo-cord transplant are also UCB derived. Of interest, the NIH group observed immediate UCB-derived T-cell reconstitution, without detectable adult donor-derived T cells at any moment. This strikingly different kinetic profile of immune reconstitution may be due to the use of a different formulation of ATG. Nobody has evaluated the origin of NK and B cells.

7 Conclusion and Perspectives

The cumulative experience with this approach corroborated by us and others indicates that for the large majority of patients, hematopoietic recovery of both myeloid and megakaryocytic lineages is rapid and that this is an efficient and tolerable procedure. Our matched control comparison with double cord blood transplantation strongly suggests that this enhanced recovery results in improved survival. Increasing experience has allowed us to address certain issues of supportive care and have raised intriguing possibilities for further development. Traditionally cord blood transplantation has been limited by the necessity of a large CBU infusate and has therefore been more extensively utilized in children. In adults, cell dose barriers resulted in ineligibility of many patients. Those undergoing transplant often had prolonged admissions and delayed recovery, increasing the risk and expense of the procedure. These barriers to transplant have been largely overcome by haplo-cord transplantation. While many centers limit dUCB SCT to patients below a certain age limit or weight, no such barriers have been used in our programs.

In contrast to experience with single or double UCBT, the umbilical cell cord dose does not correlate with time to neutrophil or platelet recovery after haplo-cord transplant. Even very low CBU doses of $< 1 \times 10^6$ Nucleated blood cells (NBC)/kg have reliably engrafted after initial recovery was provided by the haplo graft. The ability to reduce the CBU dose may in fact constitute one of the greatest advantages of the haplo-cord procedure. The lower UCB threshold dose effectively increases the cord blood inventory by several folds and this may allow one to identify appropriately matched units for a higher percentage of recipients. This may be especially important for African American patients since UCB units of AA descent, presumably on average better matched, tend to have considerably lower cell content. Ongoing studies are attempting to identify the lowest acceptable dose of UCB cells that can be used in this setting. For patients with access to multiple UCB units, choice between donors may no longer be guided by cell dose, but by desirable UCB characteristics such as KIR type or National Integrated Medical Association (NIMA) matching [24].

Numerous options now exist for patients lacking a matched related donor. The largest experience has been accrued with transplantation from adult unrelated donors and this remains the de facto standard. But preliminary data suggest that haplo-cord transplantation may represent an equally effective alternative. The Spanish group compared the outcomes of allogeneic transplantation using haplo-cord transplant with that of those undergoing unrelated donor transplant and found similar survivals [27]. Preliminary analysis of outcomes at our own center in patients over 50 finds outcomes with haplo-cord transplant that are at least similar and likely superior to those with adult unrelated donor transplant. The low rates of chronic GvHD, combined with low rates of disease recurrence after cord transplantation, may indeed have their highest impact in older patients who often have high-risk leukemia and are particularly vulnerable to the ravages of chronic GVHD. Prospective studies are required to address this issue.

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Chapter 17
Studies Comparing Haploidentical and Cord Blood Transplantation

Christopher G. Kanakry and Ephraim J. Fuchs

1 Introduction

1.1 The Need for Alternative Donor Transplantation

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative modality for treating otherwise incurable hematologic disorders. Although a human leukocyte antigen (HLA)-matched sibling donor has been the preferred donor stem cell source due to worse outcomes historically using other donors [1–4], only 30 % of patients have an HLA-matched sibling. Therefore, there has been a huge need to develop safe and effective alternative donor strategies for allo-HSCT. Alternative donor options include HLA-matched or partially HLA-mismatched unrelated donors, partially HLA-mismatched related donors, and umbilical cord blood (UCB) units. As unrelated donors cannot be identified for many patients and the search for an unrelated donor can be time-consuming and delay life-saving treatment with allo-HSCT, partially HLA-mismatched related donors and unrelated UCB units are the most universally available options.

While partially HLA-mismatched donors can take many forms, the most readily available for most patients are HLA-haploidentical (haplo) related donors. A haplo donor shares with the patient an identical haplotype of chromosome 6 but has an unshared form of the other haplotype. This unshared haplotype may be completely different or may share a variable number of common loci when assessed by high-resolution typing. As each patient on average will have two to three related haploidentical potential donors among parents, siblings, or children, nearly all

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patients will have ready access to an available and dedicated haplo donor. The use of a haplo donor for allo-HSCT (haplo-HSCT) also allows for a ready source of cells for future adoptive cell-therapeutic approaches, if necessary, to treat or prevent relapsed disease in the patient.

With UCB banks now containing over 600,000 UCB units, UCB has become another viable and readily available donor source for most patients undergoing allo-HSCT [5]. As there is no requirement for contacting donors, further HLA typing, or clinical evaluation and harvesting of the most suitable donor, unrelated UCB units are much more quickly and reliably available than are unrelated donors of bone marrow (BM) or peripheral blood stem cells (PBSCs). These advantages have allowed more than 30,000 individuals to undergo UCB allo-HSCT (UCBT) over the past 25 years [5].

As we will describe in this chapter, there is no decisive answer yet as to which of these alternative donor strategies is preferable. No prospective direct comparison has yet been completed, although the first multicenter phase III randomized study (Blood and Marrow Transplant, BMT, Clinical Trials Network, CTN, study 1101) comparing allo-HSCT using haplo-HSCT or UCBT is currently ongoing. While we await accrual and outcomes for this study, we will describe the history of these two approaches, available data that can be used to compare the relative benefits and drawbacks of each approach, and what we expect to learn from the BMT CTN 1101 study.

2 Umbilical Cord Blood Transplantation

2.1 History of the Umbilical Cord Blood (UCB) Approach

Early work showed that UCB contained sufficient numbers of progenitor cells for engraftment [6]. Furthermore, UCB could be kept at 4 or 25 °C for at least 3 days and still maintain viability and functionality of the progenitor cells [6], thus allowing routine transportation and processing of UCB to be performed. UCB units could be cryopreserved for extended periods of time, maintaining function more than 20 years after cryopreservation [7]. Such properties led to the establishment in the Broxmeyer laboratory of the first UCB bank, which processed grafts for the first five UCBTs starting in 1988 [5, 8].

The first UCBTs were HLA-matched related pediatric transplants, and early studies showed that younger age and transplantation from HLA-matched related donors were favorable prognostic factors for UCBT [9]. Initially, UCBT was restricted to use in children as it was believed that the numbers of cells in an UCB unit would be insufficient to engraft in adult patients. Although the use of double unit UCBTs in adults has since become popular to address this concern, adequately sized single UCB units are reported to be sufficient for engraftment in adults with similar outcomes in prospective studies compared with double unit UCBTs [10–12] with the possible exception of a higher risk of relapse after single unit UCBTs [11, 12].

The feasibility of using unrelated UCB donors for UCBTs was demonstrated in a phase I study published in 1996 [13]. Eighteen pediatric patients (13 malignant diseases, 5 nonmalignant diseases) were treated; 11 patients received HLA-mismatched UCB units. Engraftment was 100 %, and grade III–IV acute graft-versus-host disease (aGVHD) was only 11 % at 100 days. Survival at an early time point of 6 months was 65 %. A second study of 22 patients receiving unrelated UCBT (95 % HLA-mismatched) showed 100 % engraftment in all patients surviving the first 30 days [14]. However, 32 % of patients had grade III–IV aGVHD, 9 of 10 evaluable patients developed chronic graft-versus-host disease (cGVHD), and nonrelapse mortality (NRM) was 43 % at 100 days.

Despite these early results showing universal engraftment, other studies revealed that UCBT in adults receiving myeloablative conditioning was associated with high NRM [15–17]. A study of 68 patients receiving unrelated HLA-mismatched UCBTs after myeloablative conditioning showed engraftment of 90 %, grade II–IV aGVHD of 60 %, grade III–IV aGVHD of 20 %, and cGVHD of 38 % [15]. Unfortunately, 47 % of patients died within the first 3 months due to toxicity or infection. Another prospective study of high-risk patients receiving myeloablative conditioning before unrelated UCBT showed delayed and incomplete engraftment and poor survival with only 30 % of patients alive at 6 months and only two long-term survivors (6 %) [16].

As the toxicity associated with this approach seemed prohibitive, many investigators turned their attention to exploring reduced intensity conditioning (RIC) approaches to UCBT. A number of studies have refined this approach and shown the efficacy of RIC for facilitating HLA-mismatched UCBT with sustained engraftment rates of 76–94 %, grade III–IV aGVHD of 9–25 %, and NRM of 14–38 % [18–21].

2.1.1 Umbilical Cord Blood (UCB) Transplantation Compared with Other Approaches

Several studies have retrospectively assessed outcomes after UCBT and suggested similar overall outcomes to that seen using other donor sources for allo-HSCT. One large retrospective registry study examined outcomes for children less than 16-years-old with acute leukemia treated with allo-HSCT using either UCB or BM allografts that were either HLA-matched or one- or two-antigen HLA-mismatched [22]. Engraftment was 97 % in patients receiving BM allografts but was lower (59–85 %) in patients receiving UCB of varying HLA-matching or cell dose. Grade III–IV aGVHD rates were similar between UCBT and BM allo-HSCT, but were higher with increasing HLA-mismatching of either stem cell source. NRM was low in HLA-matched UCBT but was higher in HLA-mismatched UCBT compared with HLA-matched BM allo-HSCT, with NRM of 32 % and 46 % for one-antigen and two-antigen HLA-mismatched UCBT, respectively. In contrast, relapse rates were lower for all UCBT groups and HLA-mismatched BM allo-HSCT compared with HLA-matched BM allo-HSCT, suggesting enhanced graft-versus-tumor activity resulting from the HLA-mismatching. For most of the graft sources assessed, these

opposing effects of higher NRM but decreased relapse seen with increasing HLA-mismatching balanced each other such that most groups had similar leukemia-free survival rates. However, the group receiving HLA-matched UCBT had significantly improved leukemia-free survival. These results suggested that, apart from lower engraftment, HLA-matched UCBT could be a preferred graft source in this population; on the other hand, HLA-mismatched UCBTs neither had a strong survival advantage nor disadvantage compared with BM allografts. A second pediatric study retrospectively compared UCBT (92 % HLA-mismatched) with HLA-matched unrelated (MUD) allo-HSCT and again showed that HLA-mismatched UCBT was associated with higher risk of NRM (38 %) and lower rate of engraftment, despite conferring lower aGVHD and cGVHD rates and similar relapse rate to MUD allo-HSCT [23]. A third retrospective study in a predominantly pediatric population confirmed these results, demonstrating that HLA-mismatching of UCB units continued to remain a risk factor for NRM [24].

A few studies have examined outcomes for adult patients receiving RIC UCBT compared with MUD PBSC allo-HSCT. One study examined the outcomes of patients with a heterogeneous group of hematologic malignancies undergoing RIC allo-HSCT using either PBSCs ($n = 52$) or double unit UCB ($n = 39$) [25]. Engraftment was lower after UCBT but the difference was not significant. Although NRM was significantly higher in patients receiving UCBT (27 % versus 6 % at 2 years), overall survival (OS) was similar due in part to a lower relapse rate in patients receiving UCBT (23 % versus 36 % at 2 years). A second study examined 645 European patients with lymphomas undergoing alternative donor allo-HSCT (104 UCB and 541 MUD PBSC) [26]. Two-thirds of UCBTs involved a two-antigen mismatched UCB unit. Engraftment was significantly lower after UCBT (81 % versus 97 %). aGVHD rates were similar but cGVHD was less frequently seen after UCBT (26 % versus 52 %). There were no significant differences seen in NRM, relapse, or OS.

Another study explored outcomes after myeloablative conditioning for HLA-mismatched double unit UCBT compared with HLA-matched or one-antigen-mismatched related or unrelated allo-HSCT [27]. Again, engraftment was much lower after UCBT as were rates of grade II–IV aGVHD and cGVHD. However, similar to previous studies, NRM was higher but relapse was lower, leading to an overall similar leukemia-free survival between the groups. Overall, the findings of these several retrospectively comparative studies support that HLA-mismatched UCBT has similar OS outcomes to that seen using MUD allo-HSCT and potentially HLA-matched related allo-HSCT.

3 HLA-Haploidentical Transplantation

3.1 Approaches to HLA-Haploidentical Transplantation

Early attempts at haplo-HSCT after myeloablative conditioning were met with unacceptably high risks of graft failure, GVHD, and NRM, resulting from intense

bidirectional alloreactivity [2–4, 28, 29]. However, recent advances have led to several viable approaches to controlling this alloreactivity and facilitating haplo-HSCT: (1) T cell depletion (TCD), (2) aggressive pharmacologic immunomodulation, and (3) posttransplantation cyclophosphamide.

The TCD approach, developing out of work done by Reisner and colleagues [30–34], was directed toward removing the main culprit cell type responsible for GVHD and graft failure. Although TCD reduced GVHD, graft failure remained a problem in early clinical studies of TCD haplo-HSCT, and it was unclear whether this was due to host-versus-graft alloreactivity or to limited niche space in the BM for donor hematopoietic stem cells. Therefore, the Perugia group adopted a two-pronged strategy of augmented conditioning followed by allografting with "megadoses" of granulocyte colony stimulating factor (G-CSF)-primed peripheral blood progenitors and BM [35–37]. The rate of graft failure was quite low (5–7 %) as were rates of grade III–IV aGVHD and cGVHD (each < 6 %), but unfortunately NRM was quite high at 37–53 % mostly attributable to infections. Current research in this field focuses on trying to decrease complications resulting from impaired immune reconstitution after TCD.

Aggressive pharmacologic immunomodulation has been tried in various formats, but the most tested is the GIAC protocol developed by the Peking group [38, 39]. Built on preclinical data suggesting that G-CSF can have an immunomodulatory effect in preventing GVHD induction [40–43], this strategy pretreated donors with G-CSF followed by intense immunosuppressive treatment of patients with methotrexate, mycophenolate mofetil (MMF), cyclosporine, and anti-thymocyte globulin. Graft failure rarely occurred (< 1 %) with the GIAC protocol. However, aGVHD and cGVHD still occurred at relatively high rates (grade II–IV aGVHD 43–56 % and cGVHD 54–74 %), and NRM was 23–26 %. At 2 years, relapse rates were low (12 % for standard-risk and 39 % for high-risk patients), leading to favorable OS rates of 68 and 42 %, respectively [38]. Interestingly, the degree of HLA mismatching was not associated with outcomes for this approach [44].

The preclinical use of posttransplantation cyclophosphamide (PTCy) to induce immunologic tolerance was developed over a 40-year period largely through mouse studies where it was found to be successful in facilitating skin allografting [45–50]. Building on this work, investigators at Johns Hopkins adapted this approach to facilitating haplo-HSCT in mice after RIC [51, 52] followed by a phase I study showing that this approach of RIC, BM allografting, and GVHD prophylaxis with PTCy, tacrolimus, and MMF could facilitate haplo-HSCT in humans [53]. While graft failure did occur in two of ten patients (20 %) in cohort 2 of this study, surprisingly only one patient developed cGVHD. Based on this study, a phase I–II study enrolled 68 patients at two institutions using the same approach. Graft failure occurred in nine patients (13 %), though, given the low intensity conditioning, all but one experienced rapid autologous reconstitution with median neutrophil recovery occurring at 15 days posttransplant. The cumulative incidences of severe grade III–IV aGVHD and cGVHD were 5 and 7.5 % in patients receiving two doses of PTCy. While the relapse rate in these high-risk patients was 58 %, given a low NRM of 16 %, OS was 36 % at 2 years. Similar to that seen with the GIAC protocol, the use of PTCy obviated any impact of degree of HLA-mismatching on outcomes [54].

3.2 Comparisons of HLA-haploidentical Transplantation Approaches

Similar to that performed for UCBT, a few retrospective studies have been performed to compare the outcomes of the GIAC approach for haplo-HSCT with outcomes seen for HLA-matched sibling allo-HSCT [55, 56]. Engraftment was high and similar between the haplo-HSCT and HLA-matched related allo-HSCT cohorts. Both studies showed higher rates of grade II–IV aGVHD with haplo-HSCT but no difference in cGVHD. However, the two studies differed on the impact of donor type on relapse and OS. One study showed small but statistically significant differences favoring HLA-matched allo-HSCT despite still very favorable outcomes for haplo-HSCT [55]; the other study actually showed that haplo-HSCT patients had better survival due to a lower rate of relapse [56].

There are no prospective data that directly compare these various haplo-HSCT approaches. However, one retrospective study compared outcomes for the TCD and PTCy approaches to haplo-HSCT using the same conditioning regimen [57]. The patients studied had very aggressive disease with 60 % of each cohort having active disease at the time of allo-HSCT. Improved outcomes were seen using the PTCy approach with higher engraftment (94 % versus 81 %), lower NRM (16 % versus 42 %), and better progression-free and OS (50 % versus 21 % and 64 % versus 30 %, respectively) compared with TCD. Moreover, grade III–IV aGVHD and cGVHD rates were lower with PTCy, and relapse rates were similar. Thus, this single retrospective study suggested that PTCy may be preferred over TCD when using haplo-HSCT to treat patients with advanced disease.

4 Optimal Alternative Donor Strategy

4.1 Retrospective Comparison of Umbilical Cord Blood Transplantation (UCBT) and Haplo-HSCT

The European Society for Blood and Marrow Transplantation (EBMT) together with Eurocord has retrospectively examined 1121 adult patients with acute leukemia who underwent either UCBT ($n = 796$) or haplo-HSCT ($n = 325$) in EBMT centers from 2007–2011 (Ruggeri et al. personal communication). Among the UCBT, 363 patients received a single unit UCBT and 433 received a double unit UCBT. Haplo-HSCT utilized BM in 146 patients, PBSCs in 172 patients, and both in 7 patients. Haplo-HSCT grafts in this analysis were all non-TCD but ranged in their pharmacologic approach to haplo-HSCT; only 56 (17 %) utilized PTCy as GVHD prophylaxis. Conditioning was approximately evenly split between myeloablative conditioning and RIC. Compared with haplo-HSCT, UCBT was associated with delayed neutrophil recovery but lower incidence of cGVHD. However, other outcomes including NRM, relapse, leukemia-free survival, and OS were not different between UCBT and haplo-HSCT.

4.2 *Blood and Marrow Transplant Clinical Trials Network Protocols 0603 and 0604*

Unfortunately, there are no prospective comparative studies to assess outcomes for any alternative donor approach. Nevertheless, two parallel phase II studies were conducted by the BMT CTN to study the reproducibility and thus wider applicability of promising alternative donor strategies [58]. Double unit UCBT was assessed in BMT CTN 0604, while PTCy haplo-HSCT was assessed in BMT CTN 0603. The goal of carrying out these prospective studies in parallel was to generate data that might provide further impetus for a larger randomized phase III study.

The study populations for both trials were identical: patients of age 70 years or younger with advanced or high-risk leukemia or lymphoma and lacking an HLA-matched or one antigen-mismatched related donor. Patients with acute leukemia had to be in morphologic complete remission, while aggressive lymphoma patients had to have achieved at least a partial remission before transplant. Patients with low-grade lymphomas had to have failed two prior lines of therapy but did not have to have chemotherapy-sensitive disease. The transplantation platforms are shown in Fig. 17.1. The conditioning was reduced intensity and in both cases involved cyclophosphamide, fludarabine, and low-dose total body irradiation (TBI; 200 cGy), although the doses of cyclophosphamide and fludarabine were slightly lower in the PTCy study. In both studies, postgrafting immunosuppression involved MMF and a calcineurin inhibitor (cyclosporine for UCBT and tacrolimus for PTCy), though of course PTCy also was used for BMT CTN 0603 (haplo-HSCT). Fifty patients were enrolled to each study.

Patient characteristics were relatively similar between the two studies with a few notable differences despite the identical eligibility. Median age was 10 years older in the UCBT study (age 58 versus 48). The UCBT study had more patients with acute myeloid leukemia (AML) and these leukemia patients were more likely to be in first complete remission than leukemia patients on the PTCy study. In contrast, lymphoma patients on the UCBT study were slightly more heavily pretreated than lymphoma patients on the PTCy study although more PTCy patients had had a prior autologous transplantation. Of the two UCB units used for each UCBT, HLA-matching for the least matched unit was 4/6 in 33 cases (66 %), 5/6 in 14 cases (28 %), and 6/6 in 3 cases (6 %). Meanwhile, more than 75 % of haplo-HSCT patients were HLA-mismatched at 4 or 5 HLA loci out of the 10 tested.

Comparative results of these parallel phase II studies are shown in Table 17.1. With the exception of relapse (45 % after PTCy and 31 % after UCBT), all other parameters favored haplo-HSCT with PTCy. Graft failure occurred in 2 % of PTCy-treated patients and 12 % of UCBT patients with the majority of graft failure events being fatal. Neutrophil engraftment at day 56 was similar between the studies (PTCy 96 %, UCBT 94 %), but platelet engraftment was lower after UCBT (PTCy 98 %, UCBT 82 %). Furthermore, the tempo of engraftment for both neutrophils and platelets was delayed after UCBT. Grade II–IV aGVHD rates were similar between the two studies (PTCy 32 %, UCBT 40 %), but both grade III–IV aGVHD (PTCy 0 %, UCBT

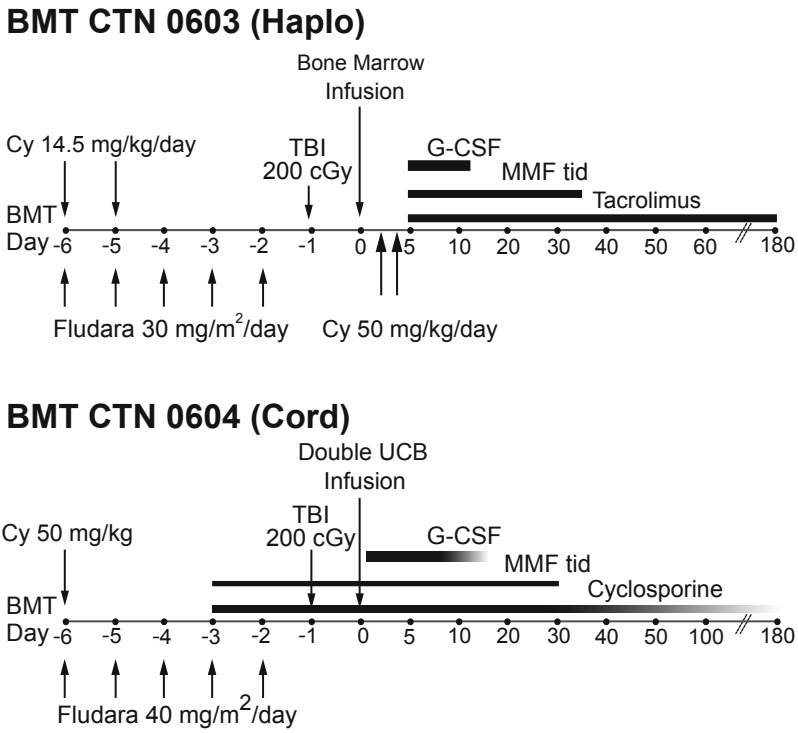

Fig. 17.1 Transplantation schema for BMT CTN Protocols 0603 and 0604. This research was originally published in Blood [58]. © the American Society of Hematology. *BMT* blood or marrow transplantation, *CTN* clinical trials network, *Cy* cyclophosphamide, *Fludara* fludarabine, *G-CSF* granulocyte colony stimulating factor, *MMF* mycophenolate mofetil, *TBI* total body irradiation, *UCB* umbilical cord blood

21 %) and cGVHD (PTCy 13 %, UCBT 25 %) were higher after UCBT. Four patients treated with UCBT died of GVHD, while none did who received haplo-HSCT. Grade 3–4 toxicities occurred in 30 % of PTCy patients but 56 % of UCBT patients. NRM was 7 % for PTCy and 24 % for UCBT. OS at 6 months, the primary endpoint of each study, was 84 % after PTCy haplo-HSCT and 74 % after UCBT. OS at 1 year was 62 % after PTCy and 54 % after UCBT.

However, a direct comparison was not the intent of these studies. Rather, as above, the approach was intended to reproduce in a multi-institutional cooperative group setting the results seen in single-institutional studies. Therefore, these studies confirmed that the outcomes of each approach were similar enough to that reported using MUD allo-HSCT to warrant direct comparison of PTCy haplo-HSCT and UCBT in a randomized phase III study of alternative donor strategies for allo-HSCT.

Table 17.1 Outcomes of parallel phase II BMT CTN studies 0603 and 0604

	UCBT	PTCy haplo-HSCT
Median follow-up (in days)	365	357
Neutrophil engraftment day + 56	94 %	96 %
Platelet engraftment day + 56	82 %	98 %
Graft failure Primary Secondary	12 % 10 % 2 %	2 % 2 % 0 %
Grade 3–4 toxicities up to day + 180	56 %	30 %
Grade II–IV Acute GVHD at 100 days	40 %	32 %
Grade III–IV Acute GVHD at 100 days	21 %	0 %
Chronic GVHD at 1 year	25 %	13 %
Nonrelapse mortality at 1 year	24 %	7 %
Relapse/progression at 1 year	31 %	45 %
Progression-free survival at 1 year	46 %	48 %
Overall survival at 6 months	74 %	84 %
Overall survival at 1 year	54 %	62 %

BMT blood or marrow transplantation, *CTN* clinical trials network, *UCBT* umbilical cord blood transplantation, *PTCy* post-transplantation cyclophosphamide, *haplo-HSCT* HLA-haploidentical allogeneic hematopoietic stem cell transplantation, *GVHD* graft-versus-host disease.

4.3 The Randomized Phase III Study (BMT CTN 1101)

This randomized phase III study is now open and accruing patients. The target sample size is 410 patients with a target accrual period of 4 years. Eligibility includes adult patients, 18–70-years-old, with acute leukemia in remission or chemotherapy-sensitive lymphoma and the presence of both two partially HLA-matched (4–6 out of 6 HLA matched) UCB units (minimum per unit precryopreserved total nucleated cell counts of 1.5×10^7/kg for red blood cell depleted units or 2.0×10^7/kg for non-red blood cell depleted units) and a partially HLA-mismatched (4–6 out of 8 HLA-matched) related donor. The transplantation platforms are identical to those used in the parallel phase II studies. The primary outcome is progression-free survival at 2 years posttransplantation.

BMT CTN 1101 is the first trial that randomizes patients to either of two different alternative donor graft sources, and thus this study could help define the standard of care in alternative donor allo-HSCT. However, the results may reveal that progression-free survival, the primary endpoint, is equivalent between the two approaches. If so, then other factors may be used in determining the optimal approach, such as toxicity profiles, cost, ease of administration, and global applicability. Moreover, subgroup analyses may suggest that one approach versus the other may be preferable for certain patient populations and thus warrant follow-up confirmatory studies. Ultimately, upon establishment of the optimal approach, if one exists, we

anticipate the continued refinement of that strategy towards improved patient outcomes, whether through preventing or better treating relapse after haplo-HSCT or by improving immune reconstitution and rates of GVHD and NRM after UCBT [59, 60].

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Chapter 18
Comparison of Umbilical Cord Blood to Adult Related and Unrelated Donors

Areej El-Jawahri and Yi-Bin Chen

1 Introduction

The use of umbilical cord blood (UCB) as a donor source offers several hypothetical advantages over hematopoietic stem cells derived from, peripheral blood or bone marrow from related or unrelated donors. Due to the immunologic immaturity of UCB, a higher degree of human leukocyte antigen (HLA) mismatch is tolerated without a significant increase in the risk of graft-versus-host disease (GVHD) [1, 2]. Additionally, the relative ease of obtaining previously collected and stored UCB can expedite the transplantation process, which can often be delayed by the time required for donor search, evaluation, stem cell mobilization, and collection. On the other hand, UCB units have significantly lower numbers of hematopoietic progenitor cells compared to conventional adult donor sources, leading to delayed engraftment and immune reconstitution [2]. Several studies have shown that higher total nucleated cells/kg is associated with improved survival after UCB hematopoietic stem cell transplantation (HSCT) [3, 4]. Since the average nucleated cell dose of a cord blood unit is approximately 1×10^9, most single UCB units may be acceptable only for children and small adults further limiting their utilization [5].

In order to gain a better understanding of the utility of UCB transplantation, it is important to compare its efficacy, safety, and overall outcomes relative to related and unrelated donor allogeneic HSCT. To date, there have been no prospective studies evaluating the impact of different donor stem cell sources on outcomes in patients undergoing allogeneic HSCT [2, 6]. This is not surprising given the inherent differences in donor options between individual patients. However, multiple retrospective studies have been reported comparing outcomes of UCB, matched related donor (MRD), and unrelated donor (URD) allogeneic HSCT in the context of both myeloablative and reduced intensity/nonmyeloablative conditioning [1, 2].

In this chapter, we will review the available data on the impact of donor source (UCB vs. MRD vs. URD) on outcomes after allogeneic HSCT including engraftment,

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graft failure, nonrelapse mortality (NRM), acute and chronic GVHD, disease relapse, disease free survival (DFS), and overall survival (OS) in adult patients. We will then summarize general trends observed with the use of UCB transplantation compared to related and unrelated donor allogeneic HSCT. Finally, we will discuss future directions in terms of general strategies being used to optimize the use of UCB transplantation with the hopes of improving its utility as a donor stem cell source.

2 Myeloablative Conditioning

2.1 Comparison of UCB to Related Donors

Table 18.1 depicts the major retrospective analyses to date comparing UCB vs. related donor adult myeloablative allogeneic HSCT. In this setting, UCB transplantations are generally associated with longer times to neutrophil and platelet engraftment, higher rates of graft failure and NRM, but lower rates of chronic GVHD compared to related donor HSCT. DFS and OS appear to be similar between the two donor strategies.

In a single-center retrospective study conducted in Japan, Takahashi and colleagues compared the outcomes of 171 adults with hematologic malignancies undergoing single UCB transplantation vs. related donor HSCT [7]. The median time to neutrophil and platelet engraftment was longer in patients receiving UCB vs. MRD HSCT (neutrophils 23 vs. 17 days, $p < 0.01$); platelets 56 vs. 25 days, $p < 0.01$). Furthermore, there was a higher rate of graft failure (5.2 % vs. 0 %) with the use of UCB HSCT although this was not statistically significant. The incidence of severe acute GVHD and extensive chronic GVHD were lower in patients undergoing UCB HSCT (Table 18.1). One hundred-day NRM and 3-year DFS was similar between the two groups [7].

In a joint retrospective analysis conducted by the Fred Hutchinson Cancer Research Center (FHCRC) with the University of Minnesota between 2001 and 2008, investigators compared clinical outcomes of patients undergoing double UCB, MRD, and URD (matched, MUD and mismatched, MMUD) HSCT after receiving myeloablative conditioning [8]. Once again, double UCB recipients had a statistically significant longer time to neutrophil and platelet engraftment compared to those receiving MRD HSCT, and lower rates of chronic GVHD at 2 years (26 % vs. 47 %, $p = 0.03$; Table 18.1). However, the 5-year NRM was higher in patients undergoing double UCB vs. MRD SCT (34 % vs. 24 %, $p < 0.01$). Interestingly, the cumulative incidence of relapse at 5 years was significantly lower in recipients of double UCB (15 % vs. 43 %, $p < 0.01$), resulting in similar 5 year DFS between the two donor sources [8].

Other studies have also repeatedly shown higher rates of NRM in patients undergoing myeloablative UCB transplantation compared to MRD HSCT [9–11]. In a recent study of patients with Philadelphia chromosome negative acute lymphoblastic leukemia, the 3-year NRM for UCB HSCT recipients was 27 % vs. 13 % for MRD

Table 18.1 Comparison of myeloablative matched-related donor SCT vs. umbilical cord SCT

Study	Arms myeloablative	Neutrophil engraftment	Platelet engraftment	Graft failure	Acute GVHD	Chronic GVHD	NRM	DFS	OS
Brunstein, Blood 2010 $n = 204$ MRD, $n = 152$ MUD, $n = 52$ MMUD, and $n = 128$ dUCB	Time MRD dUCB p-value	45-d 16 % 26 % $p < 0.01$	100-d 20 % 53 % $p < 0.01$		100-d III-IV 13 % 22 % $p = 0.57$	2-year 47 % 26 % $p = 0.03$	5-year 24 % 34 % $p < 0.01$	5 year 33 % 51 % $p = 0.59$	
Kumar, BBMT 2008 Patients with ALL: $n = 90$ MRD, $n = 15$ MUD, $n = 14$ MMUD, $n = 19$ UCB	Time MRD UCB p-value				1 year III–IV 20 % 32 % $p = 0.64$	1-year 22 % 16 % $p = 0.01$	3 year 26 % 5 % $p = 0.09$	3 year 26 % 61 % $p < 0.01$	3-year 27 % 66 % $p < 0.01$
Majhail, BBMT 2009 $n = 67$ myeloablative MRD/$n = 54$ non-myeloablative MRD; $n = 63$ myeloablative UCB/$n = 110$ non-myeloablative UCB	Time MRD UCB p-value	Median 17 days 23 days $p < 0.01$	Median 25 days 56 days $p < 0.01$	3 % 19 % $p < 0.01$	100d III–IV 15 % 24 % $p = 0.45$		100-d 21 % 29 % $p = 0.55$		100-d 81 % 70 % $p = 0.95$
Nishiwaki, Annals Oncol 2013 Philadelphia negative ALL patients: $n = 684$ MRD, $n = 809$ MUD, $n = 233$ UCB	Time MRD UCB p-value	100-d 98 % 72 % $p < 0.0001$	1-year 95 % 81 % $p < 0.0001$		100-d III–IV 8 % 11 % NS	3-year 34 % 31 % $p = 0.52$	3-year 13 % 27 % $p = 0.0001$		4-year 65 57 $p = 0.11$

Table 18.1 (continued)

Study	Arms myeloablative	Neutrophil engraftment	Platelet engraftment	Graft failure	Acute GVHD	Chronic GVHD	NRM	DFS	OS
Ponce BBMT 2011 $n = 108$ MRD (89 ablative), $n = 184$ MUD (156 ablative), $n = 75$ dUCB (53 ablative)	Time MRD dUCB *p*-value	Median 11 days 24 days $p < 0.001$	Median 17 days 51 days $p < 0.001$		100-d I–IV 27 (12–42) 43 (31–54) $p = 0.326$	1-year 31 % 28 % $p = 0.04$	180-d 8 % 21 % $p = 0.01$	2-year 66 % 55 % $p = 0.573$	2-year 70 % 65 % $p = 0.602$
Takahashi, Blood 2007 $n = 100$ UCB, $n = 71$ MRD 24 % MRD with mismatches vs. 40 % UCB	Time MRD UCB *p*-value	Median 17 days 22 days $p < 0.01$	Median 22.5 days 40 days $p < 0.01$	0 % 5.2 % NS	100-d III–IV 13 % 6 % $p = 0.04$	3-yr Exten 30 % 23 % $p = 0.01$	100-d 8 % 9 % $p = 0.13$	3-year 60 % 70 % $p = 0.26$	
Zheng, BBMT 2013 CML in advanced stage: $n = 16$ MRD, $n = 16$ UCB	Time MRD UCB *p*-value	Median 12.5 days 22.1 days $p < 0.001$	Median 16.3 days 43.7 days $p < 0.001$	0 % 0 % NS	100-d III–IV 13.8 % 26.3 % $p = 0.15$	2-yr Exten 0 % 6.3 % $p = 0.09$	180-d 12.5 % 37.5 % $p = 0.01$	5-year 40.5 % 50 % $p = 0.12$	5-year 48.6 % 62.5 % $p = 0.10$

recipients ($p = 0.0001$) [10]. Similarly, Ponce and colleagues reported a higher rate of 180-day NRM in recipients of UCB vs. MRD HSCT (21 % vs. 8 %, $p = 0.01$) in patients with various hematologic malignancies [11]. The higher rates of nonrelapse deaths occurred in most of these studies early within the first 100 days with most deaths attributed to graft failure, infection, and hemorrhage. However, only one of these studies showed a statistically significant difference in graft failure rates between UCB and MRD recipients (19 % UCB vs. 3 % MRD, $p < 0.01$) [12], although the sample size needed to detect statistical significance is a potential limitation in the other studies.

The observed lower relapse rate in the FHCRC/ University of Minnesota series is compelling, and one potential explanation is the higher degree of HLA-mismatch in the setting of double UCB transplantation, potentially leading to more effective graft-versus-malignancy [8]. In a small retrospective study of patients with advanced chronic myelogenous leukemia (CML), with the majority (62.5 %) receiving single UCB transplantation, lower relapse rates were also noted for UCB vs. MRD recipients [9]. Others have also shown a nonstatistically significant trend towards lower relapse rate with the use of UCB transplantation [7]. Taken together, these findings are suggestive of potentially stronger graft-versus-malignancy effect with the use of UCB vs. MRD HSCT, especially with the use of double UCB, although the precise mechanism remains unclear, especially with the significantly lower rates of chronic GVHD observed with UCB HSCT.

In terms of GVHD, one consistent finding across all studies is a lower cumulative incidence of chronic GVHD in recipients of UCB compared to MRD transplantation (Table 18.1). In contrast, only the Japanese study showed a lower cumulative incidence of significant acute GVHD [7]. In fact, other studies have shown a pattern towards higher rates of acute GVHD in patients receiving UCB transplantation compared to MRD HSCT [8, 9, 11–13]. The favorable results seen in the Japanese study could be attributed to the homogeneity, and thus limited haplotypes, of the Japanese population leading to relatively lower alloreactivity [14–16]. Particularly, lower polymorphism in non-HLA immune mediators and host defense genes could affect the severity of acute GVHD [16].

2.2 Comparison of UCB to Unrelated Donors

Multiple single-center and registry-based retrospective studies have compared outcomes of patients undergoing myeloablative UCB vs. MUD or MMUD transplantation as illustrated in Table 18.2. Generally, UCB transplant recipients have longer time to neutrophil and platelet engraftment, higher risk of graft failure and NRM, with lower cumulative incidence of acute and chronic GVHD. Studies have reported conflicting results when it comes to DFS and OS.

In a registry-based study reported by the Eurocord and the European Blood and Marrow Transplant Group (EBMT), outcomes were compared between 98 patients receiving single UCB and 584 patients receiving unrelated myeloablative bone marrow transplants for acute leukemia [17]. All bone marrow transplants were HLA

Table 18.2 Comparison of myeloablative unrelated donor SCT vs umbilical cord SCT

Study	Arms myeloablative	Neutrophil engraftment	Platelet engraftment	Graft failure	Acute GVHD	Chronic GVHD	NRM	DFS	OS
Atsuta, Blood 2009	Time	100-d	4-months		100-d II–IV	1yr Exten	2-year	2-year	2-year
patients with acute leukemia	MUD AML	94 %	85 %		35 %	20 %	22 %	54 %	59 %
AML MUD $n = 311$ UCB $n = 173$	UCB AML	77 %	59 %		32 %	8 %	33 %	42 %	48 %
	p-value	$p < 0.001$	$p < 0.001$		$p = 0.23$	$p = 0.004$	$p = 0.08$	$p = 0.004$	$p = 0.01$
	MUD ALL	97 %	83 %		42 %	17 %	25 %	53 %	53 %
ALL MUD $n = 222$ UCB $n = 114$	UCB ALL	80 %	61 %		28 %	10 %	24 %	53 %	52 %
	p-value	$p < 0.001$	$p < 0.001$		$p = 0.02$	$p = 0.14$	$p = 0.98$	$p = 0.41$	$p = 0.99$
Burnstein, Blood 2010	Time	45-d	100-d		100-d III–IV	2-year	5-year	5-year	
$n = 204$ MRD, $n = 152$ MUD, $n = 52$ MMUD, $n = 128$ dUCB	MUD	19 %	21 %		14 %	43 %	14 %	48 %	
	MMUD	18.5 %	21 %		37 %	48 %	27 %	38 %	
	dUCB	26 %	53 %		22 %	26 %	34 %	51 %	
	p-value	$p < 0.01$	$p < 0.01$		$p = 0.57$	$p = 0.03$	$p < 0.01$	$p = 0.59$	
Eapen, Lancet Oncol 2010	Time	42-d			100-d II–IV	2-year	2-year	2-year	
Patients with acute leukemia: MUD BM $n = 632$, MUD PBSC $n = 332$, MMUD BM $n = 256$, MMUD PBSC $n = 140$, UCB $n = 165$	MUD	96 %			45 %	48 %	23 %	57 %	
	MMUD	96 %			50 %	41 %	35 %	63 %	
	UCB	80 %			30 %	24 %	33 %	59 %	
	p-value	$p < 0.001$			$p = 0.001$	$p < 0.0001$	$p < 0.0001$	$p = 0.09$	
Kumar, BBMT 2008	Time				1-year III–IV	1-yr Exten	3-year	3-year	3-year
$n = 90$ MRD, $n = 15$ MUD, $n = 14$ MMUD, $n = 19$ UCB	MUD				10 %	47 %	20 %	13 %	13 %
	MMUD				7 %	21 %	0 %	14 %	14 %
	CBT				32 %	16 %	5 %	61 %	66 %
	p-value				$p = 0.64$	$p = 0.01$	$p = 0.09$	$p < 0.01$	$p < 0.01$

Table 18.2 (continued)

Study	Arms myeloablative	Neutrophil engraftment	Platelet engraftment	Graft failure	Acute GVHD	Chronic GVHD	NRM	DFS	OS
Laughlin, NEJM 2004 adults with leukemia MUD $n=367$, MMUD $n=83$, UCB $n=150$ Extensive chronic GVHD lower in CBT Compared to MUD and MMUD	Time MUD MMUD UCB p-value	Median 18 days 20 days 27 days $p<0.001$	Median 29 days 29 days 60 days $p<0.001$		100-d II–IV 48 % 52 % 41 % $p=0.17$	2-year 35 % 40 % 51 % $p=0.02$	3-year 46 % 65 % 63 % $p<0.001$	3-year 33 % 19 % 23 % $p=0.001$	
Marks, Haematologica 2013 Patients with ALL in CR1 or CR2 $n=546$ MUD, $n=140$ MMUD, $n=116$ UCB or dUCB Less extensive chronic GVHD with UCB	Time MUD MMUD UCB p-value	28-day 95 % 96 % 57 % $p<0.001$	100-day 86 % 82 % 56 % $p<0.001$	 3 % 2 % 8 % $p=0.01$	100-d III–IV 16 % 24 % 9 % $p<0.001$	3-year 42 % 45 % 39 % $p=0.23$	3-year 31 % 39 % 42 % $p=0.56$		3-year 44 % 43 % 44 % $p=0.98$
Nishiwaki, Annals Oncol 2013 Philadelphia negative ALL patients: $n=684$ MRD, $n=809$ MUD, $n=233$ UCB	Time MUD UCB p-value	100-day 98 % 72 % $p<0.0001$	1-year 91 % 81 % $p<0.0001$		100d III–IV 18 % 11 % $p=0.008$	3-yr Exten 38 % 31 % $p=0.52$	3-year 23 % 27 % NS		4-year 64 % 57 % $p=0.11$
Ponce BBMT 2011 $n=108$ MRD (89 ablative), $n=184$ MUD (156 ablative), $n=75$ dUCB (53 ablative)	Time MUD dUCB p-value	Median 11 days 24 days $p<0.001$	Median 18 days 51 days $p<0.001$		100d I–IV 39 % 43 % $p=0.326$	1 yr Exten 44 % 28 % $p=0.01$	180-d 13 % 21 % $p=0.123$	2-year 55 % 55 % $p=0.573$	2-year 62 % 65 % $p=0.602$

Table 18.2 (continued)

Study	Arms myeloablative	Neutrophil engraftment	Platelet engraftment	Graft failure	Acute GVHD	Chronic GVHD	NRM	DFS	OS
Rocha NEJM 2004	Time	Median			100-d II–IV	2-year	2-year	2-year	2-year
Adult with leukemia	MUD	19 days		7 %	39 %	46 %	38 %	38 %	42 %
UCB $n = 98$ MUD BM $n = 584$	UCB	26 days		20 %	26 %	30 %	44 %	33 %	36 %
	p-value	$p < 0.001$		NS	$p = 0.008$	$p = 0.07$	$p = 0.13$	$p = 0.06$	$p = 0.08$
Takahashi, Blood 2004	Time	Median	Median		100d III–IV	1-yr Exten	1-year	2-year	
$n = 45$ MUD, $n = 68$	MUD	18 days	25 days	0 %	12 %	14 %	29 %	44 %	
UCB 87% MUD full match 1 mismatch cord 2–3 mismatches	UCB	22 days	40 days	8 %	4 %	13 %	9 %	74 %	
	p-value	$p < 0.01$	$p < 0.01$	NS	$p < 0.01$	$p = 0.18$	$p = 0.02$	$p < 0.01$	

matched, whereas 94 % of cord blood grafts were mismatched ($p < 0.001$). Cord blood transplant recipients had delayed neutrophil recovery (26 days vs. 19 days, $p < 0.001$) and lower incidence of acute GVHD (26 % vs. 39 %, $p = 0.008$), but other outcomes including relapse, DFS, OS, and incidence of chronic GVHD were similar to recipients of MUD bone marrow transplantation (Table 18.2) [17].

In contrast, data from the International Bone Marrow Transplant Registry and the National Cord Program Blood Center suggest superior outcomes with the use of MUD bone marrow HSCT compared to UCB and MMUD transplantation [18]. Laughlin and colleagues compared outcomes of adults with leukemia undergoing MUD, MMUD, and single UCB transplantations. Similar to prior studies, there was a delay in median time to neutrophil and platelet engraftment with the use of UCB (neutrophil engraftment: MUD 18 days, MMUD 20 days, UCB 27 days, $p < 0.001$; platelet engraftment: MUD 29 days, MMUD 29 days, UCB 60 days, $p < 0.001$). Higher rates of chronic GVHD and NRM were seen in patients receiving UCB or MMUD stem cell transplant (SCT) compared to MUD transplant recipients (Table 18.2). Interestingly, UCB recipients had a lower incidence of extensive chronic GVHD compared to MUD and MMUD recipients [18]. The 3-year DFS was superior in patients receiving MUD HSCT compared to UCB or MMUD transplantation (MUD 33 % vs. MMUD 19 % vs. UCB 23 %, $p = 0.001$). The rates of relapse and acute GVHD were similar between all groups [18].

The discrepancies in findings between these studies may be explained by differences in patient populations and methodological issues. Laughlin and colleagues included patients with myelodysplastic syndrome, chronic myeloid leukemia, and acute leukemia, while the European study focused on patients with acute leukemia. Additionally, Laughlin and colleagues included earlier transplantations prior to 1998, when there was much less experience with UCB transplantation, including knowledge regarding the relevance of cell dose and HLA matching for UCB HSCT, and lack of modern antifungal and antiviral therapies. These issues may explain the worse outcomes with UCB transplantations in that study compared to the study by Rocha and colleagues, which limited the analyses to transplantations after 1998 [17, 18]. Lastly, the degree of HLA disparity in UCB grafts was different between the two studies with over 77 % of grafts having more than one HLA antigen mismatch in the Laughlin study compared to only 43 % in the European study.

The Japanese group have also reported more favorable outcomes with the use of UCB transplantation compared to unrelated donor strategies [19]. Takahashi and colleagues reported slower hematopoietic recovery, lower NRM, and lower incidence of acute GVHD in patients undergoing UCB compared to MUD SCT (Table 18.2) [19]. In this study, the 2-year DFS in patients undergoing UCB was 74 % compared to only 44 % in MUD recipients ($p < 0.01$) [19].

More recent studies have confirmed lower cumulative incidences of acute GVHD [10, 20–22] and chronic GVHD [8, 11, 13, 22] in patients undergoing UCB compared to MUD and MMUD patients. Furthermore, recent studies continue to show higher rates of NRM in UCB recipients compared to unrelated donors. [8, 13, 21, 22] Overall similar rates of relapse, DFS, and OS are observed (Table 18.2).

3 Reduced Intensity Conditioning

3.1 Comparison of UCB to Related Donors

Reduced intensity and nonmyeloablative conditioning HSCT relies primarily on a graft-versus-malignancy effect in its efficacy against hematologic malignancies [23]. The advent of reduced intensity and nonmyeloablative conditioning has clearly expanded the use of allogeneic HSCT for older patients with hematologic malignancies over the past decade, with the majority of experience using peripheral-blood-derived hematopoietic stem cells [23–26]. Early on, there was concern that the inherently lower stem cell dose in UCB would limit its use with reduced intensity regimens, however, several series have consistently shown reliable engraftment with UCB in this setting. Nevertheless, there is limited comparative experience of alternative donor strategies compared to related donor transplantation in the context of reduced intensity conditioning [2].

There have been a few recent small retrospective studies comparing UCB vs. MRD HSCT in older patients undergoing reduced intensity or nonmyeloablative conditioning for hematologic malignancies (Table 18.3). Majhail and colleagues compared the outcomes of older patients (> 55) with hematologic malignancies undergoing MRD HSCT ($n = 47$) or UCB transplantation ($n = 63$, 88 % double UCB transplantation) [27]. There was a statistically significant higher rate of graft failure seen in UCB recipients (8 % vs. 0 %, $p < 0.01$), but lower rates of chronic GVHD at 1 year (17 % vs. 40 %, $p = 0.02$) [27]. There were no significant differences in the cumulative incidence of acute GVHD, NRM, DFS, or OS between the two groups [27].

Another study in patients older than the age of 50 with acute myeloid leukemia also showed comparable outcomes between UCB- and MRD-reduced intensity HSCT [28]. Similar to the myeloablative studies reported earlier, UCB recipients (88 % double UCB transplantation) had longer time to hematopoietic recovery, but similar rates of acute GVHD (10 % UCB vs. 14 % MRD, $p = 0.59$), chronic GVHD (23 % UCB vs. 40 % MRD, $p = 0.14$), NRM (24 % UCB vs. 18 % MRD, $p = 0.22$), 3-year DFS (33 % UCB vs. 48 % MRD, $p = 0.73$), and 3-year OS (43 % UCB vs. 55 % MRD, $p = 0.26$) [28].

Interestingly, in the reduced intensity setting, only one study has reported a significantly higher rate of NRM with the use of UCB compared to MRD [29]. This study primarily compared outcomes of patients undergoing peripheral blood HSCT (MRD and MUD) with recipients of double UCB transplantation. Although UCB recipients had a longer time to hematopoietic recovery and higher NRM, death due to relapse was lower compared to peripheral blood MRD or MUD HSCT (44 % UCB vs. 80 % peripheral blood, nonsignificant) with a trend towards lower relapse incidence with the use of UCB (23 % UCB vs. 35.5 % peripheral blood) [29].

The limited number of studies with small numbers of patients analyzed in a retrospective fashion makes it difficult to draw firm conclusions. Generally, however, the use of reduced intensity UCB transplantation is associated with a longer time to

Table 18.3 Comparison of RIC/NMA matched related donor SCT vs umbilical cord SCT

Study	Arms RIC/NMA	Neutrophil engraftment	Platelet engraftment	Graft failure	Acute GVHD	Chronic GVHD	NRM	DFS	OS
Le Bourgeois, BBMT 2013 Peripheral blood $n=52$ (30 MRD, 20 MUD, 2 MMUD), $n=29$ dUCB	Time PBSCT dUCB *p*-value	Median 17 days 16 days NS	Median 0 days 38 days $p<0.001$	 0 % 6.9 % NS	100-d I–IV 15 % 8 % NS	2-year 35 % 26 % NS	100-day 0 % 13 % $p=0.02$	2-year 58 % 50.5 % $p=0.43$	2-year 62 % 61 % $p=0.51$
Latour, BBMT 2013 Acute myeloid leukemia, over 50 $n=82$ MRD, $n=35$ MUD, $n=80$ UCB	Time MRD UCB *p*-value	28-day 99 % 85 % $p=0.003$	3-months 94 % 73 % $p<0.001$	 0 % 0 % NS	100-d I–IV 10 % 14 % $p=0.59$	3-year 40 % 23 % $p=0.14$	3-year 18 % 24 % $p=0.22$	3-year 48 % 33 % $p=0.73$	3-year 55 % 43 % $p=0.26$
Majhail, BBMT 2008 $n=47$ MRD, $n=63$ UCB (88 % dUCB)	Time MRD UCB *p*-value			42-d 0 % 5 % $p=0.05$	180-d II–IV 42 % 49 % $p=0.20$	1-year 40 % 17 % $p=0.02$	180-d 23 % 28 % $p=0.36$	3-year 30 % 34 % $p=0.98$	3-year 43 % 34 % $p=0.57$
Majhail, BBMT 2009 $n=67$ MA MRD $n=54$ NMA MRD $n=63$ MA UCB, $n=110$ NMA UCB	Time MRD Cord *p*-value	Median 7 days 13 days $p<0.01$	Median 15 days 47 days $p<0.01$	 0 % 8 % $p<0.01$	100-d III–IV 22 % 17 % $p=0.45$		100-d 20 % 19 % $p=0.55$		100-d 78 % 78 % $p=0.95$
Sawada, BBMT 2013 T- or NK-cell lymphoproliferative diseases $n=17$ BMT (11 MRD, 6 MUD), $n=15$ UCB	Time BMT UCB *p*-value				100-d III–IV 25 % 8.3 % $p=0.25$	 50 % 8 % $p=0.02$		 86.9 % 80.0 % $p=0.51$	 92.9 % 93.3 % $p=0.87$

hematopoietic recovery, higher rates of graft failure, but lower incidence of chronic GVHD compared to MRD HSCT (Table 18.3). Other outcomes including NRM, DFS, and OS appear to be similar between the two groups.

3.2 Comparison of UCB to Unrelated Donors

Table 18.4 depicts a summary of all comparative studies of reduced intensity/nonmyeloablative UCB vs. URD HSCT. Patients undergoing UCB transplantation have a longer time to neutrophil and platelet recovery, higher NRM, but lower incidence of chronic GVHD. All studies report similar DFS and OS outcomes between URD and UCB transplantation in the reduced intensity/nonmyeloablative setting.

In a retrospective registry-based study, Brunstein and colleagues compared the use of double UCB with peripheral blood from URD (MUD or MMUD) donors [30]. Four treatment groups were evaluated in this study: double UCB-TCF ($n = 120$ total body irradiation 200 cGy + cyclophosphamide + fludarabine), double UCB-other reduced intensity regimens ($n = 40$), MUD peripheral blood HSCT ($n = 313$), and MMUD peripheral blood HSCT ($n = 111$). Similar to previous studies, longer time to neutrophil and platelet recovery and higher rates of graft failure were seen with UCB transplantation [30]. The double UCB-TCF group had a lower 2 year cumulative incidence of chronic GVHD compared to MUD HSCT (Table 18.4). TRM, DFS, and OS were similar in the double UCB-TCF group compared to MUD HSCT [30]. In fact, NRM in recipients of double UCB-TCF was lower than recipients of MMUD (19 % UCB-TCF vs. 28 % MMUD, $P = 0.04$) [30]. In recipients of UCB with other reduced intensity regimens, higher NRM, lower OS and DFS were observed compared to the other groups suggesting that the conditioning regimen or center experience may play an important role in terms of clinical outcomes.

In a retrospective analysis performed at the Dana-Farber Cancer Institute and Massachusetts General Hospital between 2004 and 2008, investigators reported on outcomes of patients with a variety of hematological malignancies comparing reduced intensity conditioning (RIC) double UCBT ($n = 64$) to RIC MUD SCT ($n = 221$). The 2-year NRM was significantly higher in the UCB group (26.9 %) compared to the MUD group (10.4 %), but the 2-year cumulative incidence of chronic GVHD was significantly lower at 21.9 % in the UCB group vs. 53.9 % in the MUD group ($p < 0.0001$). Despite the lower incidence of chronic GVHD, there was a trend towards lower incidence of relapse in patients after UCBT resulting in similar DFS and OS between the two groups [31]. While only one other analysis has reported a significantly higher NRM with the use of UCB transplantation compared to MUD in the context of reduced intensity [29, 31], all studies, as seen in Table 18.4, reported a consistent trend towards higher NRM in the UCB group. Similarly, three of these studies have also confirmed lower rates of chronic GVHD in UCB recipients compared to MUD recipients [29, 31, 32].

Table 18.4 Comparison of RIC/NMA matched unrelated donor vs umbilical cord SCT

Study	Arms RIC/NMA	Neutrophil engraftment	Platelet engraftment	Graft failure	Acute GVHD	Chronic GVHD	NRM	DFS	OS
Brunstein, Blood 2012	Time	28-day	6-month		100-d I–IV	2-year	2-year	2-year	2-year
Acute leukemia	MUD	93 %	90 %	3 %	14 %	56 %	21 %	35 %	44 %
$n = 120$ dUCB TCF, $n = 40$ uUCB other conditioning, $n = 313$MUD, $n = 111$	MMUD	92 %	89 %	3 %	23 %	54 %	28 %	29 %	37 %
	dUCB T	83 %	66 %	9 %	17 %	34 %	19 %	31 %	37 %
	dUCB O	83 %	58 %	10 %	18 %	36 %	52 %	15 %	19 %
	p-value	$p < 0.0001$	$p < 0.0001$	$p < 0.001$	NS	dUCB T vs. MUD $p < 0.001$	dUCB T vs. MMUD $p = -0.04$	dUCB O vs. MUD $p = 0.04$	dUCB O vs. MUD $p = 0.004$
Chen, BBMT 2012	Time	Median	Median		100-d II–IV	2-year	2-year	3-year	3-year
$n = 221$ MUD, $n = 64$ dUCB	MUD	13 days	19 days		20.3 %	53.9 %	10.4 %	40 %	50 %
	UCB	21.5 days	41 days		14.1 %	21.9 %	26.9 %	30 %	46 %
	p-value	$p < 0.001$	$p < 0.0001$		$p = 0.32$	$p < 0.0001$	$p = 0.0009$	$p = 0.47$	$p = 0.49$
Latour, BBMT 2013	Time	28-day	3-months		100d III–IV	3-year	3-year	3-year	3-year
Acute leukemia, over 50	MUD	88 %	86 %	0 %	15 %	41 %	18 %	57 %	45 %
$n = 82$ MRD, $n = 35$ MUD, $n = 80$ UCB	UCB	85 %	73 %	0 %	14 %	23 %	24 %	33 %	43 %
	p-value	$p = 0.003$	$p < 0.001$		$p = 0.59$	$p = 0.14$	$p = 0.22$	$p = 0.73$ (adjusted)	$p = 0.26$
Le Bourgeois, BBMT 2013	Time	median	Median		100-d I–IV	2-year	100-day	2-year	2-year
$n = 52$ (30 MRD, 20 MUD, 2 MMUD)	PBSCT	17 days	0 days	0 %	15 %	35 %	0 %	58 %	62 %
Peripheral blood, $n = 29$ dUCB	dUCB	16 days	38 days	6.9 %	8 %	26 %	13 %	50.5 %	61 %
	p-value	NS	$p < 0.001$	NS	NS	NS	$p = 0.02$	$p = 0.43$	$p = 0.51$
Rodrigues, Haematologica 2013	Time	60-day	60-day		100-d I–IV	3-year	3-year	3-year	3-year

Table 18.4 (continued)

Study	Arms RIC/NMA	Neutrophil engraftment	Platelet engraftment	Graft failure	Acute GVHD	Chronic GVHD	NRM	DFS	OS
Mature lymphoid malignancies $n = 541$ MUD, $n = 104$ UCB	MUD UCB p-value	97 % 81 % $p < 0.001$	91 % 61 % $p < 0.0001$		32 % 29 %	52 % 26 % $p = 0.0005$	28 % 29 %	36 % 41 % NS	49 % 56 % NS
Sawada, BBMT 2013 T- or NK-cell lymphoproliferative Diseases, $n = 17$ bone marrow (11 MRD, 6 MUD), $n = 15$ UCB	Time BMT UCB p-value				100-d III–IV 25 % 8.3 % $p = 0.25$	1-year 50 % 8 % $p = 0.02$		1-year 86.9 % 80.0 % $p = 0.51$	1-year 92.9 % 93.3 % $p = 0.87$

4 General Trends

General themes emerge in summarizing outcomes of patients undergoing UCB HSCT compared to related and unrelated donor HSCT. UCB transplantation is clearly associated with a longer time to hematopoietic recovery, higher rates of graft failure or rejection, and a general trend towards increased rates of NRM. On the other hand, recipients of UCB experience a lower incidence of chronic GVHD compared to related or unrelated adult donors. Relapse rates with UCB appear to be similar, if not slightly lower compared to related and unrelated donor strategies. Most importantly, long-term DFS and OS are comparable between donor groups. Therefore, UCB can be considered an acceptable source of donor stem cells in both the myeloablative and reduced intensity settings for patients who lack a fully matched adult related or unrelated donor.

Clearly, there are major limitations to drawing conclusions from retrospective comparative analyses, which likely explain some of the discrepancies in the findings between the different studies. Results must be interpreted with caution due to the following factors: heterogeneity in (1) patient selection, (2) disease status at transplant, (3) timing of transplantation, (4) conditioning regimen used, (5) GVHD prophylaxis regimen, (6) use of ATG, (7) single vs. double UCB transplantation, and (8) comparison to peripheral vs. bone marrow graft source. Prospective randomized studies are the ideal way to compare different donor stem cell sources, however, due to logistical issues with HLA-matching requirements, financial constraints, donor availability, and delays in donor evaluation, prospective randomized studies concerning this issue are difficult to conduct.

Comparison of adult UCB transplantation with related and unrelated donors is consistent with the experience of UCB HSCT in the pediatric population. UCB in pediatrics is associated with slower engraftment, lower rates of acute and chronic GVHD, but similar relapse rates, DFS, and OS compared to related and unrelated donors [33–37]. While the experience in the reduced-intensity/nonmyeloablative setting is more limited, similar findings were observed compared to adult patients undergoing myeloablative allogeneic HSCT.

The delayed hematologic and immunological reconstitution seen with UCB transplantation likely leads to increased early NRM and prolonged hospitalization [12]. This is driven in part by the lower stem cell dose and immunological immaturity of the UCB graft compared to adult bone marrow or peripheral blood stem cell sources [38]. Recently, two studies have compared the healthcare costs of UCB HSCT with other graft sources [12, 39]. In one study, the absolute 100 day costs of myeloablative and nonmyeloablative UCB HSCT were higher than myeloablative and nonmyeloablative MRD transplantation [12]. Posttransplant complications, graft failure, and prolonged inpatient hospitalization were the primary causes of increasing costs with UCB HSCT [12]. Strategies to enhance UCB engraftment and improve immune reconstitution are clearly warranted to improve outcomes after UCB HSCT and are current active areas of investigation.

In addition to the increase in early NRM seen with UCB HSCT, the delayed immune reconstitution contributes to a higher degree of morbidity and mortality. In one

study comparing double UCB HSCT to matched related and unrelated donors, there was a higher transplant-related mortality after day 100 in the UCB recipients [40]. The 2-year NRM was 29 % for those receiving UCB compared to 9 % in unrelated donor and 8 % in matched related donors HSCT. Infections were the leading cause of late transplant-related mortality [40]. Other studies have also confirmed higher risk of viral infections and late posttransplant complications in UCB recipients [41, 42].

UCB appears to be associated with a lower incidence of chronic GVHD compared to related and unrelated donors in both the myeloablative and reduced-intensity settings. Findings when it comes to acute GVHD are more mixed although, generally, also favor UCB transplantation especially compared to unrelated donors. The use of single vs. double UCB transplantation may explain some of the differences observed with respect to acute GVHD as the use of double UCB has been shown to have higher rates of acute GVHD [43–45].

The reasons for lower rate of chronic GVHD with UCB transplantation likely can be attributed to reduced alloreactivity due to functional and phenotypic immaturity of UCB lymphocytes [46–49] and/or the reduced T-cell dose infused with UCB [50–53]. Compared to adult bone marrow or peripheral blood stem cells, UCB contains a much more naïve T-cell population that is unexposed to prior antigenic stimulation, and this likely leads to enhanced tolerance and less allogeneic reactivity [47].

Overall, the higher NRM with UCB appears to be balanced by a lower incidence of chronic GVHD, leading to the similar DFS and OS seen with UCB compared to related and unrelated donors. It remains unclear, however, whether relapse rates are significantly lower in patients receiving UCB transplantation compared to related or unrelated donors. Interestingly, the graft-versus-malignancy effect in UCB recipients appears to be preserved despite lower rates of chronic GVHD, which is not what has been observed with conventional adult donor grafts [50]. The graft-versus-malignancy effect after UCB transplantation could be explained by intact UCB natural killer cell function, which has been implicated in mediating graft antitumor effect [54–56]. In animal models, the infusion of donor-derived alloreactive natural killer cells not only provided a graft-versus-malignancy effect, but was also protective against GVHD by targeting recipients' antigen-presenting cells [56]. Given that UCB contains similar levels of natural killer cells compared to adult peripheral blood grafts, this may explain the preserved graft-versus-malignancy effect even with a much lower dose of mature T cells [46, 58, 59]. It remains unknown whether the antitumor activity associated with UCB HSCT has the same physiological mechanisms as graft-versus-malignancy witnessed with conventional donor transplantation.

Some of the most impressive results with UCB HSCT have been reported by the Japanese experience [7, 19]. While some of the advantages with the Japanese population including immunologic homogeneity and smaller recipients' size contribute to improved outcomes after UCB HSCT [2], other factors are also important to consider. Better selection of patients for UCB HSCT, the use of conditioning regimens without ATG, and increased early utilization of UCB as a donor source may also explain the superior clinical outcomes with UCB transplantation observed in Japan [2].

5 Future Directions

The biggest limitations of UCB transplantations are the high rates of NRM due to delayed engraftment and immune reconstitution and higher incidence of graft failure or rejection compared to related and unrelated donors. Future strategies should focus on expediting engraftment and improving immune reconstitution in order to improve outcomes of patients undergoing UCB transplantation. Strategies such as injection of UCB directly into marrow space [60], stem cell expansion techniques [1, 61–64], modification of the stem cell niche [65, 66], or enhancement of stem cell homing [67] are all the subject of ongoing clinical investigations with definitive results eagerly awaited.

Another strategy to improve outcomes after UCB transplantation is to select UCB units of sufficient cell dose and HLA match [1]. Over the past few years, investigators have identified additional factors such as HLA antibodies, matching at the noninherited maternal alleles, and high resolution HLA matching, which may further impact outcomes in patients undergoing UCB transplantation [68–71]. As we gain a better understanding of these factors and the complexity of their interactions and as we build more robust UCB banks, we can better optimize our selection of UCB units, thereby improving outcomes.

While prospective studies are challenging to carry out due to many logistical limitations, retrospective studies comparing the efficacy of various graft sources have significant limitations as noted earlier. Collaborative multi-institutional studies in homogenous patient populations comparing alternative donor strategies are clearly needed.

Due to the higher morbidity and mortality during the first year after UCB HSCT, we suspect that UCB will remain an alternative donor source to be utilized if a matched related or unrelated donor is unavailable in many transplant centers across the world in the immediate future. However, the lower rates of GVHD and the intriguing possibility of a more potent graft-versus-malignancy effect are attractive features of UCB HSCT. With new innovations focused on enhancing cell engraftment and immune reconstitution, we may see a marked reduction in NRM following UCB HSCT over the next decade, thereby favoring UCB as a donor source over matched related or unrelated donors for specific patient populations. Younger patients and those with higher risk of disease relapse may benefit the most from the potentially more potent graft-versus-malignancy effect and the lower rates of morbidity due to chronic GVHD.

6 Conclusions

In this review, we compared clinical outcomes of adult patients undergoing myeloablative or reduced intensity/nonmyeloablative UCB with related and unrelated donor transplantations. Comparative studies in this arena are all retrospective with notable limitations in terms of heterogeneity in patient-, transplant-, and methodology-related

considerations. Despite this heterogeneity, important themes emerged. Compared to related and unrelated donors, UCB HSCT recipients have longer time to neutrophil and platelet engraftment, higher rates of graft failure and NRM, but lower incidences of acute and chronic GVHD. UCB transplants are associated with at least similar, if not lower, relapse rates compared to related and unrelated donors. Taken together, these comparisons have generally yielded similar DFS and OS in patients undergoing UCB transplantation compared to recipients of related and unrelated donor transplants, validating UCB as a viable stem cell source for adult patients lacking well-matched adult donors.

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Chapter 19
Disease Specific Analysis of Cord Blood Transplantation for Adults and Clinical Results of Single and Double Umbilical Cord Blood Transplantation

Vanderson Rocha

1 Introduction

Umbilical cord blood transplantation (UCBT) from human leukocyte antigen (HLA) mismatched unrelated donors have been used in the last two decades. The first decade of UCBT was important in defining critical total nucleated cell dose (TNC) and CD34 + cell dose thresholds required for acceptable clinical outcomes, and in moving from related to unrelated donor UCBT and from pediatric to adult patients. However, the limitations of this approach were also quickly defined, with low cell dose available being the critical barrier. Recipients of UCBT receive on average 10 times less the number of CD34 + cells compared to conventional bone marrow (BM) grafts and almost 20 times less of that received in a mobilized peripheral blood (PB) stem cell (MPBSC) graft, resulting in increased risk of graft failure and early nonrelapse mortality (NRM). This may also be part of the reason for a significant delay in neutrophil and platelet engraftment and immune cell reconstitution. Despite this, the second decade of UCBT was marked by improved outcomes, especially in adults, as the knowledge of TNC dose requirements led to improved collection and to availability of cord blood (CB) units with higher cell doses. Improvements in supportive care also contributed. Initial reports were published showing outcomes for recipients of UCBT comparable with conventional donors [1, 2]. Finally, the use of double UCBT (dUCBT) was pioneered. Importantly, this procedure demonstrated possibility of hematopoietic stem cell transplantation with CB donors for essentially all patients without suitable donors [3]. However, as it will be discussed, dUCBT did not result in faster neutrophil or platelet recovery, or immune cell reconstitution.

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2 Malignant Hematological Diseases

2.1 Adults with Acute Lymphoblastic Leukemia (ALL)

Most of the data published in the literature regarding the use of UCBT in adults with ALL includes patients with acute myeloid leukemia (AML). Single center experience has shown interesting results in UCBT in adult recipients with high-risk ALL, but reports analyze small series of patients. One report [4] described 22 patients who received reduced intensity conditioning regimen (RIC) followed by allogeneic transplantation with either matched related ($n = 4$) or CB ($n = 18$) donor grafts. Overall survival (OS), NRM, and relapse were 50, 27, and 36 % at 3 years, respectively. Another paper [5] published the results on 27 patients who received a single UCBT (sUCBT) after myeloablative conditioning regimen (MAC). With a median follow-up of 47 months, the probability of leukemia-free survival (LFS) at 5 years was 57 %. A more recent study reported outcomes after UCBT for 256 adults with ALL transplanted from 1997–2006 in Japan [6]. The cumulative incidence (CI) of neutrophil engraftment at day 100 was 78 %. Infused CD34 + cell dose ($> 1 \times 10^5$/kg) was associated with successful neutrophil engraftment. CI of grade II–IV acute graft-versus-host disease (aGVHD) at day 100 was 37 %. Two-year LFS and OS rates were respectively 36 and 42 %. Multivariate analysis showed that in elderly patients (older than 51 years), nonremission at the time of the UCBT, presence of grade III–IV aGVHD, and absence of chronic GVHD (cGVHD) were negatively associated with OS.

The Eurocord group has performed a survey and risk factor analysis of the outcomes after UCBT for adults with ALL [L. Tucunduva & V. Rocha for Eurocord, BMT in press]. From 2000 to 2011, 421 adult patients received a UCBT for ALL in European centers. Median age at time of UCBT was 32 years (18–76 years) and 59 % of patients ($n = 247$) were older than 35 years. At time of the transplant 46 % ($n = 195$) were in first complement receptor type 1 (CR1), 32 % ($n = 136$) in CR2, and 22 % ($n = 90$) of patients were not in remission. Of 314 patients with available karyotype at diagnosis, 229 had abnormalities. They were analyzed according to the presence of t(9;22) as Philadelphia positive (Ph +, $n = 129$) and negative (Ph −, $n = 185$). dUCBT was performed in 173 patients (41 %), and the median TNC dose at freezing was 3.9×10^7/kg. Most patients received CB units with one (24 %, $n = 103$) or two (55 %, $n = 231$) HLA disparities. MAC was given to 314 patients (75 %), and 103 patients (25 %) received RIC. Median follow-up was 27 months. The CI of 60-day neutrophil recovery was 78 %. CI of aGVHD and cGVHD was 33 and 26 %, respectively. CI of NRM at 2 years was 42 % and it was lowered in young patients (age < 35 years) in computed radiography (CR) at transplant and using RIC. Two-year relapse incidence (RI) was 28 % and the factors associated with lower RI were the use of MAC and CR at the time of transplant. Estimated 2-year LFS was 39 % for patients in CR1 ($n = 195$), 31 % for CR2 ($n = 136$), and 8 % for advanced disease ($n = 90$). There was no difference in outcomes regarding the presence or absence of the Ph chromosome. Three factors were associated with improved LFS:

age < 35 years, CR at transplant, and not using antithymocyte globulin as part of the conditioning regimen.

Eurocord has also analyzed the impact of minimal residual disease (MRD) on outcomes after UCBT for 98 adults with Ph + ALL [L.Tucunduva & V.Rocha for Eurocord, unpublished data]. Seventy-nine patients were transplanted in first complete CR remission and 19 on 2nd CR for whom MRD was available before transplantation (92 % analyzed by reverse transcription polymerase chain reaction, RT-PCR). Median age was 38 years and median follow-up was 36 months; 63 % of patients received MAC and 42 % double-unit UCBT. Eighty-three patients were treated with at least one tyrosine kinase inhibitor (TKI) before UCBT. MRD was negative (−) in 39 and positive (+) in 59 patients. Three-year CI of relapse was 34 %; it was 45 % in MRD + and 16 % in MRD − patients ($p = 0.013$). CI of 3-year NRM was 31 % and it was increased in patients older than 38 years ($p = 0.02$). LFS at 3 years was 36 %; it was 27 % in MRD + and 49 % in MRD − patients ($p = 0.05$); it was 41 % for CR1 and 14 % for CR2 ($p = 0.008$). In a multivariate analysis, only CR1 was associated with improved LFS. As it has been already described in children, MRD + before UCBT is associated with increased relapse after transplantation. Strategies to decrease relapse in UCBT adult recipients with Ph + ALL with MRD + are needed.

2.2 Adults with Acute Myeloid Leukemia (AML)

Patients with high-risk AML have few chances of cure without an allogeneic hematopoietic stem cell transplantation (HSCT). HSCT can be used in first remission for patients with poor-risk cytogenetics, as a rescue for AML refractory to chemotherapy, and at first relapse or in second and subsequent remission. In spite of various reports and retrospective comparisons with other hematopoietic stem cell (HSC) sources, reports on outcomes and risk-factor analysis on larger series of AML patients given UCBT with longer follow-up and more homogenous populations are still missing. Some single center experiences and registry data have been reported encouraging results [7–10]. In those studies, LFS varied from 40 to 46 % in CR1, from 26 to 39 % in CR2, and from 11 to 20 % in advanced phase of the disease. However, RIC and MAC as well as sUCBT and dUCBT have been analyzed together; these reports have not separately analyzed these conditions.

Eurocord has conducted a survey on outcomes of UCBT for adults with AML. Six hundred and four adult patients with de novo AML in complete remission 1st CR ($n = 229$), 2nd CR or 3rd CR ($n = 228$) and advanced disease ($n = 147$) who received UCBT as first transplant were retrospectively analyzed [V. Rocha for Eurocord, unpublished data]. UCBT was performed from 2000–2010 in 131 European centers. Median age of recipients was 41 years, and 18 % of the patients had previous autologous transplants. Based on available cytogenetic and molecular markers at diagnosis ($n = 339$), 56 % were in intermediate risk and 31 % in unfavorable risk groups. CB grafts were composed of single ($n = 361$) or double ($n = 243$) CB units;

39 % of CB units were identical to recipient or had 5/6 HLA matched (antigen level for HLA-A and B allelic level for DRB1), while 61 % had 4 or even 3/6–3 HLA compatibilities. At infusion median, TNC dose was 3.1×10^7/kg (2.4×10^7/kg with single CB and 3.7×10^7/kg with double CB) and median CD34 + cell count was 1.2×10^5/kg (1×10^5/kg with single and 1.3×10^5/kg with double). MAC was given to 51 % and RIC to 49 % of recipients. The most common regimens used were Busulphan + Fludarabine + Thiotepa (TBF) for MAC, and Cyclophosphamide (CY) + Fludarabine (Flu) + TBI2 Gy for RIC. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine A (CSA) associated or not to mycophenolate mofetil (MMF) in 58 % of patients and CSA ± steroids in 32 %. Median follow-up was 13 months. The CI of neutrophil recovery, aGVHD (II–IV) and 1-year NRM was 80, 26, and 21 %, respectively. The CI of 2-year relapse was 38 % (1st CR 27 %, 2nd CR and 3rd CR 29 %, and non remission 56 %); it was 31 % for those patients transplanted with MAC ($n = 291$) and 30 % with RIC ($n = 282$). For those patients given MAC, 2-year LFS was 50 % for 1st CR, 27 % for 2nd CR and 3rd CR, and 17 % for those patients transplanted in nonremission, whereas it was 35, 44, and 18 % for RIC respectively. In this large series of patients, but still short follow-up time, UCBT appears to be an option for treatment of adults with high-risk AML after MAC or RIC without an HLA identical donor.

2.3 Adults with Myelodysplastic Syndrome (MDS)

Up to now, studies reporting results of UCBT for patients with MDS have been scarce [11–13]. Through the Eurocord and EBMT registries, outcomes and risk factors have been evaluated for adult patients who underwent sUCBT or dUCBT for MDS or secondary AML [14]. One hundred and eight adults with MDS ($n = 39$) or secondary AML ($n = 69$) were analyzed. Median age was 43 (18–72) years. Seventy-seven patients (71 %) received a sUCBT. MAC was given to 57 (53 %) patients. Median numbers of nucleated and CD34 + cells at freezing were respectively 3.6×10^7 and 1.1×10^5 kg. At 60 days, the CI of neutrophil recovery was 78 % and was independently associated with numbers of CD34 + cells/kg ($> 1.1 \times 10^5$/kg) and advanced disease status (blasts < 5 % at time of UCBT). Two-year NRM was significantly higher after MAC (62 vs. 34 %, $p = 0.009$). Two-year disease-free survival (DFS) and OS were 30 and 34 %, respectively. In multivariate analysis, patients with high risk disease (blasts > 5 % and international prognostic scoring system, intermediate-2 or higher in MDS) had significantly poorer DFS. In this study, neutrophil recovery seemed to be inferior to that in other malignant disorders in adults, and a CB unit containing a high number of CD34 + cells or a dUCBT should be recommended. Interestingly, in agreement with data in children [15] DFS of 30 % appears to be comparable to that after HSCT using other sources of donor cells in large multicentric studies in high risk MDS patients. However, no retrospective comparisons with other sources of donor cells have been made in this disease-specific setting.

2.4 Adults with Lymphoid Malignancies

The role of UCBT for patients with lymphomas or chronic lymphocytic leukemia (CLL) seems promising. However, there have only been a few isolated reports for refractory Non Hodgkins Lymphoma (NHL) [16–18] and malignant lymphoma treated by RIC-UCBT [19, 20]. Some years ago, the Eurocord group evaluated 104 adult patients (median age, 41 years) who underwent unrelated donor UCBT for lymphoid malignancies [21]. CB grafts were two antigen HLA mismatched in 68 % and were composed of one ($n = 78$) or two ($n = 26$) CB units. Diagnoses were NHL ($n = 61$), Hodgkins Lymphoma ($n = 29$), and CLL ($n = 14$) with 87 % having advanced disease and 60 % having failed a prior autologous transplant. Sixty-four percent of patients received a RIC regimen and 46 % low-dose total body irradiation (TBI). Median follow-up was 18 months. The CI of neutrophil engraftment was 84 % by day 60 with greater engraftment in recipients of higher CD34 + /kg cell dose. CI of NRM was 28 % at 1 year, with a lower risk in patients treated with low-dose TBI. CI of relapse or progression was 31 % at 1 year with a lower risk in recipients of double-unit UCBT and those with chemosensitive disease. The probability of DFS was 40 % at 1 year with improved survival in those with chemosensitive disease (49 vs. 34 %) who received higher cell doses ($\geq 2 \times 10^7$/kg) (49 vs. 21 %) and conditioning regimens containing low-dose TBI (59 vs. 23 %). Thus, UCBT is a viable treatment for adults with advanced lymphoid malignancies. Chemosensitive disease, use of low-dose TBI, and higher cell doses were factors associated with significantly better outcome. Despite the fact that most patients were transplanted in advanced phase of their disease, relatively low NRM and good survival rates were observed. Especially favorable characteristics were indolent NHL, chemosensitive disease, use of higher cell doses, and use of low-dose TBI. Based on this information, there are several important strategies to be considered for patients with malignant lymphoma and CLL: greater use of less toxic RIC regimens, such as those containing low-dose TBI, better selection of CB units, and a broader use of dUCBT. Report of comparative results with unrelated MPBSC has been performed and results are described below.

3 Results of Single Unrelated Cord Blood Transplantation (CBT) Compared to Other Graft Sources in Specific Diseases

3.1 Adults with Acute Leukemia and Other Hematological Malignancies After Myeloablative Conditioning Regimen

Three retrospective studies comparing results of unrelated CB with unrelated BM HSCT in adults [22, 23, 24] after MAC have been published. Briefly, in those studies, neutrophil and platelet recovery were delayed in CB recipients, incidence of acute or cGVHD were decreased or responsive to steroid treatment, NRM was decreased, similar or increased in the Japanese, European, and American studies, respectively. DFS was superior after unrelated UCBT when compared to unrelated

BM HSCT in the Japanese study, similar in the European study, and decreased in the American study, respectively. A meta-analysis pooling these studies has been published: 316 adults undergoing UCBT (mostly 1 or 2 antigen-mismatched) were compared to 996 adults undergoing BM HSCT (almost entirely fully matched with the recipient). NRM and DFS were not statistically different between the groups, in spite of delayed neutrophil recovery in recipients received CB [25]. Interestingly, the same Japanese team who found that unrelated CB has better outcomes than unrelated BM compared results of HLA genoidentical BM or PB transplants ($n = 71$) with HLA mismatched CB transplants ($n = 100$) for adults with hematological diseases. All patients received myeloablative regimens. Multivariate analysis demonstrated no statistically significant differences in NRM (9 % in CB and 13 % in BM/PB recipients), relapse (17 % in CB and 26 % in BM/PB recipients), and DFS (70 % in CB and 60 % in BM/PB recipients) between groups [26].

However, all the above studies compared outcomes of BM HSCT recipients using donor cells typed in low resolution techniques for HLA-A and -B and high resolution typing for HLA-DRB1. Currently the choice of unrelated donor is based on high resolution typing or allelic typing for HLA Class I and II. Therefore, the Eurocord group in collaboration with CIBMTR compared results of unrelated UCBT in adults with acute leukemia, given a CB graft with more than 2.5×10^7/kg TNC at freezing and not more than 2 out of 6 HLA disparities, with BM or PB grafts after MAC. Selection criteria used was based on recommendations for choosing CB grafts. Data were available on 1,525 patients aged > 16 years with acute leukemia transplanted between 2002 and 2006 using CB ($n = 165$), PB ($n = 888$), and BM ($n = 472$) [27]. CB units were matched at HLA-A and -B at an antigen level and HLA-DRB1 at an allele level ($n = 10$) or were mismatched for one ($n = 40$) or two antigens ($n = 115$). PB and BM grafts from unrelated adult donors were matched for allele-level HLA-A, -B, -C, -DRB1 ($n = 632$; $n = 332$) or mismatched at one locus ($n = 256$; $n = 140$). LFS after CB HSC was comparable to that observed after allele-matched (at HLA-A, -B, -C, -DRB1) and mismatched PB or BM transplantation. NRM was higher after UCBT than after allele-matched PB ($p = 0.003$) or BM ($p = 0.003$) since the engraftment rate was decreased in CB recipients. Grades II–IV acute and cGVHD were lower in UCBT recipients compared to allele-matched PB ($p < 0.001$ and $p < 0.001$, respectively). Chronic, but not aGVHD, was lower after CB compared to allele-matched BM HSCT ($p = 0.011$). Therefore, a search for CB units is recommended in the absence of a HLA matched donor or in urgent situations when time to search for a donor is limited.

3.2 Adults with Lymphoid Malignancies After RIC UCBT Compared to RIC Unrelated MPBSC Transplant

There have been encouraging results after UCBT for patients with lymphoid malignancies. Whether these outcomes are comparable to HLA-matched (8/8) unrelated MPBSC donor HSCT in a setting of RIC remains to be defined. Recently, the Eurocord group in collaboration with the Lymphoma Working Party of EBMT has studied

645 adult patients with lymphoid malignancies who received a sUCBT or dUCBT ($n = 104$) or a mismatched unrelated donor (MUD) HSCT ($n = 541$) after a RIC regimen [28]. Median follow-up time was 30 months. The CI of engraftment at day 60 was 81 % after CB and 97 % after MUD HSCT, whereas at day 100, the CI of grade II–IV aGVHD was 29 and 32 %, respectively. At 36 months, the CI of NRM was 29 % after CB and 28 % after MUD HSCT, relapse or progression was 28 and 35 %, and cGVHD was 52 and 26 % ($p < 0.01$), respectively. Two-year progression-free survival (PFS) and OS were 43 and 58 % after CB and 36 and 51 % after MUD HSCT, respectively. In a multivariate analysis, NRM, relapse or progression, PFS, and OS were not statistically different between CB and MUD HSCT, but MUD HSCT was associated with higher risk of cGVHD and UCBT with delayed engraftment. Thus, UCBT is a valuable alternative for patients with advanced lymphoma and CLL who lack an HLA-matched related or unrelated donor since UCBT is associated with a lower risk of cGVHD.

4 Outcomes After Double Compared with sUCBT in Adults with Acute Leukemia

The Minnesota group has pioneered the use of double CBT [29–33] analyzed 177 patients with acute leukemia transplanted with sUCBT and dUCBT. They have shown that relapse was significantly lower for early stage (1st and 2nd CR) patients who received double CB units compared to single-unit UCBT, suggesting a possibly greater graft-versus-leukemia (GVL) effect [31]. We can speculate that the increased GVL effect after dUCBt can be associated with a phenomenon of graft-versus-graft immune interaction, which has been suggested could explain the dominance of a cord blood unit [34] or it could be explained by a higher HLA disparity of the graft. However, in this analysis, the group of patients was heterogenous, including children and different types of conditioning regimens. With the aim to analyze outcomes after sUCBT with dUCBT, Eurocord group has conducted 2 different analyses according to the type of conditioning regimen (RIC or MAC) and one analysis comparing dUCBT with single intrabone injection using MAC for hematological malignancies. Patients treated with either sUCBT or dUCBT in Europe are outlined in Table 19.1.

4.1 Outcomes After Double Cord blood Transplantation Compared to Single Cord Blood Transplantation in Adults with Acute Leukemia given a Reduced Intensity Conditioning Regimen (RIC)

With this aim, we analyzed 360 adults (> 18 years) with ALL ($n = 77$) or AML ($n = 238$) in CR1 ($n = 212$) and in CR2 ($n = 148$) transplanted with a sUCBT ($n = 131$) or a dUCBT ($n = 229$) after a RIC. Only patients transplanted with a single

Table 19.1 Adults receiving unrelated UCBT according to diagnosis and graft type in Europe

Diagnosis	Number of patients (%) dUCBT; $n = 1735$	Number of patients (%) sUCBT $n = 1752$
ALL	368 (21)	302 (17)
AML	679 (39)	655 (37)
MDS/MPN	236 (14)	239 (14)
CML	133 (8)	82 (5)
CLL	26 (1)	72 (4)
Lymphoma	198 (12)	264 (15)
Other	40 (2)	76 (4)
Nonmalignant diseases	55 (3)	62 (4)

UCBT umbilical cord blood transplantation, *dUCBT* double umbilical cord blood transplantation, *sUCBT* single umbilical cord blood transplantation, *ALL* acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *MDS* myelodisplastic syndrome, *MPN* myeloproliferative neoplasm, *CML* chronic myelogenous leukemia, *CLL* chronic lymphocytic leukemia

unit containing more than 2.5×10^7/kg TNC were included. Patients were transplanted from 2005–2011 in EBMT centers. Comparing the two groups of patients receiving an sUCBT or a dUCBT in CR1, there were no statistical differences according to age, diagnosis (AML or ALL), weight, CMV serostatus, cytogenetics risk, number of HLA incompatibilities. However, dUCBT were performed more recently (2009 vs. 2008), the time from CR1 to transplantation was longer (142 days vs. 121 days), more frequently transplanted with CY + Flu + TBI2 Gy (87 vs. 68 %), lower frequency of anti-thymocyte globulin (ATG) use (21 vs. 35 %), and finally, dUCBT recipients received higher number of TNC collected (5×10^7/kg vs. 3.9×10^7/kg) or infused (4×10^7/kg vs. 3.1×10^7/kg). Median follow-up was 23 months in both groups. CI of 60 days neutrophil recovery was 82 ± 3 % after dUCBT and 76 ± 2 % after sUCBT ($p = 0.86$) and frequency of full donor chimerism at day 100 was not statistically different between dUCBT (81 %) and sUCBT (86 %). At day 100, CI of aGVHD (grade II–IV) was 35 % in both groups; however, there was a trend of increased incidence of grade III–IV after sUCBT (19 %) compared to dUCBT (10 %, $p = 0.06$) but increased incidence of grade II aGVHD after dUCBT (28 %) compared to 17 % after dUCBT ($p = 0.05$). CI of chronic GVHD at 2 years was 21 ± 4 % after dUCBT and it was 12 ± 5 % after sUCBT ($p = 0.15$). At 2 years, CI of NRM after dUCBT was 28 ± 4 % and it was 30 ± 6 % after sUCBT ($p = 0.87$). However, CI of 2-year relapse was 21 ± 4 % after dUCBT, whereas it was 38 ± 2 % after sUCBT ($p = 0.03$). In a multivariate analysis adjusting for the differences between the two groups, dUCBT was associated with lower incidence of relapse compared to sUCBT (HR = 0.74, $p = 0.01$). Therefore, there was an improved 2-year LFS after dUCBT (51 ± 5 %) compared to sUCBT (32 ± 3 %; $p = 0.03$). This was confirmed in a multivariate analysis (HR = 0.64, $p = 0.04$). Concerning patients transplanted in CR2 ($n = 148$), there were no statistically differences of outcomes after dUCBT ($n = 93$) or sUCBT ($n = 55$). At 2 year, LFS was 40 ± 6 % after dUCBT and 48 ± 3 % after sUCBT ($p = 0.32$). In a subgroup analysis of dUCBT ($n = 118$) and sUCBT ($n = 51$)

recipients using the same conditioning regimen (CY + Flu + TBI2 Gy) 2-year LFS were 54 ± 5 % and 33 ± 7 %, respectively ($p = 0.05$). In conclusion, in this unpublished retrospective comparative based registry analysis, in AL patients transplanted in CR1, neutrophil recovery, GVHD and NRM were not statistically different after RIC-dUCBT or RIC-sUCBT, however, dUCBT recipients had decreased RI and improved LFS. For acute leukemia (AL) patients transplanted in CR2, there was no benefit of using dUCBT when compared to sUCBT recipients [V. Rocha for Eurocord, unpublished data]. This finding needs to be confirmed in a larger series, but it is speculated that probably the scheme of RIC used may be associated with different outcomes, as has been recently reported [32].

4.2 Outcomes After Double Cord blood Transplantation Compared to Single Cord Blood Transplantation in Adults with Acute Leukemia given a Myeloablative Conditioning Regimen (MAC): Impact of Type of Conditioning Regimen

We have also conducted a retrospective comparison of outcomes of dUCBT and sUCBT after MAC regimen for 239 patients transplanted for AL in CR1. All sUCBT patients received a TNC > 2.5 × 10e7/Kg. Conditioning regimen for sUCBT was TBI12 Gy or Busulfan-based ± ± Flu ($n = 68$, group 1), TBF ($n = 88$, group 2), and for dUCBT was TBI12 + CY ± Flu ($n = 83$, group 3). dUCBT recipients were younger, received higher cell dose, and less frequently ATG. In multivariate analysis we found similar neutrophil recovery among the three groups, however aGVHD II–IV was higher in dUCBT compared to others. NRM and RI were not statistically different among the three groups. LFS was decreased in sUCBT using TBI- or BU-based MAC compared to the others ($p = 0.02$ and $p = 0.03$, respectively) and it was no statistically different between sUCBT and TBF and dUCBT. In conclusion, use of sUCBT with adequate cell dose (> 2.5 × 107/kg) and the TBF conditioning regimen in the MAC setting results in similar outcomes as dUCBT. The choice of TBF conditioning regimen for sUCBT may improve results, whether this regimen may improve results in dUCBT should be further analyzed [35].

4.3 Outcomes After Single-unit Intrabone Injection Compared to Double-unit Intravenously in Patients with Hematological Malignancies

Intrabone injection of cord blood cells (IB-UCBT) and dUCBT are strategies designed to circumvent the problem of delayed engraftment after UCBT.

In a retrospective-based registry analysis, we compared outcomes of 87 IB-UCBT with 149 dUCBT recipients after MAC adjusting for the differences between the two groups. Median infused TNC was 2.5 × 10^7/kg for IB-UCBT and 3.9 × 10^7/kg

for dUCBT ($p < 0.001$). At day + 30, CI of neutrophil recovery was 76 and 62 % ($p = 0.014$) with median time to engraftment of 23 and 28 days ($p = 0.001$) after IB-UCBT and dUCBT, respectively. At day + 180, CI of platelets recovery was 74 % after IB-UCBT and 64 % after dUCBT ($p = 0.003$). In multivariate analysis, IB-UCBT was associated with neutrophil and platelets recovery and lower acute GVHD (II–IV) ($p < 0.01$). At 2 year, CI of NRM and RI were 30 and 25 % after IB-UCBT and 34 and 29 % after dUCBT and DFS was 45 and 37 %, respectively. However, after landmark analysis at 4.7 months from transplantation, in multivariate analysis, RI was reduced ($p = 0.03$) and there was a trend for better DFS after IB-UCBT ($p = 0.09$).

In conclusion, both approaches expand the possibility of offering UCBT to patients with hematopoietic malignancies; IB-UCBT is associated with faster myeloid and platelet recovery and lower aGVHD and may reduce the total cost. However, studies on cost-effectiveness are needed to compare both strategies [36].

In conclusion, in the above retrospective comparisons, dUCBT seems to be associated with better outcomes only in the setting of RIC in first CR. The platforms used, such as type of conditioning regimen and GVHD prophylaxis, are important factors associated with graft type. When single CB units are chosen with more than 2.5 × 10e7/kg, outcomes are quite comparable with double units CBT. In case of CB units with lower number, dCBT and intrabone injection are other possible approaches.

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Chapter 20
Selection of the Optimal Cord Blood Unit

Karen K. Ballen

1 Introduction

The first umbilical cord blood transplantation (UCBT) was performed in 1988 in France in a child with fanconi anemia (FA) and was performed by Dr. Eliane Gluckman and colleagues [1]. Over the last 25 years, umbilical cord blood (UCB) banking and transplantation have grown exponentially. More than 600,000 UCB units have been donated for public use worldwide, and more than 30,000 UCBT have been performed. Only 30 % of patients will have a human leukocyte antigen (HLA) matched sibling. There are approximately 20 million adult volunteer donors in the National Marrow Donor Program and affiliated registries; however, only 60 % of whites and 20 % of blacks and other minorities will have a rapidly identified suitably matched unrelated volunteer donor [2]. Thus, UCBT extends access to hematopoietic cell transplantation (HCT) to a diverse group of patients. However, the optimal UCB unit for each individual patient is not clear as cell dose and HLA typing may be only part of the important variables to consider.

2 Cell Dose

Initial studies in adult UCBT indicated the importance of cell dose; survival was improved in patients receiving UCB units with a higher CD34 + cell dose [3]. An improvement in survival results over time can be partially attributed to selecting UCB units with a higher cell dose; the Eurocord group showed an increase in disease-free survival (DFS) from 23 % prior to 2000 to 38 % in recent years in single, myeloablative UCBT [4]. Barker and colleagues explored the interaction between cell dose and HLA match in 1061 recipients of single, myeloablative UCBT [5]. The lowest

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transplant related mortality (TRM) was seen in recipients of 6/6 HLA A, B, DRB1-matched UCBT, regardless of cell dose; UCB units that were 5/6 HLA matched to the recipient with a total nucleated cell (TNC) count of $> 2.5 \times 10^7$/kg had similar TRM to a 4/6 HLA matched UCBT with a TNC of $> 5 \times 10^7$/kg. Many centers will not accept a UCB unit with TNC $< 2.5 \times 10^7$/kg with one or two mismatches as a single-unit graft [6].

Series of patients with nonmalignant diseases indicate that a higher cell dose threshold may apply to nonmalignant diseases. Children who received an UCBT with TNC $< 3.5 \times 10^7$/kg and a 2–3 HLA antigen mismatched UCBT had $< 10\,\%$ survival [7]. For nonmalignant diseases, a cell dose of TNC $> 4.0 \times 10^7$/kg is recommended.

In double UCBT, after myeloablative conditioning, a higher TNC, CD34 + cell count and colony-forming units (CFU) in the dominant unit were associated with faster engraftment [8]. Patients receiving a dominant unit with a CFU higher than median had a neutrophil engraftment of 19 days, compared to 27 days for patients receiving a CFU less than the median.

3 HLA Typing

The importance of HLA typing is discussed above in the section on cell dose. A fully matched UCBT has excellent survival, regardless of cell dose. The initial UCBT were performed with Class I typing to low resolution. Recent data suggests that allele level typing for both Class I and Class II may be important; TRM was higher with UCB units mismatched at one or two alleles compared to HLA matched UCB units, 26, 26, and 9 % respectively [9]. Matching at HLA-C may also be important to improving outcomes after UCBT. In a study of single unit, myeloablative UCBT, TRM was higher for patients who received UCB units matched at HLA A, B, and DRB1 and mismatched at HLA-C compared to patients who received fully matched UCB units [10]. Similarly, TRM was higher for patients receiving UCB units with a single mismatch at HLA A, B, or DRB1 and mismatched at HLA-C compared to patients receiving UCB units with a single mismatch at HLA A, B, and DRB1 and mismatched at HLA-C. Preliminary data in a double UCBT cohort suggests that matching at HLA-C may be important in the reduced-intensity setting as well [11].

A further question is whether the direction of the HLA mismatch impacts transplant outcomes. Mismatches can either be in the graft-versus-host disease (GVHD) direction (donor homozygous at one locus with recipient sharing one antigen with the donor) or host-versus-graft direction (recipient homozygous at one locus with donor sharing one antigen with the recipient). A large study from Eurocord analyzed 1565 patients receiving single unit, myeloablative UCBT, with a median age of 15 years [12]. The direction of HLA mismatch did not impact nonrelapse mortality or survival. In a Japanese study, however, HLA mismatch in the GVHD direction was associated with slower engraftment [13]. HLA mismatch in the host-versus-graft direction had no impact on engraftment.

HLA antibodies have recently been recognized as an important component in UCB unit selection. In both the single and double UCBT settings, the presence of preformed HLA antibodies against the selected UCB units was associated with a higher risk of graft failure and death [14–16]. In a single UCB, myeloablative UCBT study, neutrophil recovery was 82 % for the patients who were HLA antibody negative compared to only 32 % for those patients with HLA antibodies vs. the selected UCB unit [16]. In a study from Boston, patients who received double UCBT with reduced intensity conditioning (RIC), 3 year overall survival (OS) was 0 if there were preformed HLA antibodies vs. both UCB units compared with OS of 45 % for patients with no antibodies, $p = 0.04$ [14]. Many centers, including our own, have now decided not to select UCB units to which there are performed antibodies.

As explained in depth in the chapter by Scaradavou and colleagues on maternal typing, two studies demonstrated an improvement in OS (55 vs. 38 % at 5 years) when the HLA typing of the patient matched the noninherited maternal allele of the UCB donor [17, 18].

Other investigators have sought to determine if donor killer immunoglobulin receptor (KIR) ligand matching is beneficial, and results are conflicting [19, 20]. Tanaka and colleagues found no difference between KIR ligand compatible or incompatible groups in the GVHD direction for OS, disease-free survival, relapse or acute GVHD [21]. However, in the host vs. graft direction, KIR ligand incompatibility was associated with poorer engraftment in patients with acute lymphocytic leukemia (ALL). In a RIC double UCBT cohort of patients who received antithymocyte globulin, KIR ligand incompatibility was not associated with a reduction in relapse [22]. The Minnesota group found that the effect of KIR alloreactivity was dependent on conditioning intensity [20]. KIR ligand mismatch had no effect of GVHD, relapse, or survival in recipients receiving a myeloablative preparative regimen. However, after RIC, KIR ligand mismatch resulted in higher rates of severe Grades III–IV GVHD, and worse survival.

An important and unanswered question is the effect of HLA mismatch on relapse after UCBT. It is not clear if relapse is decreased in recipients of HLA mismatched UCBT compared to HLA matched UCBT [23, 24].

4 Unit Processing

UCB units can be processed by a variety of techniques including partial red blood cell depletion and manual processing as initially described by Pablo Rubinstein and colleagues, automated cell processing, and plasma depletion (RBC replete units) [25]. Plasma depleted units were studied in 120 pediatric patients with nonmalignant disorders, with a median time to engraftment of 21 days, and 70 % DFS [26]. Preliminary data by Nikiforow and colleagues showed no effect of cord blood bank of origin, processing technique (manual or automated), or RBC depletion technique on engraftment or cord unit dominance [27]. Survival was improved for the 17 patients who received two RBC replete units.

Storage time precryopreservation differs among the cord blood banks. Using the viability of CD 34 + cells as a marker for quality, the British National Health Service showed a decline in viability by approximately 7 %/day [28].

5 Cord Blood Unit Potency

The group at Duke has studied UCB potency, a composite of TNC, CD 34 + cells, and CFU. A shorter interval from collection to processing, gestational age 34–40 weeks, white race, and higher birth weight were all associated with a higher potency, as illustrated in the chapter by Page and Kurtzberg [29]. The impact on transplant outcomes is less clear; however, higher CFUs have been associated with faster neutrophil engraftment in some studies [30].

6 ABO Mismatch

Red blood cell typing (ABO) is essential for matching of solid organ transplant donors but its impact in hematopoietic cell transplantation is less clear. ABO-mismatch had no effect on acute or chronic GVHD after single or double UCBT. Therefore, ABO compatibility is not a factor in UCB unit selection [31].

7 Racial/Ethnic Match

The Center for International Blood and Marrow Transplant Research (CIBMTR) has studied the impact of race/ethnicity on UCBT outcomes [32]. Black patients had inferior OS compared to white patients after single unit UCBT with myeloablative conditioning. Black patients were also less likely to receive well-matched units of a cell dose $> 2.5 \times 10^7$ NC/kg. There was no survival advantage to selecting an UCB unit of the same race/ethnicity as the UCBT recipient. A University of Minnesota study also found that donor race matching had no effect on relapse, GVHD, or survival [33].

8 Conclusions

The choice of the optimal umbilical cord blood unit for each individual patient is an evolving field. In the 1990s, cell dose and HLA match were employed in UCB unit selection. Today, allele level typing, HLA C, match at the noninherited maternal allele, HLA antibodies, cord blood potency, and processing methods may all impact survival. There are few established guidelines to aid the transplant physician. Table 20.1 illustrates the current suggested strategy used at Massachusetts General

Table 20.1 Sample cord blood selection strategy

Sample cord blood selection strategy
HLA antibodies should be checked at the time of cord blood search—do not select UCB units against which patients have preformed antibodies. Antibody screen may need to be repeated just prior to ordering cords
Cord units must not be HLA identical, unless a 6/6 match with the patient
Double mismatches at any given locus should be avoided
Cord blood units should have high resolution typing performed for Class I and Class II. This can be done from an attached segment or stored DNA as available. A, B, and DR are used in the match strategy. Cord unit to patient matching should be at the allele level (high resolution) whenever possible. Data on C and DQ are collected but not used in the match strategy to date. If possible, cord blood identity should be confirmed just prior to transplant on an attached segment
Patients must have an accurate weight at the time of search
Choices regarding cell dose vs. HLA for double cord shall be as follows Total TNC must be at least 3.7×10^7 NC/kg Each unit must be at least 1.5×10^7 NC/kg Higher cell dose triumphs over HLA match when TNC is $\leq 2.0 \times 10^7$ NC/kg for each single CBU Closer HLA match triumphs over cell dose when TNC is $> 2.0 \times 10^7$ NC/kg for each single CBU unless there is a $> 50\,\%$ higher cell dose in the less well-matched cord blood unit
For selection between equivalent units choose CD 34 + dose (higher dose preferable) Greater viability (never use a unit with viability less than 90 %)
Example Cord A 4/6 match to patient, cell dose 3×10^7 NC/kg Cord B 5/6 match to patient, cell dose 1.7×10^7 NC/kg Cord C 4/6 match to patient, cell dose 5.0×10^7 NC/kg Select A and C
Example Cord A 4/6 match to patient, cell dose 3.5×10^7 NC/kg Cord B 5/6 match to patient, cell dose 3.0×10^7 NC/kg Cord C 4/6 match to patient, cell dose 5.0×10^7 NC/kg Select B and C

HLA *human leukocyte antigen,* UCB *umbilical cord blood,* TNC *total nucleated cell,* CBU *cord blood unit*

Hospital. We anticipate that with expanded knowledge, these guidelines will evolve further.

This is an exciting time in the field of cord blood transplantation and much has been accomplished. Several challenges remain including efforts to decrease infection, to expand access throughout the world, and to decrease cost. As the UCBT field matures from infancy through the first 25 years, the efforts of the investigators demonstrated here have all contributed to the improvement in patient outcomes. The next 25 years will surely bring advances in technology, cell manipulation, supportive care, and patient and cord unit selection.

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