Advances and Controversies in
Hematopoietic Transplantation and Cell Therapy
Series Editors: Syed A. Abutalib · James O. Armitage

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Nelson Chao
Editors

Cord Blood Transplantation

Springer

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Advances and Controversies in Hematopoietic Transplantation and Cell Therapy
ISBN 978-3-319-53627-9 ISBN 978-3-319-53628-6 (eBook)
DOI 10.1007/978-3-319-53628-6

Library of Congress Control Number: 2017939943

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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Eliane Gluckman and Annalisa Ruggeri

1.1 Introduction

1.1.1 Preclinical Steps

The concept of using umbilical cord blood (UCB) was developed in the late 1970s, and the first human umbilical cord blood transplant was performed in 1988 (Gluckman et al. 1989); however, it was the pivotal work of HE Broxmeyer that moved UCB from the laboratory to clinical practice. HE Boyse provided the proof of concept studies in mice, while HE Broxmeyer systematically evaluated the hematopoietic potential of human UCB *in vitro* and developed practical and efficient methods for large-volume collection and storage of UCB. It was postulated at that time that UCB collected at birth might contain enough hematopoietic stem/progenitor cells for clinical use (Broxmeyer et al. 1989). This possibility was strengthened by the knowledge that hematopoietic progenitor cells from UCB could be maintained for many weeks in long-term cultures suggesting their production from more primitive cells. Mice studies showed that small amounts of neonatal blood but not small amounts of adult blood allowed survival of approximately half of the lethally irradiated mice. This led to a multi-institutional study that addressed the following: (1) estimation of the reconstituting cellular content of cord blood by measurement of hematopoietic progenitor cell numbers and comparison with adult bone marrow, (2) collection, (3) transportation,

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_1

and (4) optimal cryopreservation of cord blood. They found on 101 samples, collected in New York and analyzed in Indiana University School of Medicine, that the number of progenitors was in the lower range of numbers associated with successful engraftment and that the numbers were improved if there was no attempt of erythrocyte removal (Broxmeyer et al. 1992). They noted that samples could be successfully frozen, stored, and thawed without major loss. At that time, it was felt, by this group, that cord blood stem/progenitor cells would be especially attractive for autologous purpose, making available for a recipient a perfectly HLA-matched set of stored cells for future use. However, it was unlikely that autologous cord blood transplantation could be tested in the near future. This concept led to a long-lasting debate on patenting cord blood for clinical use and of setting up commercial cord blood banks for personal use. Next, testing the concept of allogeneic cord blood cell transplants when an HLA-identical sibling was available was discussed. The first UCBT was made possible by an intensive collaboration between three groups: AD Auerbach from the Rockefeller University in New York (USA) described a method of prenatal diagnosis in Fanconi anemia (FA) (Auerbach et al. 1990), HE Broxmeyer from Indiana University in Indianapolis (USA) systematically analyzed the number of hematopoietic cell progenitors in cord blood for the purpose of using the cells for hematopoietic reconstitution in humans, and E Gluckman, from the Hospital Saint Louis in Paris (France), showed that the *in vivo* hypersensitivity of FA cells translated in an increased toxicity of the pretransplant conditioning regimen used in aplastic anemia, and she was the first to use a modified attenuated dose conditioning in these patients with improved short- and long-term survival (Gluckman et al. 1983).

AD Auerbach selected mothers of FA patients who were pregnant; she made a prenatal diagnosis on cultured amniotic fluid cells and was able to select five mothers who were expecting a baby who was known, before birth, to be unaffected by FA and HLA identical to the patient. Based on the preceding information, umbilical cord blood was harvested and cryopreserved at birth. It was felt by all involved and by the human subjects institutional review boards of the involved centers that the availability of cord blood in this case obviated the need for bone marrow aspiration from the infant sibling. Nevertheless, the infant sibling was available for bone marrow donation if necessary.

1.2 From the First Umbilical Cord Blood Transplant to the Development of Umbilical Cord Blood Banks

The first UCBT was performed in a patient with FA (Gluckman et al. 1989). This patient had a healthy HLA-identical sibling shown by prenatal testing to be unaffected by the disorder, to have a normal karyotype, and to be HLA identical to the patient. The cord blood was collected at birth, cryopreserved, and used after thawing for transplantation. The patient was conditioned by a procedure developed specifically for the treatment of FA patients who are extremely sensitive to the administration of alkylating agents like cyclophosphamide. The patient was conditioned with a low dose of cyclophosphamide (20 mg/kg instead of 200 mg/kg) and 5 Gy total lymphoid

irradiation (Gluckman et al. 1983). The frozen cells were hand delivered from Indiana to Paris in a dry shipper that maintained the temperature at −175 °C. The cells were thawed without further processing on day 0. Thawed cells were tested for viability, and progenitor assays and results were similar to the counts recorded before freezing. First signs of engraftment appeared on day 22 with subsequent complete hematological reconstitution and donor chimerism. The patient had no graft *versus* host disease (GvHD) and is currently more than 25 years after UCBT, healthy with a complete long-term hematological and immunological donor reconstitution.

This first success opened the way to a new field in the domain of allo-HCT as it showed that (1) a single UCB contained enough hematopoietic $CD34^+$ cells to reconstitute definitely the host lympho-hematopoietic compartment, (2) an umbilical cord blood unit could be collected at birth without any harm to the newborn infant, and (3) umbilical cord blood hematopoietic $CD34^+$ cells could be cryopreserved and transplanted in a myeloablated host after thawing, without losing their repopulating capacity. Since, our knowledge on the biological characteristics of umbilical cord blood cells has increased, emphasizing the advantages of using umbilical cord blood cells for transplant.

Several aspects were identified as subject of further questioning and investigations: Would a single cord blood unit contain enough $CD34^+$ cells to permanently engraft children and adults? Would maternal cell contamination in fetal blood engraft and give severe GvHD? Would the same results be obtained in patients transplanted for indications other than Fanconi anemia, such as leukemia? What are the immunological properties of cord blood cells? How does it interfere with graft *versus* host disease, graft *vs*. leukemia, and immune reconstitution? Is the immune immaturity of cord blood lymphocytes able to overcome the HLA barrier and authorize HLA-mismatched transplants? Is it possible to establish cord blood banks for unrelated and related transplants? What would be the criteria for collection, quality control, and cryopreservation? Would it be possible to collect cord blood not only for familial transplant but also for unrelated transplants? What would be the size of this bank if it was demonstrated that HLA incompatibilities would not be recognized because of the immaturity of the immune system at birth?

All these questions have been answered during the last 20 years, thanks to the worldwide development of intense international cooperation, in Europe with Eurocord and Netcord, in the USA with CIBMTR and NMDP, with Asia cord, Australia cord, and single transplant centers.

1.2.1 Milestones in the Development of CBT

- Optimization of UCB collection and storage (Gluckman et al. 1989; Rubinstein et al. 1993, 1995)
- First HLA-identical sibling cord blood transplant in a patient with Fanconi anemia (Gluckman et al. 1989)
- Development of CBB for related and unrelated transplants (Paris, Dusseldorf, New York, Milan)

- First unrelated mismatched cord blood transplant in children (Kurtzberg et al. 1996)
- Creation of the Eurocord Netcord network (Gluckman et al. 1997)
- Description of criteria of donor choice based on the number of cells and possibility to use mismatched cord blood (Wagner et al. 2002; Gluckman et al. 2004)
- Demonstration that, compared to HLA-identical sibling bone marrow transplants, cord blood gave delayed engraftment, less GVH, and same survival than (Rocha et al. 2000)
- Demonstration that, compared to match-unrelated bone marrow, mismatched cord blood gave similar long-term leukemia-free survival in children (Locatelli et al. 1999; Wagner et al. 1996; Eapen et al. 2007) and in adults (Laughlin et al. 2001; Cairo and Wagner 1997; Rubinstein et al. 1998; Sanz et al. 2001; Rocha et al. 2004) (Table 1.1)
- Improvement of results mostly in adults by double cord blood transplants and non-myeloablative conditioning regimens (Brunstein et al. 2007; Barker et al. 2005)
- Improvement of engraftment with ex vivo expansion
- Use of CB for immune recovery after transplant

Table 1.1 Results of comparative studies on BMT, PBHC, and UCBT in adults with hematological diseases

Series	Patients, *n*	Graft source (*n*)	Conditioning regimen	Median FU in months	Comments (UCBT *vs.* others)
UD *vs.* single UCBT for adults with acute leukemia Rocha et al. (2004)	682	BM (584) sUCB (98)	MAC	27	Delayed myeloid recovery Decreased a and cGvHD Comparable OS and DFS
UD *vs.* single UCBT for adults with acute leukemia Eapen et al. (2011)	1280	BM (364) PBHC (768) UCB (148)	MAC	26 (BM) 24 (PBHC) 29 (UCB)	Delayed myeloid recovery (*vs.* BM) Increased NRM but comparable OS and DFS (*vs.* BM)
Sibling, UD *vs.* single UCBT Brunstein et al. *Blood* (2010)	384	MRD (204) MMUD (52) dUCBT (128)	MAC	36	Compared to 8/8 UBM Delayed myeloid recovery Increased NRM, reduced relapse comparable OS and DFS
UD *vs.* double UCBT for adults with acute leukemia Brunstein et al. *Blood* (2012)	584	8/8 PBHC (313) 7/8 PBHC (111) dUCBT (160)	RIC	36 (8/8 PBHC) 24 (7/8 PBHC and dUCBT)	Comparable results with TCF regimen (*vs.* 8/8 PBHC)

Table 1.1 (continued)

Series	Patients, *n*	Graft source (*n*)	Conditioning regimen	Median FU in months	Comments (UCBT *vs.* others)
UD *vs.* UCBT Malard et al. *BBMT* (2015)	651	MUD (347) MMUD (99) UCB (205)	RIC	/	Delayed engraftment Similar NRM, OS, cGvHD Higher relapse (*vs.* MUD) Lower aGvHD (*vs.* MMUD)
Non-T-cell depleted haploidentical transplant *vs.* UCBT for adults with acute leukemia Ruggeri et al. *Leukemia* (2015)	1446 (918 AML; 528 ALL)	Haplo (360 AML; 158 ALL) UCB (558 AML; 370 ALL)	MAC and RIC	24	Delayed engraftment Higher graft failure Lower incidence of cGvHD Similar relapse, DFS and NRM

Legend: *UD* unrelated donor, *BMT* bone marrow transplant, *PBHC* peripheral blood hematopoietic cells, *FU* follow-up, *BM* bone marrow, *UCBT* umbilical cord blood transplant, *sUCB* single umbilical cord blood, *MAC* myeloablative conditioning regimen, *RIC* reduced intensity conditioning regimen, *cGvHD* chronic graft *versus* host disease, *OS* overall survival, *DFS* disease-free survival, *NRM* non-relapse mortality, *MRD* matched related donor, *MMUD* mismatched unrelated donor, *dUCBT* double umbilical cord blood, *UBM* unrelated bone marrow, *TCF* TBI, fludarabine, cyclophosphamide, *aGvHD* acute graft *versus* host disease, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia

1.2.2 Major Breakthrough in the Field of Cord Blood Transplant

1.2.2.1 Role of Cell Dose

Almost all series focusing on UCBT in children and adults have demonstrated the profound impact of cell dose, measured as pre-freezing or infused total nucleated cells (TNC), colony-forming units, and $CD34^+$ cells on engraftment, transplant-related events, and survival (Wagner et al. 2002; Gluckman et al. 2004). According to several different reports, a minimum TNC of 3×10^7/kg at cryopreservation needs to be obtained for engraftment (Gluckman et al. 2004; Scaradavou et al. 2013).

Sometimes the cell dose available in a single UCB is insufficient for transplantation. To overcome the cell dose limitation, Barker and the Minnesota group (Barker et al. 2005) pioneered the use of double UCBT (dUCBT), sequentially infusing two UCB in one transplant procedure. The results observed with the use of dUCBT were encouraging; all patients achieved neutrophil engraftment in a median of 23 days (range, 15–41) (Brunstein et al. 2007; Barker et al. 2005; Scaradavou et al. 2013).

Some authors reported that double UCBT was associated with higher risk of acute GvHD (MacMillan et al. 2009) and lower risk of relapse (Verneris et al. 2009); however, engraftment failure is still around 15% (Brunstein et al. 2007). A CIBMTR study (Scaradavou et al. 2013) to determine the efficacy of dUCBT compared to single UCBT in patients with acute leukemia showed similar results for the two

groups. Also, in a prospective trial of children with hematological malignancies after myeloablative conditioning (MAC) regimen, no difference between single and dUCBT was reported (Wagner et al. 2014; Michel et al. 2016).

1.2.2.2 Antigenic and Allele HLA Typing and Interactions with Cell Dose

Conventionally, HLA matching of UCB for allo-HCT has used low-/intermediate-resolution typing for HLA-A and HLA-B (antigenic level) and high-resolution typing for HLA-DRB1 (allelic level). HLA matching has also been identified, since earlier studies, as an important factor for engraftment. HLA matching for UCB units is generally based on three loci, and a maximum of two out of six HLA mismatches is considered acceptable due to the very high transplant-related mortality (TRM) associated with greater mismatch (Barker et al. 2010). In a study analyzing the role of the HLA-C on UCBT, Eurocord and NMDP/CIBMTR (Eapen et al. 2011) reported higher TRM in patients receiving an UCB unit with a mismatch at HLA-C level; also, contemporary mismatching at HLA-C and HLA-DRB1 was associated with the highest risk of mortality.

1.2.2.3 HLA High-Resolution Typing in Pediatric and Adult UCBT Setting

The COBLT study reported 179 patient–donor pairs using high-resolution typing for HLA-A and HLA-B and HLA-DRB1. Matching at high-resolution typing was associated with decreased incidence of acute GvHD but not with length to neutrophil or platelet engraftment. There was a trend to better survival for patients given a 6/6 high-resolution typing and worse survival rates for those with 3/6, but the numbers were too small to reach statistical significance (Kurtzberg et al. 2008).

Subsequently, a joint Eurocord and CIBMTR study analyzed the effect of high-resolution typing (HLA-A, HLA-B, HLA-C, and HLA-DRB1) on the outcomes of 1,568 MAC single UCBT for hematological malignancy (Eapen et al. 2014). Day 28 neutrophil recovery was significantly lower for transplants mismatched at three or more alleles compared with UCBT both fully-matched UCB (8/8) or CB mismatched at one/two alleles. No significant differences in neutrophil recovery were observed when considering mismatching at specific HLA loci. NRM was associated with HLA matching with the lowest risk reported in 8/8 UCBT. Single allele mismatches at HLA-A, HLA-C, or HLA-DRB1 were associated with increased NRM [HR, 3.05 (95% CI, 1.52–6.14), $p = 0.02$; HR, 3.04 (95% CI, 1.28–7.20), $p = 0.01$; HR, 2.93 (95% CI, 1.38–6.25), $p = 0.005$, respectively]. Notably, the use of a UCB with TNC $<3 \times 10^7$/kg was associated with higher NRM, independently of HLA matching. However, further increases in TNC dose above the minimum threshold of 3×10^7/kg were not associated with a reduction in NRM. Therefore, single UCBT should have a minimum pre-cryopreserved TNC of 3×10^7/kg. The best HLA-allele-matched UCB should then be selected, although mismatches at one or two alleles are acceptable. However, UCB with mismatches at three or more alleles should be used with caution due to the higher rates of graft failure and NRM. Given

Table 1.2 Criteria for the choice of cord blood unit, on behalf of Eurocord

1. Screening for *antibodies against HLA antigens* of the cord blood unit
2. *Number of cells* in the cord blood unit for malignant disease:
(a) >2.5 – 3 × 10^7 TNC/kg and/or ≥1 × 10^5 $CD34^+$/kg[a]
3. *HLA mismatches*:
(a) Prefer class I than class II mismatch
(b) Avoid cord blood unit with more than two HLA mismatches
4. *HLA typing*:
(a) HLA-A and HLA-B typing at antigenic level, HLA-DRB1 at allelic level
(b) If possible, include HLA-C typing, avoiding HLA-C mismatches when present[b]
5. Adapt cell dose to *graft indication*:
(a) Malignant diseases: cell dose is the best prognostic factor, because HLA differences reduce relapse.
(b) Nonmalignant diseases: increase cell dose (>4.0 × 10^7 TNC/kg) and find the best HLA match.
6. If several cord blood units are available, choice of the best one should be also guided by:
(a) Cord blood bank accreditation status and location
(b) ABO compatibility
(c) NIMA and KIR status[c]

Legend: *TNC* total nucleated cell dose, *sUCBT/dUCBT* single/double umbilical cord blood transplantation, *NIMA* non-inherited maternal antigen

[a]If the minimum number of cells for a sUCBT is not achieved, a dUCBT should be considered

[b]Especially when both HLA-C and HLA-DRB1 mismatches

[c]No sufficient data to support unit selection on NIMA or KIR-L status

the results of the different studies, Eurocord proposed recommendations for the selection of UCB (Table 1.2).

1.2.3 Current Trends in the Field of Cord Blood Transplantation

1.2.3.1 Improving Outcomes After UCBT

The main problem using UCB unit for transplantation is the relatively low number of $CD34^+$ cells compared with bone marrow or peripheral blood $CD34^+$ cell grafts, which translates into increased risk of graft failure (GF), delayed hematopoietic engraftment (Laughlin et al. 2004; Eapen et al. 2010), and delayed immune reconstitution (Komanduri et al. 2007; Ruggeri et al. 2011). The CI of non-engraftment after UCBT varies from 10 to 20%, and the median time to neutrophil recovery varies from 22 to 27 days.

Anti-HLA antibodies in the recipient are a contributing factor for GF after allo-HCT. Patient anti-HLA antibody testing to detect antibodies directed against HLA of the available UCB unit using standardized methodology should be included in the algorithm of donor choice and has to be considered when selecting a UCB unit (Takanashi et al. 2010; Cuttler et al. 2011; Ruggeri et al. 2013; Brunstein et al. 2011;

Delaney et al. 2010). The use of a UCB unit with donor-specific antibodies should be avoided, in order to reduce the risk of GF and TRM.

As for donor selection using other cell sources, ABO compatibility should be considered when many cord blood units are available, preferring the UCB unit that is ABO compatible or with minor incompatibilities, especially in the setting of dUCBT.

1.2.3.2 Other Experimental Approaches

Currently, multiple strategies under clinical investigation aimed to overcome the low total cell and $CD34^+$ cell dose provided by a single or double cord blood graft. These strategies focus primarily, but not exclusively, on methods to increase the cell dose of a cord blood graft and include the use of ex vivo expanded CB units (Horwitz et al. 2014; de Lima et al. 2012; Farag et al. 2013; Ruggeri et al. 2015), the systemic addition of mesenchymal stem cells, and the use of agents to enhance CB homing to the marrow. Promising results have been reported with all the abovementioned strategies (Table 1.3); however, they remain experimental, and a definitive conclusion cannot yet be made regarding their reproducibility, cost-efficacy, or the long-term outcomes.

Table 1.3 Platform of cord blood expansion

Modality/clinical strategies	Clinical trial	Institution	Ref
Ex vivo expansion			
Nicotinamide (NiCord)	Yes	Duke University/Gamida cell	Horwitz et al. (2014)
Copper chelation (TEPA, StemEx)	Yes	Gamida cell/Teva	de Lima et al. (2008)
Notch ligand	Yes	FHCRC	Delaney et al. (2010)
MSC coculture (Mesoblast)	Yes	MDACC/Mesoblast	de Lima et al. (2012)
Aryl hydrocarbon receptor antagonist (StemRegenin 1)	Yes	University of Minnesota/ Novartis	Boitano et al. (2010), Wagner et al. (2014)
Cytokine (SCF, TPO, Flt-3L, G-CSF, MGDF)	Yes	MDACC	Shpall et al. (2002)
Continuous perfusion culture (HS, EPO, Flt-3L)	Yes	Duke University/Aastrom Biosciences	Jaroscak et al. (2003)
Enhancing homing			
Inhibition of CD26/DPP-4	Yes	Indiana University	Farag et al. (2013)
PGE-2 exposure	Yes	DFCI/Fate Therapeutics	Cuttler et al. (2013)
Fucosylation	Yes	MDACC	Robinson et al. (2014), Xia et al. (2004)
C3a complement coculture	Yes	University of Minnesota	Brunstein et al. (2013)

Legend: *FHCRC* Fred Hutchinson Cancer Research Center, *DFCI* Dana-Farber Cancer Institute, *MDACC* MD Anderson Cancer Center, *TEPA* tetraethylenepentamine, *SCF* stem cell factor, *TPO* thrombopoietin, *Flt-3L* Flt-3 ligand, *G-CSF* Granulocyte-colony stimulating factor, *MGDF* megakaryocyte growth and development factor, *EPO* erythropoietin, *HS* horse serum, *DPP-4* Dipeptidyl peptidase IV, *PGE-2* prostaglandin E-2

Conclusion

Much has been learned in a relatively short time on cord blood hematopoietic progenitor properties and their clinical applications. All these results show that mismatched allo-HCT is feasible, and there is no donor shortage. This is an evolving field, which must be evaluated carefully through multicenter and registries studies.

Recently, the number of cord blood transplantations has declined, probably, due to the increased use of unmanipulated cells from family haploidentical donors. Preliminary comparative results show (Ruggeri et al. 2015) similar early outcomes, but follow-up is still too short to give recommendations for choosing between unrelated cord blood transplant and family haploidentical mismatched transplants.

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Current Cord Blood Banking Concepts and Practices

2

Kristin M. Page and Joanne Kurtzberg

2.1 Introduction

Umbilical cord blood (CB) is firmly established as an unrelated donor source for hematopoietic cell transplantation (HCT) and has the potential to play an important role in the evolving fields of regenerative medicine and cellular therapies. Currently, there are over 160 public banks with a global inventory of over 700,000 fully characterized, high-quality cord blood units (CBUs) (http://www.bmdw.org n.d.). Family (or private) CB banks are also available for those families electing to pay a fee to store their baby's CB for their own use. Approximately four million CBUs have been banked at an estimated 215 family banks through this mechanism (Ballen et al. 2015). As indications for autologous and allogeneic CB continue to expand, the decision facing pregnant women, whether to altruistically donate CB or bank privately as a form of "medical insurance," will become more complex. In this chapter, we review the history and current state of CB banking as well as challenges confronting the banking community.

2.2 The Historical Perspective

Over 30 years ago, it was recognized that CB was a rich source of hematopoietic stem and progenitor cells (HSPCs). In a pivotal series of experiments, Dr. Ted Boyce, working with Dr. Hal Broxmeyer and colleagues, demonstrated that CB HSPCs showed high proliferative potential, which could successfully repopulate hematopoiesis in murine models, and tolerated cryopreservation and thawing with

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_2

efficient HSC recovery (Broxmeyer et al. 1989). This critical work provided the scientific rationale to evaluate CB as a potential donor source of HSPCs in humans. The first patient to undergo a CB transplant (CBT) was a 5-year-old boy with Fanconi anemia and whose mother was pregnant with an unaffected, fully human leukocyte antigen (HLA)-matched sibling. In preparation for the transplant, the sibling's CB was collected into a sterile bottle containing preservative-free heparin. The CB was then transported to Dr. Broxmeyer's laboratory, diluted with tissue culture media and dimethyl sulfoxide (DMSO), cryopreserved, and stored under liquid nitrogen until it was transported in a dry shipper to Paris, France. In 1988, Dr. Eliane Gluckman performed the first CBT in the world using the sibling's CB as the donor (Gluckman et al. 1989). He successfully engrafted with his sister's cells and remains healthy with full donor chimerism 28 years later. Building on this initial success, additional related donor CBTs were performed in selected centers over the next 5 years (Wagner et al. 1992, 1995; Kohli-Kumar et al. 1993; Broxmeyer et al. 1991). Supported by a pilot grant from the National Heart, Lung, and Blood Institute (NHLBI), Dr. Pablo Rubinstein established the first unrelated donor CB bank at the New York Blood Center in 1992. In the following year, Dr. Joanne Kurtzberg performed the first unrelated donor CBT at Duke University in a 4-year-old child with relapsed T-cell leukemia. The early experience with this child and 24 additional patients transplanted over the next 2 years at Duke demonstrated that partially HLA-mismatched, banked unrelated donor CB could successfully restore hematopoiesis. Engraftment was associated with the total nucleated cell (TNC) dose available relative to the recipient body size, and the incidence of GvHD was lower than expected (Kurtzberg et al. 1996). Shortly thereafter, Wagner et al. published their experience using banked, unrelated CB in 18 recipients with similar findings reported (Wagner et al. 1996). In 1996, the NHLBI funded the cord blood transplantation (COBLT) study to prospectively test the use of unrelated donor CBT in children and adults with diseases commonly treated with HCT. Through this program, three additional unrelated donor banks were established. Standard operating procedures (SOPs) were created for donor recruitment, collection, shipping, processing, testing, long-term storage, and distribution of CBUs (Fraser et al. 1998). Over 11,000 well-characterized diverse CBUs were banked to support multicenter transplantation protocols (Cairo et al. 2005; Kurtzberg et al. 2005). Over the next 5 years, CB was tested as a donor source for children with leukemia, congenital immunodeficiency syndromes, and inherited metabolic diseases and adults with leukemia (Kurtzberg et al. 2005, 2008; Martin et al. 2006; Cornetta et al. 2005; Wall et al. 2005).

With the extension into the unrelated donor setting, the fields of CBT and banking expanded rapidly. In 1995, EUROCORD was established by Dr. Eliane Gluckman and continues to operate on behalf of the European Group for Blood and Marrow Transplantation as an international registry of CBT. In 1996, the parent organizations, International Society for Cellular Therapy (ISCT) and the American Society of Blood and Marrow Transplantation (ASBMT), established the Foundation for the Accreditation of Cellular Therapy (FACT). In 1997, the International NetCord Foundation was established to serve as a registry for international public CB banks. The members of NetCord subsequently created the first international

standards for public CB banking. FACT and NetCord established a joint collaboration to produce the first international standards for accreditation for public CB banks in 1999. In the USA, the National Marrow Donor Program (NMDP) established the Center for Cord Blood in 1998, adding CBUs to their listings on the unrelated donor registry. In 2005, legislation was passed in the US Congress to establish the CW Bill Young Cell Transplantation Program. This program created coordinating centers for CB and adult donors, a single point of access donor registry (both administered through the NMDP), a stem cell outcome database (administered by the Center for International Blood and Marrow Transplant Research, CIBMTR), and the National Cord Blood Inventory (a US network of public banks, NCBI) administered through the Health Resources and Services Administration (HRSA) of the Department of Health and Human Services.

2.3 Overview of Donor Recruitment and Consent

Donor recruitment begins with the identification of potentially eligible mothers as defined by the individual bank based on maternal and infant characteristics. For example, the Carolinas Cord Blood Bank (CCBB) will accept donations from healthy mothers (≥18 years old) who are carrying a healthy term or near-term (≥34-week gestation) singleton gestation. Eligible mothers willing to donate must sign informed consent prior to collection. Some banks use a "mini consent" which grants permission for CB collection and is signed prior to active labor followed by a more extensive informed consent addressing usage of the CBU obtained after delivery and collection. Consent includes permission to collect and potentially bank the CBU for public use or utilize for research if the unit doesn't meet specifications for banking; provide a medical and family history, for the mother to provide a blood specimen to screen for certain communicable diseases; and review medical records of the infant and maternal donors.

2.4 Overview of Collection Techniques

Cord blood can be collected from either vaginal or cesarean births, either prior to delivery of the placenta (in utero) by obstetrical (OB) staff or after delivery of the placenta (ex utero) allowing for trained CB staff to perform collections. Reports have generally observed higher collection volumes after cesarean compared to vaginal deliveries (Kurtzberg et al. 2005; Jones 2003; Santos et al. 2016) and when CB is collected in utero compared to ex utero (Solves et al. 2003a), although reports have been conflicting (Lasky et al. 2002). In an analysis of collections facilitated by the CCBB ($n = 59{,}794$), cesarean deliveries yielded higher collection volume (average 14 ml higher (95% CI 13.6–14.5), $p < 0.0001$) compared to vaginal deliveries after controlling for collection method. Contrary to other reports, ex utero collections resulted in higher collection volumes (average 5 ml higher (95% CI 4.9–5.8), $p < 0.0001$) compared to in utero after adjusting for delivery method. We also

observed an interaction between delivery type and collection method. Delivery by cesarean section yielded collection volumes that were on average 15 ml higher when collected ex utero and 10.5 ml higher when collected in utero, as compared to vaginal deliveries ($p < 0.0001$). Our results also demonstrated that cesarean deliveries collected ex utero had a median volume 19 ml higher than in utero collections from vaginal deliveries. While the delivery method is dictated by the clinical status of the mother and infant, the method of collecting is determined by staffing and collection site practices. Currently, both collection methods continue to be routinely used, but in utero collections are more common, likely due to the additional personnel expenses associated with ex utero collections.

Cord blood is typically collected by cannulating the umbilical vein to allow the placental blood to be removed by gravity into collection containers with anticoagulant, most commonly citrate phosphate dextrose (CPD). While collections occurred in open systems in the early days of CB banking, closed system collection bags were shown to reduce bacterial contamination rates (Bertolini et al. 1995) and are now routinely utilized.

Utilization of publicly banked unrelated donor CBUs in patients is based on delivery of a minimal TNC/kg dose of CB cells. Banks establish thresholds for banking based on the total nucleated cell count (TNCC) and estimate whether or not a particular unit will meet this threshold at various time points during the collection and banking process. After the collection is completed, most banks measure the weight of the collection bag to estimate the collection volume. It is well established that collection volume and TNCC are closely correlated (Fig. 2.1; Table 2.1), and many banks have established minimal volume thresholds to determine which CBUs are shipped to the processing lab. Units with low volume are unlikely to have sufficient TNCC and therefore are discarded at the site. Alternatively, some banks will measure TNCC at the collection site, or use other criteria, to determine which units should be shipped to the processing laboratory.

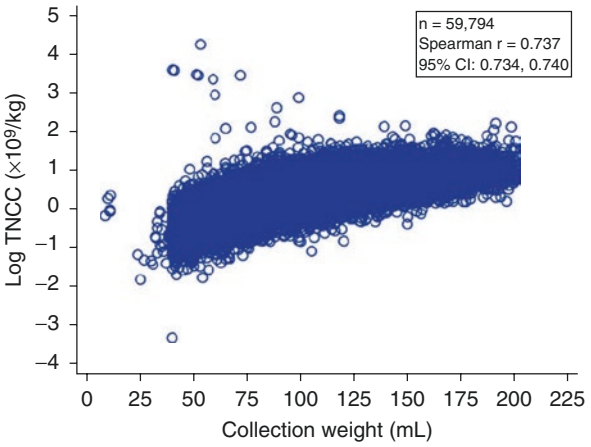

Fig. 2.1 Comparison of pre-processing TNCC and collection volume. In the scatterplot, the pre-processing TNCC (transformed logarithmically) and collection volume for cord blood units collected by the Carolinas Cord Blood Bank ($n = 59{,}794$ collections) are compared (Spearman's correlation coefficient = 0.737, $p < 0.0001$)

Table 2.1 The probability of donated cord blood units containing $<1 \times 10^9$, $1–1.75 \times 10^9$ or $>1.75 \times 10^9$ total nucleated cell content (TNCC) pre-processing based on the collection volume

	Pre-processing TNCC					
	$<1 \times 10^9$		$1 \times 10^9 – 1.75 \times 10^9$		$>1.75 \times 10^9$	
Collection weight (mL)	*N*	Proportion (95% CI)	*N*	Proportion (95% CI)	*N*	Proportion (95% CI)
<60	28,592	0.552 (0.547–0.556)	1,404	0.046 (0.043–0.048)	39	0.003 (0.002–0.004)
60–80	16,193	0.312 (0.309–0.317)	9,396	0.305 (0.300–0.311)	762	0.052 (0.048–0.056)
>80–100	4,991	0.096 (0.094–0.099)	11,420	0.371 (0.366–0.377)	3,299	0.224 (0.218–0.231)
>100–125	1,493	0.029 (0.027–0.030)	6,777	0.220 (0.216–0.225)	5,285	0.359 (0.352–0.367)
>125–150	385	0.007 (0.007–0.008)	1,494	0.049 (0.046–0.051)	3,346	0.228 (0.221–0.234)
>150	165	0.003 (0.003–0.004)	272	0.009 (0.008–0.010)	1,970	0.134 (0.129–0.140)
TOTAL	*51,819*		*30,763*		*14,701*	

TNCC total nucleated cell content, *CI* 95% Clopper-Pearson confidence interval

Efforts to increase collection volume have focused on two general approaches: identifying donations likely to have higher collection volume or developing techniques to obtain the maximal volume from an individual donation. Multiple reports have demonstrated relationships between characteristics of the mother, infant, or delivery with increased collection volume, TNCC, $CD34^+$, or colony-forming units (CFU) content of CBUs. Increased donor birth weight and older gestational age have been closely associated with higher collection volume and TNCC (Askari et al. 2005; George et al. 2006; Ballen et al. 2001), although our data which showed collections from younger infants (34–37 weeks gestation) were more likely to have higher progenitor cell content as measured by $CD34^+$ and CFU content (Fig. 2.2) (Page et al. 2014). While several studies have demonstrated comparable collection volumes among donors of races or ethnicities, the TNCC, $CD34^+$, and CFU content, all adjusted for collection volume (counts/mL), were significantly lower in African-American donors compared to Caucasian donors even after adjusting for other clinical factors (Fig. 2.3) (Kurtzberg et al. 2005; Page et al. 2014). This is likely due to differences in cellular adherence between Caucasian and African-American individuals (Reiner et al. 2011). Other clinical factors, such as gender and maternal age, have been investigated, but results have been less conclusive (Jones 2003; Page et al. 2014; Jan et al. 2008;

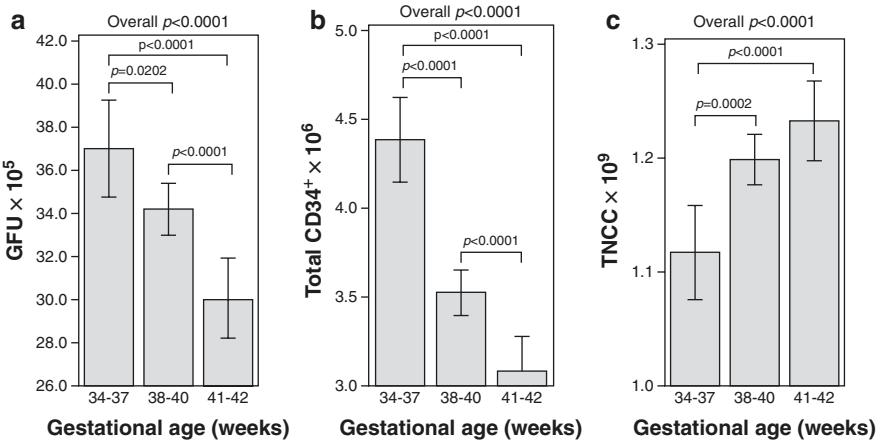

Fig. 2.2 Impact of infant-estimated gestational age on the CFU, CD34⁺, and post-TNCC content. In (**a**–**c**), the adjusted mean CFU (**a**), CD34⁺ (**b**), and post-TNCC (**c**) is shown in relationship to infant gestational age after adjusting for infant race/ethnicity, birth weight, sex, collection volume, delivery type, and maternal age. Only significant *p* values are shown. Whisker plots represent the 95% CIs (Used with permission) (Page et al. 2014)

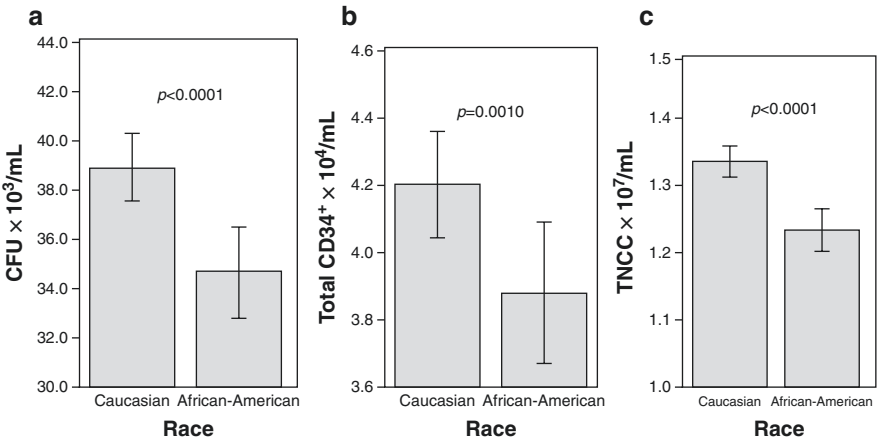

Fig. 2.3 Comparison of the CFU, CD34⁺, and post-TNCC concentrations for Caucasian and African-American infants. In (**a**–**c**), the adjusted mean CFUs/mL (**a**), CD34⁺/per mL (**b**), and post-TNCC/mL (**c**) is shown in relationship to race for infants of Caucasian and African-American race, respectively, after adjusting for infant gestational age, birth weight, sex, collection volume, delivery type, and maternal age. Only significant *p* values are shown. Whisker plots represent the 95% CIs (Used with permission) (Page et al. 2014)

Solves et al. 2012). Understanding these relationships between clinical characteristics and collection volume or other CB measurements, while typically not modifiable, can help to inform practical decisions such as banking eligibility, staffing at collection sites, etc.

Technical approaches to increasing CBU collection volume include increasing perfusion of the placenta to collect additional blood (Bornstein et al. 2005; Tan et al. 2009), but these approaches remain experimental. The timing of cord clamping also affects the volume of blood collected from a placenta. The practice of delayed cord clamping, defined by the American Congress of Obstetricians and Gynecologists (ACOG) as occurring >30 s after delivery (ACOG 2017), is becoming more common. While studies have shown benefits of delayed cord clamping for preterm infants, the benefits in term infants appear to be marginal (McDonald et al. 2014). Delays in collections have been associated with smaller volumes (Frändberg et al. 2016) and corresponding TNCCs and do increase collection failures due to clotting (Jones 2003; Allan et al. 2016; Solves et al. 2003b). Furthermore, there is ample evidence that the blood flow within the umbilical vessels immediately after birth is influenced by multiple physiologic factors, most notably infant lung aeration (Hooper et al. 2016). Therefore, it is not a simple time-dependent process. While it is apparent that further studies are needed to better understand the impact of cord clamping on the neonate, it is also clear that this will be an ongoing discussion with important obstetric, perinatal, and banking implications.

2.5 Overview of Current Processing and Cryopreservation Techniques

Currently, many banks receive collections from distant sites, and, therefore, delays in processing related to travel might exist. Results of the COBLT study indicated that TNCC and $CD34^+$ content remained relatively stable at room temperature for >48 h leading to the practice that cryopreservation of a processed CBU must begin within 48 h of collection (Kurtzberg et al. 2005). Others have demonstrated decreases in viability and cell content when aliquots were tested from 24–96 h after collection (Pereira-Cunha et al. 2013; Louis et al. 2012; Solomon et al. 2010; Guttridge et al. 2014). Our own experience has demonstrated small but significant losses of TNCC, $CD34^+$ cells, and CFU content at even earlier time points (Fig. 2.4) (Page et al. 2014) with similar findings reported recently by others (Wu et al. 2015; Dulugiac et al. 2014). We have therefore modified our standard operating procedures at the CCBB to prioritize processing of CBUs within 24 h of collection.

The overall approach to processing CB is similar between banks, although variations in technique do exist. Rubinstein et al., in their pivotal work, demonstrated that volume reduction achieved through plasma and red blood cell (RBC) depletion allowed for more efficient processing, cryopreservation, and cell recovery after thaw (Rubinstein et al. 1995). To this day, most CB banks employ these processing methods, or variations of it, to achieve plasma and RBC depletion. While manual CB processing continues to be performed in some banks, an increasing number of banks are using automated systems for plasma and RBC reduction. A comparison between two automated systems, Sepax© (Biosafe, Switzerland) and AutoXpress Platform or AXP© (Cesca Therapeutics, Rancho Cordova, CA), was performed at the Valencia CB bank. Both systems demonstrated acceptable cell recovery. The

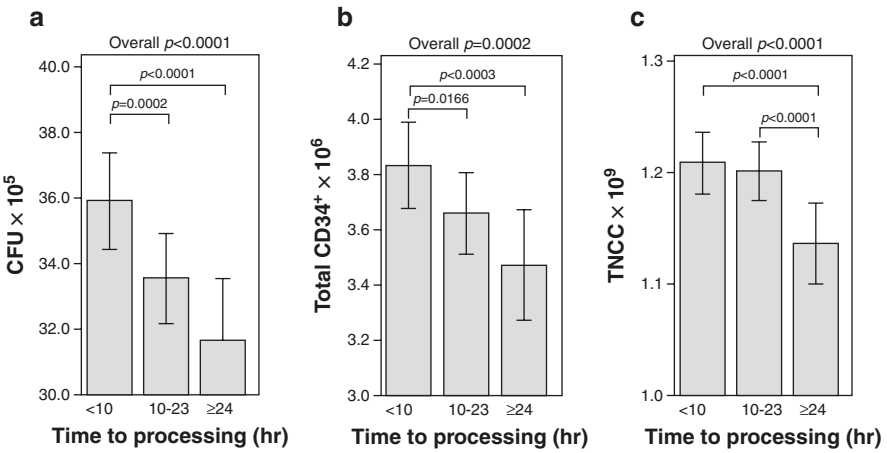

Fig. 2.4 Impact of time to processing on CFU, CD34⁺, and post-processing TNCC content. In (**a**–**c**), the adjusted mean CFUs (**a**), CD34⁺ (**b**), and post-processing TNCC (**c**) by time to processing is presented after adjusting for infant race/ethnicity, sex, gestational age, birth weight, collection volume, delivery type, and maternal age. Only significant *p* values are shown. Whisker plots represent the 95% confidence intervals (Used with permission) (Page et al. 2014)

Sepax© system, using hydroxyethyl starch (HES) as a sedimentation agent, did have improved TNCC recovery, whereas the AXP© system was especially efficient in RBC removal without HES. Recent issues with HES availability in Europe led Schwandt et al. to develop and validate a non-HES Sepax© protocol (Schwandt et al. 2016). Comparable post-processing recoveries were achieved although lower post-thaw CD34⁺ viability was noted with the non-HES protocol supporting the recommendation that HES protocols are preferred. Other automated systems for CB processing include PrepaCyte-CB (BioE, St. Paul, MN) and Macopress Smart (Macopharma, Mouvaux, Fr). Post-processing, DMSO, typically in a final concentrations of 10% along with 5% dextran or HES, is added as a cryoprotectant (Fry et al. 2013; Lecchi et al. 2016). Other concentrations of DMSO and other agents (i.e., trehalose) have been investigated, but DMSO (10%) in dextran continues to be the most commonly used cryoprotectant (Motta et al. 2014). Cryopreservation occurs via controlled-rate freezing before storage in the liquid or vapor phase of liquid nitrogen for long-term storage at less than −180 °C.

2.6 Cord Blood Banking Standards and Regulations

To ensure quality, CB products are available for patient use; standards have been developed by accrediting agencies, e.g., FACT/NetCord and AABB (formerly the American Association of Blood Banks), for CB collection, processing, and banking. These standards are the result of evidence-based consensus and establish minimal acceptable practices. Although participation is considered voluntary, many public CB banks are required to receive accreditation from FACT/NetCord or AABB to

participate in registries, receive reimbursements, etc. Many countries now regulate CB products in an effort to ensure quality and safety. In the USA, the Food and Drug Administration (FDA) regulates unrelated donor CB as biological product and issued final guidance for public banks to obtain a Biological License Agreement (BLA) in 2011. Currently six public banks in the USA have obtained a BLA.

2.7 Assessing Quality and Potency of a CBU

Banking standards require that CB products be extensively tested and characterized to assess purity, potency, and sterility of the CB unit. Testing in most banks includes assessing post-processing viability, TNCC, viable $CD34^+$ cells, growth of CFUs, and sterility. In the sections below, we discuss different methods of assessing quality and potency, review benefits and disadvantages to the assays, and briefly review the clinical impact of these measures.

2.7.1 Viability

Assessing viability is included in the banking standards for accreditation and is required for unit licensure. Guidelines require at least 85% viable cells as measured on post-processed samples. While fresh CB generally has high viability, insults to cells that can decrease viability include temperature excursions, longer time to processing, and prolonged exposure to DMSO prior to cryopreservation (Solomon et al. 2010; Dulugiac et al. 2014; Fry et al. 2013). The various cell populations contained in CB tolerate these stressors differently (Solomon et al. 2010). For example, decreases in viability may simply reflect cell death of mature granulocytes and may not reflect loss of HSPCs.

Historically, viability has been measured by staining for dying cells with trypan blue (TB) and scored either manually or using automated systems. TB is difficult to standardize and is generally felt to overestimate cell viability. More sensitive methods have been developed in the past decade and include acridine orange (AO), propidium iodide (PI), and 7-amino-actinomycin D (7-AAD) or annexin V. AO and PI are nucleic acid-binding dyes used commonly to measure cell viability. AO can pass freely into nucleated cells generating a green fluorescence. Whereas PI enters cells with compromised membranes, the red fluorescence emitted indicates a dying or necrotic cell. Automated systems allow for images to be captured and viability to be calculated. While the TB and AO/PI assays are rapid and technically easy to perform, both assays may overestimate the viability of samples by measuring only necrotic cells that have lost cell surface integrity. Nonviable cells that are earlier in the apoptotic pathway will not score positive. Other methods of assessing viability include measurement of 7-AAD or annexin V by flow cytometry. 7-AAD is a fluorescent DNA dye, whereas annexin V binds to the extracellular phosphatidylserine of early apoptotic cells. Both are able to distinguish cells earlier in the apoptotic pathway allowing for more accurate assessments of viability (Solomon et al. 2010;

Radke et al. 2013; Duggleby et al. 2012). Flow cytometry-based viability assays also allow for the viability of specific subsets to be assessed, which will be discussed further below.

2.7.2 CD34⁺ Cell Content

As a surface marker of HSPCs, it is a common practice to enumerate viable CD34⁺ cells prior to cryopreservation and again after thawing for transplantation. Efforts to standardize CD34⁺ measurements led to the development of guidelines by ISHAGE (International Society for Hematotherapy and Graft Engineering) (Sutherland et al. 1996). This "dual platform" method determined the percentage of CD34⁺ cells by flow cytometry and measured the leukocyte count using an automated cell counter. Subsequently, "single platform" approaches have been developed that enumerate CD34⁺ cells using flow cytometry (Brocklebank and Sparrow 2001; Sutherland et al. 2009). Most recently, FDA-cleared kits to enumerate viable CD34 cells have become available and adopted for use by many CB banks.

The importance of CD34⁺ cell dosing in CB grafts was demonstrated early on by Wagner et al. in 102 patients with malignant or nonmalignant diseases who received a single-unit CBT. Patients who received $>1.7 \times 10^5$/kg CD34⁺ cells infused experience higher rates of engraftment, less transplant-related mortality, and improved overall (Wagner et al. 2002). As such, some transplant centers utilize the total CD34⁺ cell dose in CBU selection recognizing that significant interlaboratory variability exists (Lemarie et al. 2007; Dzik et al. 1999; Moroff et al. 2006; Wagner et al. 2006). In our series of 435 recipients of CBT, we demonstrated that the post-thaw total CD34⁺ dose measure using the ProCOUNT© assay (BD Biosciences, San Jose, CA) was a significant predictor of neutrophil engraftment in multivariate analysis ($p = 0.04$) but to a lesser degree than post-thaw CFU ($p < 0.0001$). The total CD34⁺ dose was also weakly associated with overall survival at 6 months posttransplantation (Fig. 2.5) (Page et al. 2011a).

The presence of total CD34⁺ cells in a given CBU does not assess the viability and overall potency of the unit. This led to interest in measuring the viable CD34⁺ content. Previously, banks indirectly assessed the viable CD34⁺ content using the percent viable cells to adjust the total CD34⁺ dose. More recently, multiparametric flow cytometry methods have been developed to measure CD34⁺ in the presence of a viability marker (7-AAD; Stem Cell Enumeration Kit, BD Biosciences, San Jose, CA; Stem-kit, Beckman Coulter, Brea, CA) (Sutherland et al. 2009; Preti et al. 2014). While validation studies have demonstrated that the total and viable CD34⁺ content in fresh cord correlate closely (Preti et al. 2014; Dauber et al. 2011; Massin et al. 2015), measurements performed on thawed CB samples show more variability (Dauber et al. 2011). To date, it remains unclear how the viable CD34⁺ content of a CB graft will impact clinical outcomes since very little data is available in the literature. Purtill et al. investigated the impact of the viability of CD34⁺ cells measured post-thaw in adult patients receiving double CBT. A higher viable CD34⁺ cell dose correlated with faster engraftment kinetics in the

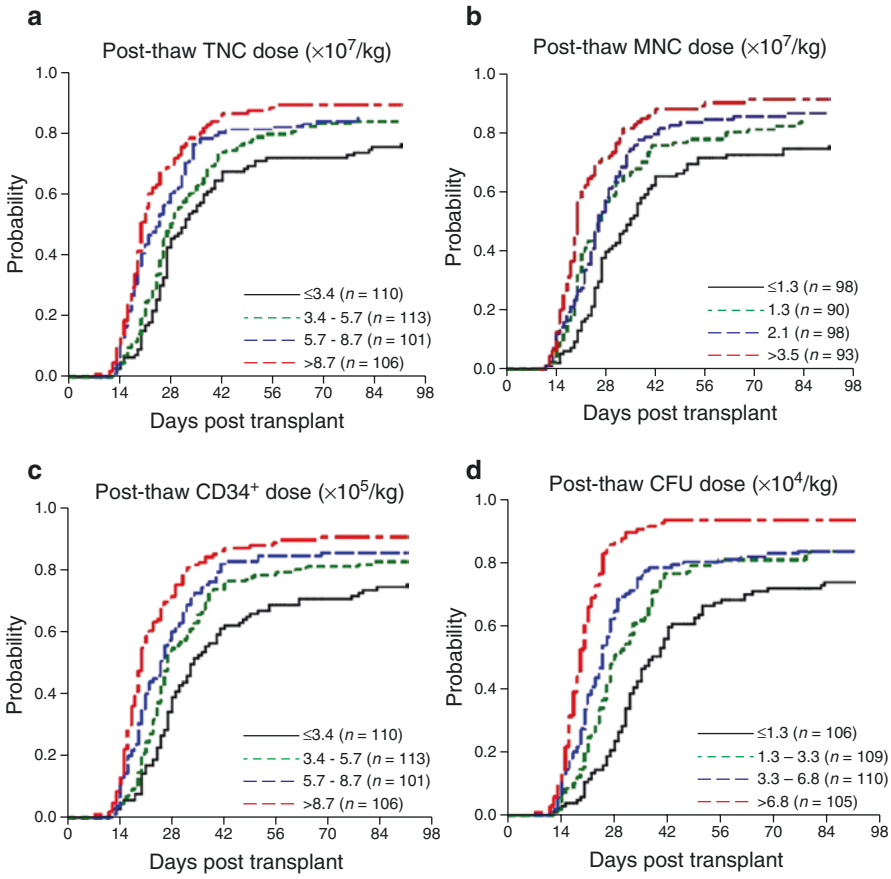

Fig. 2.5 Impact of post-thaw graft characteristics on the probability of neutrophil engraftment. Probability plates are shown for each of the four quartiles. Panels (**a**–**d**) depict the impact of post-thaw TNC ($\times 10^7$/kg recipient weight), MNC ($\times 10^7$/kg recipient weight), $CD34^+$ ($\times 10^5$/kg recipient weight), and CFU ($\times 10^4$/kg recipient weight) doses, respectively, on neutrophil engraftment (Used with permission) (Page et al. 2011a)

engrafting unit (Purtill et al. 2013, 2014). The use of the viable CD34 in CB unit selection will require further standardization of the methods for CD34 enumeration by the CB banking community.

2.7.3 Colony-Forming Units (CFUs)

CFU growth is used by many banks as a measure of potency and can be tested on a sample of processed CB or on a thawed segment post-cryopreservation. Identification and enumeration of colony types (CFU-GM, CFU-GEMM, and BFU-E) are performed by some banks, but specifications for these parameters are

unknown. Although difficult to standardize and generally perform over 14 days, the CFU assay, which requires that viable cells multiply and differentiate, is considered by many to be the best measure of CB potency. The importance of CFU dosing on clinical outcomes after CBT was first reported by Migliaccio et al. (2000) who observed that the pre-cryopreservation CFU dose better predicted neutrophil and platelet engraftment as compared to pre-cryopreserved TNCC. Post-thaw CFU content was reported by Wall et al., on behalf of the COBLT study, to best predict engraftment and OS at 2 years (Wall et al. 2005). Extending the findings of Prasad et al. (2008), we observed in a cohort of 435 primarily pediatric patients receiving single CBT that higher CFU dosing was the only pre-cryopreservation graft characteristic predictive of neutrophil ($p = 0.0024$) and platelet engraftment ($p = 0.0063$) in multivariate analysis. Likewise, post-thaw CFU content best predicted neutrophil and platelet engraftment (both $p < 0.0001$) (Page et al. 2011a). Recently, Castillo et al. demonstrated that the clonogenic efficiency (defined as the post-thaw CFU/pre-freeze $CD34^+$) along with viable $CD45^+$ cell dose was associated with faster engraftment and improved survival (Castillo et al. 2015).

Despite the ability to assess potency, the CFU assay has several issues that currently preclude its widespread use in banking and donor selection. It is a time-consuming assay that provides results weeks later. Similar to measuring $CD34^+$ content, there are also issues with standardization between laboratories (Pamphilon et al. 2013; Brand et al. 2008). Automated scoring systems and 7-day CFU assays have been developed to address these issues, and these approaches are becoming more commonly used. There have also been focused efforts to develop alternate measures of potency that would provide results rapidly. Enumeration of CFUs using a thawed contiguous segment has been shown to be a representative of the CB product and has been used to assess potency (2003).

2.7.4 Aldehyde Dehydrogenase

Aldehyde dehydrogenase (ALDH) is an intracellular enzyme found in high concentration in HSPCs scoring positive ($ALDH^{br}$) in this flow cytometry-based assay that are viable and likely to correlate with HSPC content of a graft (Balber 2011). $ALDH^{br}$ activity strongly correlated with CFUs and with speed of engraftment in autologous transplant recipients (Lee et al. 2014; Frandberg et al. 2015; Gentry et al. 2007; Fallon et al. 2003). This suggests that $ALDH^{br}$ content of a CBU may predict potency. At the CCBB, we have examined the $ALDH^{br}$ content of fresh and thawed CB. In fresh CB, $ALDH^{br}$ correlates well with TNCC, CFU, and $CD34^+$ content (Page et al. 2011b). However, potency of a CB graft is best assessed on the thawed product thereby reflecting any potential injury incurred due to cryopreservation and thaw. Therefore, we developed a potency assay for CBU release that can be performed at the time of confirmatory testing using a segment attached to a cryopreserved CBU. The assay enumerates $ALDH^{br}$, $CD34^+$,

$CD45^+$, glycophorin A^+, viability ($7\text{-}AAD^+$), and CFUs from the thawed segment (Fig. 2.6). Our study demonstrated a strong correlation between $ALDH^{br}$ and CFUs measured on the segment ($r = 0.78$). However, the correlation between $CD34^+$ (as a percentage of viable CD45 cells) and CFUs was weaker ($r = 0.25$).

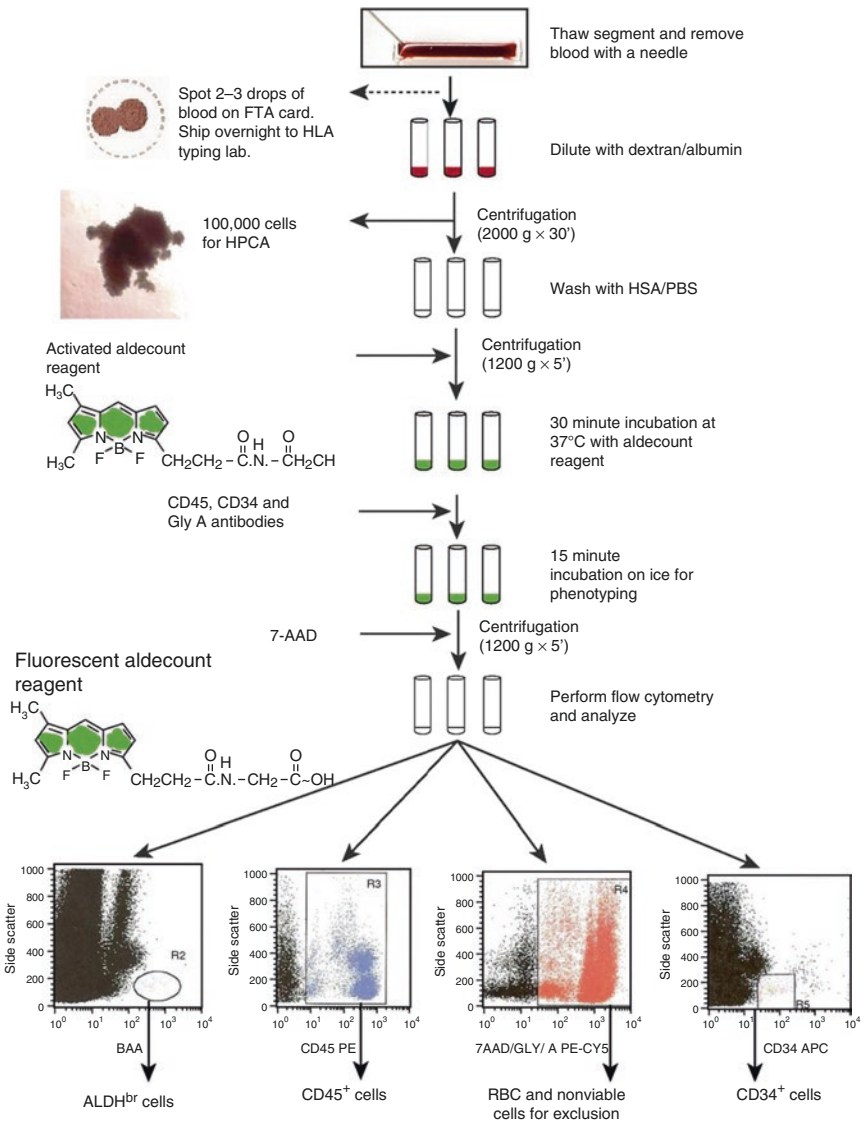

Fig. 2.6 Flowchart of the ALDH potency assay performed on attached segments of CBUs requested for CT for donor selection. *7-AAD* 7-aminoactinomycin D, *FTA* fast technology analysis, *GlyA* glycophorin A, *HSA/PBS* human serum albumin/phosphate-buffered saline, and *HPCA* hematopoietic progenitor cell assay (Used with permission) (Shoulars et al. 2016)

Comparisons between cryopreserved segments and entire unit demonstrated strong overall correlation ($r = 0.88$). We also observed faster engraftment in patients who received CB grafts with higher $ALDH^{br}$ measured on the segment ($p = 0.03$). Our findings have demonstrated that the assay can serve as a surrogate for post-thaw measurements to assess potency of a potential CBU graft. Based on these findings, we have been using this assay prior to releasing CBUs from the CCBB to the transplant centers.

2.7.5 Sterility

To prevent potential transmission of microbial agents to transplant recipients, all CB banks perform sterility assays on samples of processed cord blood units obtained prior to cryopreservation. Screening units for bacterial and fungal contamination is most commonly performed using automated culture systems with high detection capabilities (Khuu et al. 2006; Akel et al. 2013). Reported rate of contamination in the literature have been variable but generally range from 2% to 5% (Kurtzberg et al. 2005; Clark et al. 2012; Gutman et al. 2011). Considering over 13,000 processed CBUs, Clark et al. reported a contamination rate of 4%. In multivariate regression models, collections performed by OB staff, as contrasted with trained, dedicated collection staff, or after vaginal, as opposed to C-section delivery, had higher contamination rates in their series. Not surprising, vaginal and skin flora were the most common contaminates reported in the literature (Clark et al. 2012). To limit the use of CB volume for testing, investigations using pediatric culture bottles (smaller sample volume) or using processing by-products (red blood cells or plasma) have been performed. The use of pediatric culture bottles or plasma as a test sample was associated with high false-negative rates. Therefore, mixtures of RBCs and plasma spiked into adult culture bottles are considered standard practice (Ramirez-Arcos et al. 2015). CBUs screening positive in sterility assays are excluded from public bank registries. However, directed donor units that remain the best donor for a related patient which are contaminated with bacteria may be stored and used for transplantation after the recipient is covered with appropriate antimicrobial antibiotics before and after the infusion.

2.8 Finances of Public CB Banking

Public CB banking is an expensive, time-intensive endeavor. The nature of CB banking is such that extensive resources are required up front to collect, process, cryopreserve, and bank CBUs. The inventory of CBUs must be of sufficient size to provide high TNCC units representing a wide range of HLA types. Costs are partially recouped when CBUs are procured for transplantation. For most public CB banks, this is the primary source of income. Government or philanthropic

support is available for a small portion of public banks, but this may not be sufficient or reliable sources of funding. Therefore, public banks are facing a challenge to be financially self-sustainable. Individual banks can examine their practices to identify potential areas where costs can be minimized; however, many costs required for operations (i.e., supplies, equipment, and other capital costs) are fixed in nature. Since significant funds are dedicated to personnel, especially collection staff, the CCBB recently evaluated the various staffing models used at our collection sites. We found that collections performed by trained CB staff are more likely to be banked (35% *vs.* 18% of collections performed by OB staff). However, this benefit is offset by the fact that OB staff collections are more economical with respect to personnel costs. Despite this, our "best" collection site (i.e., highest proportion of collected units being banked) is fully staffed with CB bank personnel. At this site, the higher number of banked units offset the increased costs associated with additional personnel. Ultimately, individual CB banks must tailor staffing models to their own needs and the needs of their collection sites.

The banking community has actively been discussing a policy change which could lead to cost savings. The US public banking experience which provides unrelated donors for patients undergoing HCT is useful to illustrate this approach. Currently, public banks participating in the NCBI list CBUs with a minimum pre-cryopreservation TNCC of 0.9×10^9 cells. The resulting inventory contains CBUs with a median TNCC of 1.2×10^9 cells. In comparison, the median TNCC of CBUs selected for transplantation is 1.8×10^9 cells (Bart et al. 2012). Therefore, only a small portion of the inventory is likely to be selected for transplantation. It has been proposed that financial resources could be better utilized if only high TNCC units were processed and banked. Magalon et al. recently modeled a concept using registry-based data and concluded that increasing the minimum TNCC required for banking to 1.8×10^9 would be the most cost-effective strategy (Magalon et al. 2015). Our own data demonstrated that such an approach decreases operating costs but would also result in a less racially/ethnically diverse inventory (Page et al. n.d.). Until definitive data is available to show that a higher degree of HLA matching is not needed to optimize outcomes, it is important that inventory diversity is considered in this discussion. Furthermore, our data demonstrated that while these units may contain higher TNCC content, this does necessarily reflect the health or potency of the unit (Fig. 2.7). Therefore, while increasing the minimum TNCC may be a cost-effective strategy, the impact on inventory diversity, quality, and potency needs to also be considered. It is also possible that new cellular therapies using CB that are in preclinical or early phase clinical trials may provide another avenue for smaller CBUs to be used from the inventory, but this is difficult to accurately estimate. Ex vivo CB expansion techniques, discussed in an accompanying chapter, may allow for improved utilization of smaller units. In the interim, public CB banks need to explore methods, individually and collaboratively, to remain financially self-sustainable.

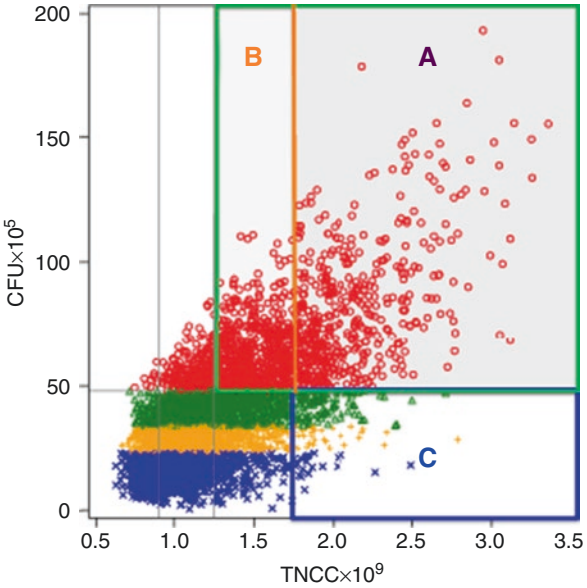

Fig. 2.7 Considering the potency (CFU content) along with post-TNCC in donor selection. The relationship between post-TNCC and the CFU content (i.e., potency) is shown for the study cohort (n = 5267 CBUs). The *vertical lines* represent (from *left* to *right*) the minimum post-TNCC required for banking (0.9×10^9), an intermediate post-TNCC (1.25×10^9), and the median post-TNCC of CBUs selected for transplantation (1.75×109). Quartiles for the CFU content are also represented (upper quartile *red circle*, second quartile *green triangle*, third quartile *gold* +, lower quartile *blue* ×). Region *A* refers to CBUs with post-TNCC of greater than 1.75×10^9 and CFU in the highest quartile. Region *B* refers to post-TNCC of 1.25×10^9 to 1.75×10^9 with CFUs in the highest quartile. Region *C* refers to CBUs with post-TNCC of more than 1.75×10^9 and CFUs in the lower three quartiles (Used with permission) (Page et al. 2014)

2.9 Family (or Private) Banking

Family banks (or private) provide an option for families wishing to store CB for personal use and willing to pay an up-front and yearly fee. Generally, these banks are "for profit" businesses and often advertise heavily to pregnant women and their providers. In actuality, the likelihood of using a privately banked CB for transplantation is quite low (Ballen et al. 2008a). Therefore, the American Academy of Pediatrics (AAP), ACOG, American Society of Blood and Marrow Transplantation, and other similar organizations worldwide do not currently recommend banking CB for personal use in a typically healthy family (Lubin and Shearer 2007; ACOG Committee Opinion No. 648: Umbilical Cord Blood Banking 2015; Ballen et al. 2008b). Guidelines from these groups stress the importance of providing pregnant women with unbiased information regarding all banking options. An exception to these guidelines is families with a history of disease (e.g., malignancy or hemoglobinopathy) that is amenable to HCT. Outcomes of CBT using sibling donors have

overall been quite successful (Gluckman et al. 2011; Screnci et al. 2016), and, therefore, banking of related CB is indicated. To facilitate this, many public and family banks offer "directed donor" programs that waive any associated banking fees. While directed donation is indicated in limited settings, continued advances in cellular therapies will likely lead to expanded indications.

Currently, family banks are not subjected to the same regulatory oversight as public banks although this varies between countries. Family banks generally use less stringent criteria for banking leading to wide variations in volume and TNCC of the private inventory. In our study of autologous CB infusions to treat acquired brain injury, CBUs from family banks were inferior to those stored in public banks with respect to collection volume, TNCC, and $CD34^+$ count (Fig. 2.8) (Sun et al. 2010). Since families bank their child's CB as "medical insurance," it is important that these banks do their utmost to ensure quality of the banked units. However, the changing landscapes of CB transplantation and regenerative medicine will likely change the indications and criteria a CBU must meet for use. In response to these changes, the role of regulatory oversight in family CB banking will need to be defined further.

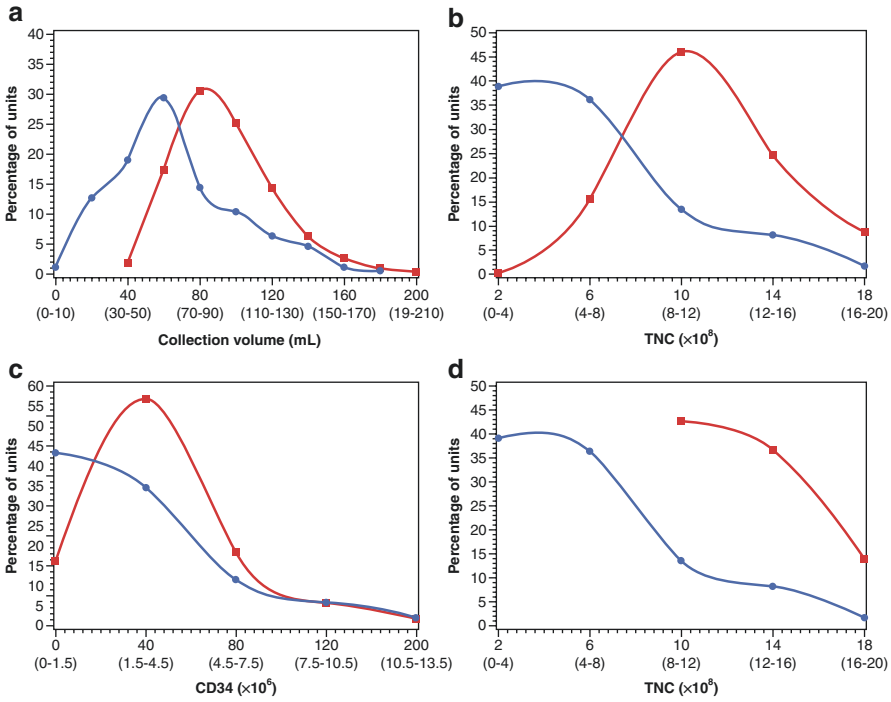

Fig. 2.8 Distributions of quality variables. In panels (**a**–**c**), the distribution of autologous CBUs is compared to the entire Carolinas Cord Blood inventory with respect to collection volume (**a**), TNC (**b**), and CD34 content (**c**). In panel (**d**), TNC of autologous CBUs [represented as *red* •] and NCBI-eligible Carolinas Cord Blood Bank CBUs [represented as *blue squares*] are compared (Used with permission) (Sun et al. 2010)

Conclusions

As the fields of CB banking and transplantation have matured into an established therapy, focus has turned to refining the use of CB for HCT and developing novel indications in the emerging field of regenerative medicine. Promising results in clinical trials using ex vivo expansion technologies will further enhance CBT and may provide an avenue for smaller units to be used. Success of these therapies relies heavily on the availability of reliable sources of healthy and potent CBUs. It is clear that CB banks are well positioned to play a major role in these exciting endeavors. However, many public CB banks are financially stressed which threatens their existence. Therefore, it is important that the banking community in partnership with regulatory bodies consider strategies that allow for financial self-sustainability while maintaining a quality and diverse inventory.

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Part I

Strategies for Cord Blood Graft Selection

Single or Double Cord Blood Unit for Transplant: What Have We Learned?

3

Jaime Sanz, Juan Montoro, José Luis Piñana, and Guillermo F. Sanz

3.1 Introduction

Umbilical cord blood transplantation (UCBT) offers an allogeneic effect for virtually all patients in need due to its unique capacity of tolerating HLA disparity and rapid availability. However, the main obstacle to a more widespread use of UCBT in adults has been the short-term risk of graft failure and delayed hematopoietic recovery attributed to the low progenitor cells content of the graft, which is over ten times lower than in a marrow harvest or peripheral blood collection. Most of the research in the field has focused on trying to overcome the cell dose limitation, which is in fact a critical determinant of outcomes (Barker et al. 2010; Rocha et al. 2009; Wagner et al. 2002). Different approaches have been used such as combining two UCB units, co-infusion of mobilized blood cells from a third-party donor (Fernandez et al. 2003), and ex vivo treatment and/or expansion of one or more UCB units (Bari et al. 2015). This chapter will focus on a critical analysis of one of the most widely used: the double cord blood transplantation (dUCBT), an approach that was originally conceived to enhance engraftment by augmenting graft cell dose with the combined transplantation of two partially HLA-matched UCB units (Barker et al. 2005).

3.2 Development of the Double Cord Blood Unit Platform

The most determinant factor associated with outcomes after UCBT is the UCB cell dose infused by recipient's weight, considering either the number of total nucleated cells (TNC) or $CD34^+$ cells. An empiric, simple, and straightforward approach to

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_3

increase cell dose was to infuse two UCB units, using standard criteria of HLA compatibility (at least four out of six HLA matches) between UCB units and the recipient. The first reported case was published by the Minnesota group in 2001 showing dual donor chimerism after transplantation of two partially HLA-matched unrelated UCB donors (Barker et al. 2001). The notion gained further attention with a report of 21 adults receiving a pretransplant conditioning with fludarabine, cyclophosphamide, and total body radiation, a graft of two partially HLA-matched cord blood units followed by graft-*versus*-host disease (GvHD) prophylaxis with cyclosporine and mycophenolate mofetil (Barker et al. 2005). This strategy proved to be safe and feasible leading to long-term engraftment of only one unit (so-called predominant unit). Compared to historical controls, receiving one UCB unit but also after different pretransplant conditioning and different GvHD prophylaxis, engraftment rates were improved. This improvement was attributed to a possible "graft-*versus*-graft" effect, in which the non-sustained unit facilitates engraftment of the predominating unit by immunological mechanisms. Since then the activity of dUCBT in adults increased dramatically and soon surpassed the number of patients transplanted with a single unit (sUCBT). dUCBT is to date the most common UCBT procedure worldwide (Rocha et al. 2010), particularly for adults, among recipients of conventional and reduced-intensity transplants for leukemia and other disorders (Brunstein et al. 2010, 2011).

3.3 Comparative Studies

Whether or not the infusion of two UCB units improves transplant outcomes is controversial and has been evaluated in a number of studies in both children and adults that are summarized in Table 3.1. Two prospective randomized trials have been recently published in children and young adults up to 35 years of age that have clearly established the lack of benefit of dUCBT over an adequately sized single UCBT (Wagner et al. 2014; Michel et al. 2016). However, in adult patients the relative role of dUCBT is much more controversial since the evidence is based on retrospective studies, including heterogeneous transplant platforms with different indications for transplant, conditioning regimens, GvHD prophylaxis, criteria for unit selection, and supportive care (MacMillan et al. 2009; Verneris et al. 2009; Rodrigues et al. 2009; Sanz et al. 2013; Ruggeri et al. 2014; Scaradavou et al. 2013).

The first comparative studies were reported by the Minnesota group in two retrospective single-center analyses (MacMillan et al. 2009; Verneris et al. 2009). Those patients with a suboptimal cell dose were allocated for dUCBT and compared with those transplanted with a sUCBT with an adequate cell dose. Although minimum acceptable cell dose for a single unit changed slightly in different time periods, after 2003 the target dose TNC was $\geq 3 \times 10^7$/kg in HLA-matched units and $\geq 4 \times 10^7$/kg in HLA-mismatched units. These investigators postulated more potent alloreactivity with an increased risk of GvHD (MacMillan et al. 2009) and a decreased risk of leukemia relapse (Verneris et al. 2009). A subsequent multicenter study using data from CIBMTR registry was unable to reproduce these findings and observed no

Table 3.1 Comparative studies of single *versus* double umbilical cord blood transplantation

	Verneris et al. (2009)	MacMillan et al. (2009)	Rodrigues et al. (2009)	Sanz et al. (2013)	Scaradavou et al. (2013)	Ruggeri et al. (2014)	Wagner et al. (2014)	Michel et al. (2016)
Study design	Retrospective Single center	Retrospective Single center	Retrospective Registry based	Retrospective Single center cohorts	Retrospective Registry based	Retrospective Registry based	Prospective randomized	Prospective randomized
Number of patients sUCBT/dUCBT	84/93	80/185	78/26	102/91	106/303	156/83	113/111	74/71
Study population	Children and adults	Children (>10 years) and adults	Adults	Adults	Adults	Adults	Children and adolescents (<22 years)	Children and young adults (<35 years)
Baseline disease	Acute leukemia	Variety of hematological diseases	Lymphoid malignancies	Acute leukemia	Acute leukemia	Acute leukemia	Hematological malignancies	Acute leukemia
Conditioning regimen	Myeloablative	Reduced intensity and myeloablative	Reduced intensity and myeloablative	Myeloablative	Reduced intensity and myeloablative	Myeloablative	Myeloablative	Myeloablative
Minimum TNC $\times 10^7$/kg	>3 (6/6 HLA match) >4 (4–5/6 HLA match)	>3 (6/6 HLA match) >4 (4–5/6 HLA match)	>1	>2	>2.5	>2.5	>2.5	>3
Outcomes of dUCBT compared to sUCBT								
Engraftment	No difference	NA	No difference	↓ Neutrophil and platelet recovery	No difference	No difference	No difference in neutrophil recovery ↓ Platelet recovery	No difference

(continued)

Table 3.1 (continued)

	Verneris et al. (2009)	MacMillan et al. (2009)	Rodrigues et al. (2009)	Sanz et al. (2013)	Scaradavou et al. (2013)	Ruggeri et al. (2014)	Wagner et al. (2014)	Michel et al. (2016)
GvHD	↑ acute grade II	↑ acute grade II	No difference	↑ acute grade II	↑ acute grade II (only in early period)	↑ acute grade II	↑ severe acute and chronic extensive	↑ chronic extensive
Relapse	↓ (only in CR2)	NA	↓	↓ (only in ALL)	No difference	No difference	No difference	No difference
NRM	No difference	No difference	No difference	No difference	No difference	No difference	No difference	No difference
Survival	No difference	NA	No difference	No difference	No difference	No difference	No difference	No difference

NA not available, *CR1* first complete remission, *GvHD* graft-*versus*-host disease, *NRM* non-relapse mortality

difference in any transplant outcome of dUCBT compared to adequately dosed sUCBT defined as TNC $\geq 2.5 \times 10^7$/kg (Scaradavou et al. 2013). Authors concluded that the infusion of two partially HLA-matched UCB units could effectively "create" an adequately dosed graft for patients lacking access to a single UCB unit containing 2.5×10^7 TNC/kg and therefore extend the application of UCBT in adults.

A further study from the Eurocord registry compared dUCBT to sUCBT with TNC $\geq 2.5 \times 10^7$/kg (Ruggeri et al. 2014). A higher incidence of aGvHD grade II–IV was observed after dUCBT and again the lack of benefit of dUCBT was confirmed for patients with an adequately dosed single unit. Interestingly, myeloablative conditioning regimen with thiotepa, busulfan, fludarabine, and antithymocyte globulin (TBF) was associated with better outcomes in the sUCBT group. The impact of conditioning regimen in the cord blood field has also been observed in the reduced-intensity conditioning setting (Brunstein et al. 2012), demonstrating that a selected conditioning regimen could influence transplant outcomes. In fact, another study was designed to compare the relative efficacy of two optimized UCBT platforms in adults with acute leukemia, which were carried out at two institutions using either a TBF conditioning with sUCBT (Valencia platform) and TBI-based conditioning with dUCBT (Minneapolis platform), as well as different UCB unit selection criteria and GvHD prophylaxis (Sanz et al. 2013). Within these specific platforms, sUCBT and dUCBT offered similar long-term outcomes. However, sUCBT was able to achieve faster engraftment using a lower TNC dose suggesting that the established minimum cell dose threshold of 2.5×10^7 TNC/kg should be reconsidered, especially in the context of specific conditioning regimens.

Although a prospective randomized study in adults would be desired, it is unlikely it will ever happen. With the abovementioned information, we will try to dissect comparative results for the different transplant outcomes.

3.3.1 Engraftment

Early studies of UCBT activity reported low engraftment rates and delayed neutrophil recovery (Rubinstein et al. 1998; Cornetta et al. 2005). Optimization of conditioning regimens, improved banking and processing procedures, high-quality UCB inventories, and better UCB unit selection criteria considering $CD34^+$ cell count have led to superior outcomes (Sanz et al. 2012). However, neutrophil and platelet recovery is slower in UCBT recipients than in bone marrow or peripheral blood transplant recipients (Atsuta et al. 2012) and is still a major limitation for the use of UCB.

In this scenario, the use of dUCBT as a method of augmenting cell dose was first designed to improve transplant outcomes and in particular engraftment. To date, none of the retrospective or prospective comparative studies have shown more rapid, complete, or sustained hematopoietic recovery of dUCBT compared to adequately dosed single-unit transplants. In fact, two studies showed a deleterious effect on engraftment. A randomized trial in children and adolescents found a poorer platelet

recovery after dUCBT than sUCBT (Wagner et al. 2014). A potential relationship with the higher rate of GvHD was proposed, but a lower cell content of the "dominant" unit is also a possible explanation. Unfortunately, additional information on the graft characteristics of the double-unit group with respect to which units finally grafted is not available. The second study was retrospective and compared two UCBT platforms in adults (Sanz et al. 2013). The sUCBT with the "Valencia platform" showed faster neutrophil and platelet recovery. More interestingly, it was achieved using lower cell doses than the current definition of an adequately dosed UCB unit making this strategy widely available for all patients in need. The reason why this strategy permitted the routine use of lower dosed single units is probably multifactorial. On one hand an optimization of the conditioning regimen with the addition of thiotepa to busulfan and ATG may have played an important role. We think however that the single most relevant measure was related to UCB unit selection, particularly the routine use of the $CD34^+$ cell count.

3.3.2 Graft-*Versus*-Host Disease

Probably the most worrying aspect of the dUCBT strategy is the reported increased risk of GvHD after grafts of two UCB units found in most studies, including the two randomized trials (Wagner et al. 2014; Michel et al. 2016; MacMillan et al. 2009; Verneris et al. 2009; Sanz et al. 2013; Ruggeri et al. 2014; Scaradavou et al. 2013). This finding is especially relevant since one of the most important advantages of UCB is the lower risk of GvHD compared to other stem cell sources.

The first study raising this issue came from a retrospective analysis of risk factors for acute GvHD (aGvHD) from the University of Minnesota (MacMillan et al. 2002). Three risk factors for grade II–IV aGvHD were identified: use of two UCB units, use of nonmyeloablative conditioning, and absence of ATG in the conditioning regimen. However, no factor was independently associated with severe acute GvHD or chronic GvHD (cGvHD). The increased risk of aGvHD was restricted to grade II aGvHD with involvement principally of the skin and showed no deleterious effect on mortality. Several retrospective single-center and registry studies described similar results in a predominantly adult population (Verneris et al. 2009; Sanz et al. 2013; Scaradavou et al. 2013). Subsequent randomized trials, balanced for GvHD prophylaxis and other risk factors, showed an increased risk of GvHD. Wagner et al. observed higher rates of grade III and IV acute and extensive chronic GvHD after double-unit transplantation (Wagner et al. 2014). The overall incidences of acute and chronic GvHD did not differ, but chronic GvHD was more frequently extensive after double-unit UCBT (Michel et al. 2016).

Although the reasons for these findings are not known, it likely reflects an increased dose of immune competent T-cell in the combined grafts. Perhaps, a higher T-cell dose was achieved in younger recipients, as compared with larger adults, which resulted in higher rates of severe acute and chronic GvHD in these younger recipients. Additionally a potential impact of graft-*versus*-graft effect, similar to an *in vivo* mixed lymphocyte reaction, cannot be ruled out.

3.3.3 Relapse

A more potent alloreactivity with a decreased risk of leukemia relapse has been postulated for dUCBT (Verneris et al. 2009). In fact, some series have shown a surprisingly low incidence of relapse (Brunstein et al. 2010; Ponce et al. 2013). Again, the Minnesota group performed a retrospective analysis trying to identify risk factors for relapse in patients with acute leukemia undergoing UCBT. Apart from the obvious impact of disease status at transplantation, they observed lower relapse for patients that received two UCB units. This apparent enhanced graft-*versus*-leukemia (GvL) effect in recipients of two units was however restricted to patients transplanted in CR2, while no difference was observed for patients in CR1. This perception was further amplified after a small series of adults with lymphoid malignancies also suggested a possible enhanced GvL effect associated with double UCBT (Rodrigues et al. 2009). Reasons for a decreased risk relapse with two UCB units are difficult to analyze critically. A higher number of immune competent T-cells in the combined graft or increased HLA disparities between two donors and the recipient were hypothesized. However, all subsequent studies, including two randomized trials, refuted this theory and relapses were shown to be no different in double or single-unit UCBT (Wagner et al. 2014; Michel et al. 2016; Ruggeri et al. 2014; Scaradavou et al. 2013).

3.3.4 Non-relapse Mortality and Survival

To date, no single retrospective or prospective study has shown any benefit of dUCBT over sUCBT in terms of NRM or survival (Wagner et al. 2014; Michel et al. 2016; MacMillan et al. 2009; Verneris et al. 2009; Sanz et al. 2013; Scaradavou et al. 2013). The double-unit transplantation is therefore ineffective at improving survival, at least when a single-unit UCB containing more than 2.5×10^7 TNC/kg can be used.

Conclusions

There seems little or no theoretical, experimental, or clinical data supporting using two *versus* one unit of umbilical cord blood cells. In children, two randomized trials have demonstrated no benefit and possible additional risks. In adults, most transplant experts recommend using two umbilical cord blood cell units when an adequately dosed single UCB unit is not available, extending the use of UCBT. This raises the question of what adequately dosed means. Often, and without strong scientific support, an adequate cell dose is defined as a TNC dose $>2.5 \times 10^7$/kg recipient body weight. With this definition some adults would not have had an adequate single unit, but should however be reevaluated in light of reports of successful strategies with lower cell doses. A randomized, double-blind trial is obviously needed. Unfortunately, this seems unlikely to be done.

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4 Cell Dose and Immunogenetic Considerations in Cord Blood Transplantation

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4.1 Cell Dose in Single-Unit CBT

4.1.1 Total Nucleated Cell (TNC) Dose

Multiple studies have established the critical effect of TNC dose on engraftment. In an early Eurocord study, pediatric patients infused with TNC >3.7 × 10^7 cell/kg had a higher rate of neutrophil and platelet engraftment by day 60 and improved survival (Gluckman et al. 1997). These findings were substantiated by subsequent reports (Rocha et al. 2001; Locatelli et al. 1999). A pre-cryopreservation TNC dose of 4 × 10^7 cells/kg was shown to correlate with neutrophil engraftment, although not with TRM (Gluckman et al. 2004). Moreover, Rubinstein et al. (Rubinstein et al. 1998) observed that the speed and success of neutrophil recovery was directly related to the pre-cryopreserved TNC count and that a TNC <2.5 × 10^7 cells/kg was also associated with increased TRM. Similar findings were observed in the prospective COBLT pediatric single-unit CBT (sCBT) trial (Kurtzberg et al. 2008), and a cryopreserved TNC dose >2.5 × 10^7 cells/kg was independently associated with a significantly higher overall survival (OS). Based on the above, a cryopreserved TNC dose of 2.5 × 10^7 cells/kg has typically been accepted as the minimum acceptable cell dose for successful engraftment in sCBT, although a higher threshold has been proposed by others especially in the setting of CBT for nonmalignant diseases (Gluckman et al. 2007; Gluckman and Rocha 2006). Notably, sCBT in children has shown comparable long-term DFS to matched or mismatched BM transplants; therefore it is considered a standard alternative for children who lack suitable unrelated donors (Rocha et al. 2001; Eapen et al. 2007).

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_4

Early studies in adult patients undergoing sCBT were characterized by slow engraftment and high rates of graft failure and TRM (Laughlin et al. 2001; Long et al. 2003; Cornetta et al. 2005), although other small series showed more promising results (Takahashi et al. 2004; Sanz et al. 2001; Ooi et al. 2004). Two registry studies published in 2004 compared outcomes after sCBT and unrelated BM in adult patients with leukemia (Laughlin et al. 2004; Rocha et al. 2004). Both studies showed slower hematopoietic recovery in sCBT recipients. The study by the International Bone Marrow Transplant Registry (IBMTR) and New York Blood Center (NYBC) found worse TRM, progression-free survival (PFS), and OS in sCBT recipients compared to matched BM transplants, although outcomes were similar to mismatched BM transplant recipients (Laughlin et al. 2004). In contrast, the Eurocord study found no significant difference between sCBT and BM recipients with regard to TRM, PFS, and OS (Rocha et al. 2004).

Eapen et al. compared the outcomes of 1525 adults with acute leukemia who underwent sCBT, unrelated BM or PBHC transplants (Eapen et al. 2010). All sCBT patients received CB units with cryopreserved TNC dose >2.5×10^7 cells/kg. TRM was higher in the CBT group compared to the 8/8 HLA-matched BM and PBHC groups, but PFS after 4–6/6 HLA-matched CBT was comparable with that after 7–8/8 HLA-allele matched PBHC or BM transplantation. All of these studies highlight the challenge of limited cell dose in sCBT. Nonetheless, despite cell dose limitations, sCBT can be considered as a valid alternative option for patients who lack HLA-matched adult donors provided that units with TNC dose of at least 2.5×10^7 cells/kg are available.

4.1.2 $CD34^+$ Cell Dose

The TNC content of CB grafts is comprised of hematopoietic stem and progenitor cells, leukocytes, and a variable number of nucleated red blood cells (NRBCs), and will be influenced by the processing method. The $CD34^+$ cell count, however, is the most clinically important determinant of engraftment potential. The $CD34^+$ cell content of CB grafts was not routinely obtained prior to cryopreservation in the early years of CB banking, and techniques for $CD34^+$ quantification have not always been standardized resulting in interlaboratory variability. Consequently, the TNC dose has been most widely used for CB unit selection. Interestingly, it has been shown that the NRBC count correlates with the TNC and $CD34^+$ cell content of CB grafts and is predictive of myeloid engraftment; therefore the inclusion of NRBCs in the pre-cryopreservation TNC count does not reduce its use as a quality index of the graft (Stevens et al. 2002).

CB $CD34^+$ cell dose is superior to TNC in predicting engraftment. In a cohort of 102 primarily pediatric patients who underwent sCBT at the University of Minnesota, the likelihood and speed of neutrophil and platelet engraftment were associated with the infused $CD34^+$ cell dose and were significantly inferior in patients who received units with $CD34^+$ dose of less than 1.7×10^5 cells/kg (Wagner et al. 2002). Notably, while the infused $CD34^+$ dose correlated with the infused TNC count, the TNC only weakly correlated with engraftment and the $CD34^+$ count was

of greater prognostic significance. Furthermore, patients who received $<1.7 \times 10^5$ $CD34^+$ cells/kg had higher TRM and worse OS. Several subsequent studies have corroborated the prognostic significance of infused $CD34^+$ cell dose on engraftment and clinical outcomes after sCBT (Kurtzberg et al. 2008; Rodrigues et al. 2009; Page et al. 2011; Terakura et al. 2007).

The above observations suggest that a minimum $CD34^+$ cell content in the CB graft is required. Importantly, the post-thaw $CD34^+$ cell count strongly correlates with the pre-cryopreservation $CD34^+$ cell count and is an important predictor of hematopoietic recovery after CBT (Page et al. 2011; Purtill et al. 2014; Lemarie et al. 2007; Sanz et al. 2010). In contrast, the correlation between post-thaw $CD34^+$ cell count and pre-cryopreservation TNC dose is weak (Purtill et al. 2014). Therefore, many transplant centers prioritize the pre-cryopreservation $CD34^+$ cell dose over TNC dose in CB graft selection. In addition, it has been found that post-thaw $CD34^+$ recovery and viability are influenced by variations in banking practices (Purtill et al. 2014) with lack of FACT-Netcord accreditation and nonstandard cryopreservation volumes being the most important factors adversely impacting post-thaw viability. Based on the above, both pre-cryopreservation $CD34^+$ cell dose and banking practices (as predictors of unit quality) should be incorporated into CB unit selection (Purtill et al. 2014).

4.2 Cell Dose in Double-Unit CBT

Based on the TNC dose parameters presented above, many larger children and adults do not have available CB units with acceptable TNC or $CD34^+$ cell doses. To address this limitation, the University of Minnesota pioneered the use of dCBT in patients who lack an adequately sized CB unit (Barker et al. 2001, 2005, 2003). Since then, dCBT has successfully extended the application of CBT in adults (Scaradavou et al. 2013). Furthermore, some centers routinely perform dCBT to exploit the potential reduced risk of relapse with dCBT that has been demonstrated in retrospective studies, predominantly in adult patients (Rodrigues et al. 2009; Verneris et al. 2009; Brunstein et al. 2010; Kindwall-Keller et al. 2012; Michel et al. 2016).

4.2.1 Unit Dominance

Laboratory studies suggest unit dominance is the result of graft-*versus*-graft immune reactions mediated by the $CD34^-$ cells (Eldjerou et al. 2010; Yahata et al. 2004). Clinical data also suggest dominant unit T-cells mediate rejection of the non-engrafting unit, although prediction of which unit will reject the other is not possible. Gutman et al. (2010) have detected $CD8^+$ effector T-cells originating from naïve precursors of the dominant unit capable of producing IFN-γ in response to the non-engrafting unit. $CD4^+$ T-cells have also been implicated in graft-*versus*-graft alloreactivity and might mediate the rejection of the non-engrafting unit (Lamers et al. 2016). Furthermore, higher $CD3^+$ cell content (Barker et al. 2005; Milano et al.

2013; Ramirez et al. 2012; Avery et al. 2011), and more specifically the naïve $CD8^+$ T-cell subset (Milano et al. 2013), is an important predictor of the engrafting unit after dCBT. In contrast, post-thaw $CD34^+$ or TNC counts were both correlated with unit dominance after ATG-based RIC dCBT (Haspel et al. 2008) suggesting that $CD34^+$ cell dose may be important when graft-*versus*-graft immune reactions are abrogated by *in vivo* T-cell depletion. In addition to the above factors, the $CD34^+$ and $CD3^+$ viability has also been found to influence unit dominance after dCBT but only when one unit has low viability (reflecting poor engraftment potential) (Avery et al. 2011; Scaradavou et al. 2010). In this scenario, the high viability unit will likely engraft. It is interesting to speculate that the enhanced engraftment observed with dCBT (*vs.* sCBT with an inadequate single unit) may, at least in part, be explained by dCBT recipients having a higher probability of receiving at least one CB unit with adequate viable dose and thus with the potential to engraft.

4.2.2 Cell Dose and Engraftment Success in dCBT

Compared to sCBT, dCBT is associated with enhanced engraftment and speed of neutrophil recovery as compared to infusion of inadequately dosed single CB units (Barker et al. 2005). Interestingly, this is not due to the transient engraftment of both CB units. In the majority of dCBT recipients, reconstitution of hematopoiesis is provided by one unit only and single-unit dominance is established as early as 3–4 weeks post transplant, often before neutrophil recovery (Barker et al. 2005; Brunstein et al. 2007). Furthermore, neither the likelihood nor the speed of neutrophil recovery after dCBT is improved by the presence of dual engraftment (Purtill et al. 2014; Avery et al. 2011; Avery et al. 2012). Consequently, other immunologic mechanisms related to graft interactions, but not co-engraftment, account for this observation.

From the standpoint of time to neutrophil engraftment, higher infused total graft $CD34^+$ cell dose has been associated with faster time to neutrophil engraftment and lower risk of TRM (Avery et al. 2011; Bejanyan et al. 2015), but the total infused TNC and $CD3^+$ cell doses also have a significant impact on engraftment (Avery et al. 2011). The latter finding is in agreement with observations that T-cells may have graft-facilitating properties beyond overcoming immune barriers (Hexner et al. 2007). Notably, the post-thaw $CD34^+$ cell dose of the dominant unit determines neutrophil and platelet recovery and is of greater predictive value when compared to total graft cell doses (Avery et al. 2011; Haspel et al. 2008). Moreover, it has recently been shown that the most critical determinant of myeloid engraftment after dCBT is the infused viable $CD34^+$ cell dose of the dominant unit (Purtill et al. 2014) (Fig. 4.1) which in turn depends on both $CD34^+$ post-thaw recovery and viability. Interestingly, however, the TNC dose of the non-dominant unit has also been independently associated with improved neutrophil engraftment, especially when the infused viable $CD34^+$ cell dose of the dominant unit is low (Purtill et al. 2015) (Fig. 4.2). This association was related to the nonviable TNC component rather than the viable TNC dose in this study. These findings suggest that the presence of the non-engrafting unit mediates a dose-dependent facilitation of

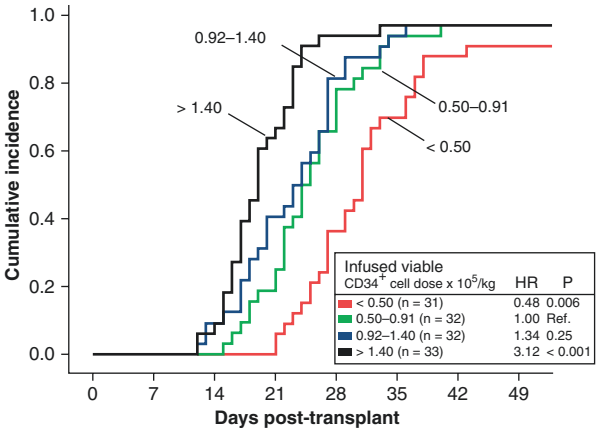

Fig. 4.1 Sustained neutrophil engraftment after myeloablative double-unit CBT by infused viable CD34⁺ cell dose per kilogram of the dominant unit ($n = 128$). Neutrophil engraftment was significantly associated with the dominant unit infused viable CD34⁺ cell dose (Purtill et al. 2014)

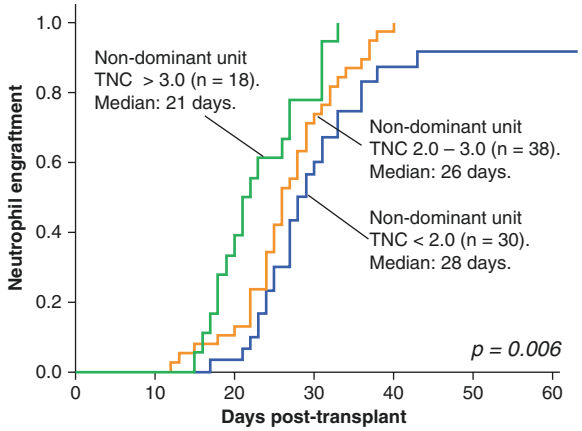

Fig. 4.2 Cumulative incidence of neutrophil engraftment according to the infused TNC dose (10^7/kg) of the non-dominant unit in dCBT recipients with a dominant unit CD34⁺ cell dose $<1.2 \times 10^5$/kg ($n = 86$) (Purtill et al. 2015)

engraftment after dCBT in the setting of a low dosed dominant CB unit, although the exact mechanism is not known.

To determine if dCBT is of benefit in children, the CTN 0501 study randomized pediatric patients to receive either single- or double-unit CBT (Wagner et al. 2014) provided that the TNC of the primary unit was at least 2.5×10^7 TNC/kg. The median total cryopreserved TNC doses were 4.8×10^7 *vs.* 9.1×10^7 cells/kg (double units combined), respectively. Similar rates of neutrophil recovery, TRM, and one-year survival were observed in the two groups, with slower platelet engraftment and higher incidence of severe GvHD noted in the dCBT arm. These findings were corroborated in a more recent randomized study from France

comparing sCBT *vs.* dCBT in children and young adults with acute leukemia or myelodysplasia (Michel et al. 2016), although a higher TNC dose of at least 3×10^7 cells/kg for the primary unit was required in this study. Survival outcomes were similar in the two groups, although higher extensive chronic GvHD (cGvHD) and lower relapse rates were observed in dCBT recipients who did not receive ATG. It should be noted that while the above studies support the use of sCBT in children and young adults who will receive high dose conditioning and have an adequately sized single CB unit, these findings are not applicable to older children or adults who do not have adequately sized CB grafts or those who will receive lower intensity conditioning. In dCBT, an arbitrary pre-cryopreservation TNC dose of $\geq 1.5 \times 10^7$/kg and $CD34^+$ cell dose of $\geq 1 \times 10^5$/kg have been used at Memorial Sloan Kettering Cancer Center (MSKCC) and other transplant centers as the minimum accepted cell doses for each unit. As the engrafting unit cannot be predicted at the time of unit selection, the same unit selection criteria must apply to both units.

4.3 HLA Match in Single-Unit CBT

4.3.1 6-Loci (HLA-A, HLA-B Antigen, HLA-DRB1 Allele-Level) Match Grade

In addition to the graft cell dose, the HLA matching between the CB graft and the recipient is an important determinant of CBT outcomes. Conventionally, HLA matching has been based on antigen-level typing for HLA-A and HLA-B and allele-level typing for HLA-DRB1. In contrast to the impact of the cell dose upon neutrophil recovery, donor-recipient HLA mismatch has more influence on TRM and relapse in many reports. However, as these outcomes are also influenced by diagnosis, disease status, GvHD prophylaxis, and the use of *in vivo* T-cell depletion, it is not surprising that early series reported conflicting results (Gluckman et al. 1997; Laughlin et al. 2001; Wagner et al. 2002; Sanz et al. 2014).

In the first 562 unrelated CBT facilitated by the NYBC, Rubinstein et al. (1998) observed that myeloid engraftment success was inversely associated with the number of HLA mismatches at HLA-A, HLA-B, and HLA-DRB1 loci. The risk of severe acute GvHD (aGvHD) was lower for patients who received 6/6 HLA-matched grafts, but did not otherwise correlate with the number of mismatches. PFS also correlated with the extent of HLA disparity. In a Eurocord analysis of 550 pediatric and adult CBT recipients (Gluckman et al. 2004), the number of HLA disparities at the HLA-A, HLA-B, and HLA-DRB1 loci was independently associated with inferior neutrophil recovery but a decreased risk of relapse. Acute GvHD was not associated with the number of HLA mismatches but rather the coexistence of class I and II disparities, and the number of HLA mismatches did not have an effect on OS. Similarly, in the prospective COLBT study (Kurtzberg et al. 2008), ≥5/6 HLA matching was independently associated with improved neutrophil

recovery and a lower rate of grade II–IV aGvHD, but HLA mismatch had no effect on relapse. There was also a trend for improved OS for recipients of 6/6 HLA-matched units and a trend for poorer survival for recipients of 3/6 matched grafts. Notably, not all registry studies have shown deleterious effects of HLA mismatch upon TRM, GvHD, or survival outcomes after sCBT (Arcese et al. 2006; Cohen et al. 2011).

4.3.2 Vector of HLA Mismatch (6 Loci)

While most studies of sCBT agree that better HLA match is associated with better outcomes, the majority of patients in need of CBT receive mismatched CB units at one or more HLA loci. An NYBC analysis (Stevens et al. 2011), including 1202 recipients of up to 4/6 HLA-matched sCBT, showed that the presence of one to two HLA mismatches in the GvH direction only was associated with improved neutrophil and platelet engraftment and no significant increase in the risk of grade III–IV aGvHD or cGvHD, compared to the reference group of one bidirectional mismatch. Furthermore, one to two HLA mismatches in the GvH direction only were independently associated with lower risk of TRM and resulted in comparable survival to HLA-matched sCBT. In contrast, the presence of one to two HLA mismatches in the host-*versus*-graft (HvG) direction only was associated with delayed and lower rate of neutrophil engraftment and higher risk of relapse.

Contradicting the above findings, other groups have reported either a negative impact of HLA mismatch in the GvH, but not in the HvG, direction on engraftment kinetics (Matsuno et al. 2009), or no impact of unidirectional HLA mismatch on engraftment, GvHD risk, relapse, or survival after sCBT (Cunha et al. 2014). Therefore, whether the vector of HLA mismatch needs to be considered in the CB unit selection remains controversial. Additionally, such considerations are only potentially applicable to a small number of patients, as most CB grafts have bidirectional HLA mismatches. Furthermore, there are no data regarding the significance of the vector of HLA mismatch in the setting of high-resolution typing and how to trade off against cell dose.

4.3.3 Interaction Between TNC Dose and Six Loci HLA Match

It has been suggested that a higher CB cell dose may compensate for the negative impact of HLA mismatch on engraftment and other outcomes and, conversely, better HLA match may compensate for units with a lower cell dose (Rubinstein et al. 1998; Eapen et al. 2007; Barker et al. 2010). In a Center for International Blood and Marrow Transplant Research (CIBMTR) analysis of children with acute leukemia undergoing sCBT, Eapen et al. (2007) found that TNC dose below the threshold of 3×10^7 cells/kg in the presence of one HLA mismatch (considering antigen-level HLA-A and HLA-B and allele-level HLA-DRB1 matching) was associated with

slower neutrophil recovery and possibly increased TRM compared to HLA-matched CBT, which was associated with superior outcomes regardless of cell dose. However, such a cell dose effect was not observed for 4/6 HLA-matched sCBT.

The interaction between cell dose and degree of donor-recipient HLA-A, HLA-B antigen, and HLA-DRB1 allele mismatch has been most clearly demonstrated in an analysis of 1061 children that underwent sCBT for acute leukemia or myelodysplasia (Barker et al. 2010). Neutrophil engraftment was associated with TNC count in a dose-response relationship and absence of HLA mismatch, but not the number of mismatches. TRM increased with increasing number of HLA mismatches and decreased with increasing TNC doses. When the two parameters were considered in combination, TRM was the lowest in recipients of HLA-matched units regardless of TNC dose. However, a TNC dose $>5 \times 10^7$ cells/kg was required for recipients of units with two HLA mismatches to achieve comparable TRM with recipients of units with one HLA mismatch and TNC dose $>2.5 \times 10^7$ cells/kg. Recipients of units with two mismatches and TNC count $<5 \times 10^7$/kg had worse TRM. Therefore, this study suggested that the cell dose requirement depends on the degree of HLA disparity.

4.3.4 Addition of HLA-C Locus Typing

More recently, studies have examined the effect HLA matching including HLA-C and high-resolution typing. The importance of antigen-level matching at the HLA-C locus was investigated in a combined CIBMTR and Eurocord analysis, including 803 patients who underwent myeloablative CBT for acute leukemia or myelodysplasia (Eapen et al. 2011). HLA-A and HLA-B typing was also performed at the antigen level, with allele-level typing only for the HLA-DRB1 locus. Mismatching at the HLA-C locus was independently associated with higher TRM in patients who received grafts with no or single mismatches at the HLA-A, HLA-B, or HLA-DRB1 loci, but had no additive effect in more mismatched transplants. There was also an association between HLA-C mismatch and worse OS in the presence of a single additional mismatch at HLA-A, HLA-B, or HLA-DRB1 loci. The overall degree of HLA disparity did not affect OS, although there was increased TRM in transplants mismatched at ≥2 loci and a lower rate of neutrophil engraftment in the setting of ≥3-locus mismatch.

4.3.5 High-Resolution HLA Allele-Level Match Grade

Typing units only at HLA-A and HLA-B antigens and HLA-DRB1 alleles and permitting up to two-locus donor-recipient mismatch allows transplantation of CB grafts with a very high degree of HLA disparity (Kurtzberg et al. 2008; Cornetta et al. 2005; Kogler et al. 2005; Dahi et al. 2014a) (Fig. 4.3). A CIBMTR-Eurocord study examined the impact of allele-level HLA-matching at HLA-A, HLA-B, HLA-C, and HLA-DRB1 on transplantation outcomes in 1568 recipients of sCBT (Eapen et al. 2014). Only 54% of patients who received 6/6 HLA-matched CB units at

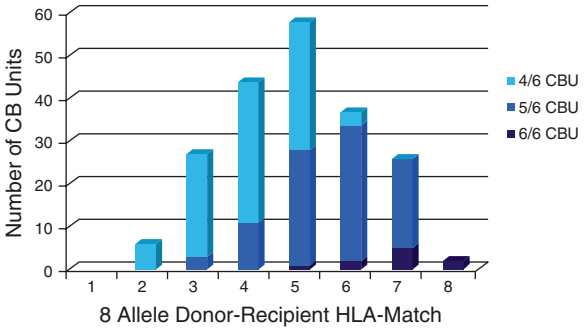

Fig. 4.3 Demonstration of the extent of mismatch at eight HLA alleles of CB units that are selected based on 4–6/6 HLA-A, HLA-B antigen, and HLA-DRB1 allele donor-recipient HLA match (n = 377). The 4–6/6 HLA-A, HLA-B antigen, and HLA-DRB1 allele donor-recipient match to the patient is shown. At high resolution the median donor-recipient HLA match at HLA-A, HLA-B, HLA-C, HLA-DRB1 alleles was 5/8 (range 2–8/8). While 6/6 matched units (n = 10) were at least 5/8 HLA allele matched, 5/6 units (n = 94) were as low as 3/8 matched, and 4/6 units (n = 96) were as low as 2/8 allele matched to the recipient (Dahi et al. 2014a)

lower resolution remained HLA-matched by eight-locus allele-level typing (Eapen et al. 2014). Compared with eight-allele HLA-matched transplants, neutrophil recovery was significantly lower in recipients of units mismatched at three to five, but not one to two, HLA alleles, regardless of the specific loci. Mismatched CBT at more than two alleles was associated with higher risk of grade II–IV aGvHD but possibly a lower risk of relapse. The best outcomes were observed in 8/8 HLA-matched transplants, and the degree of HLA mismatch was an independent factor associated with risk of TRM. The risk was heightened with ≥3 mismatches, with no additive effect of more mismatches. However, compared to HLA-matched transplants, OS was only significantly worse in transplants mismatched at five alleles.

Another important observation in this study was that a TNC dose >3×10^7 cells/kg was independently associated with lower TRM, regardless of the number of mismatches or patient's age. There was no added benefit from a higher TNC dose beyond that cell dose threshold. This observation has challenged the notion that a higher cell dose may compensate for mismatch, and the authors recommend that a closer eight-allele HLA match should take precedence over TNC beyond a minimum TNC threshold of 3×10^7 cells/kg.

4.4 HLA Match in Double-Unit CBT

4.4.1 6-Loci (HLA-A, HLA-B Antigen, HLA-DRB1 Allele-Level) Match Grade

Whether the same unit HLA-matched principles apply to the graft selection in the setting of dCBT is unknown. While registry studies are lacking, several single center studies have investigated the impact of graft-graft and graft-recipient HLA match

on dCBT outcomes. Considering antigen-level matching at HLA-A and HLA-B and allele-level matching at HLA-DRB1 loci, donor-recipient HLA match is not predictive of the dominant unit (Barker et al. 2005; Avery et al. 2011; Haspel et al. 2008; Brunstein et al. 2007). Furthermore, the extent of six-locus unit-recipient HLA mismatch (considering the dominant or more mismatched unit) does not appear to impact rate or speed of neutrophil engraftment and treatment failure (Avery et al. 2011; Brunstein et al. 2007). A previous study from the University of Minnesota found no discernible effect of HLA mismatch on incidence of grade II–IV aGvHD after dCBT when the worse matched unit was considered (Bejanyan et al. 2015). However, in a more recent report, the investigators showed that the risk of grade III–IV aGvHD is increased in dCBT recipients who engraft with a 4/6 (and possibly 5/6) HLA-matched unit, although the development of grade II–IV or III–IV aGvHD is not associated with increased TRM or worse OS (Lazaryan et al. 2016). Moreover, while a closer unit-unit HLA match has been associated with initial co-engraftment of both units, the degree of unit-unit HLA disparity has no effect on neutrophil engraftment (Avery et al. 2011) or other outcomes of dCBT (Brunstein et al., personal communication 2016) and therefore need not be considered in unit selection.

4.4.2 High-Resolution HLA Allele-Level Match Grade

Allele-level typing has provided further insight into the clinical significance of HLA matching in dCBT. Consistent with previous findings, a closer ten-allele HLA (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQ) unit-recipient match is not predictive of unit dominance and does not influence rate and speed of myeloid recovery after dCBT (Purtill et al. 2014; Avery et al. 2011; Brunstein et al. 2016). With regard to GvHD risk, better six-allele ($\geq$4/6) HLA match between the dominant unit and the CBT recipient is associated with lower risk of grade III–IV aGvHD (Ponce et al. 2013). However, eight- or ten-allele matching is either less, or not, predictive of GvHD severity after dCBT compared to conventional or six-allele HLA matching (Brunstein et al. 2016; Ponce et al. 2013; Oran et al. 2015).

Oran et al. (2015) reported that higher eight-allele-level mismatch between the engrafting unit and the recipient was associated with increased risk of two-year TRM, with no TRM observed in patients who engrafted with 7–8/8 HLA allele-matched units and TRM as high as 60% in patients engrafting with $\leq$4/8 HLA allele-matched units. There was also a trend toward worse OS although PFS was unaffected by the degree of eight-allele-level HLA mismatch, likely due to a trend for reduced relapse with more HLA mismatch. In contrast, a larger study by the University of Minnesota including 342 dCBT recipients showed that ten-allele-level HLA matching between the recipient and CB units (considering either the better matched, worse matched, or engrafting CB unit) had no impact on TRM and PFS (Brunstein et al. 2016). Interestingly, in a subset of 174 patients with acute leukemia, higher ten-allele HLA mismatch between the dominant unit and the recipient was associated with significantly lower risk of relapse. Differences between these

two studies may be due to different conditioning and immunosuppressive regimens, especially inclusion of ATG resulting in *in vivo* T-cell depletion.

Overall, taken together with the critical role of TNC and $CD34^+$ cell dose upon engraftment (Purtill et al. 2014; Avery et al. 2011), these findings suggest that cell dose and HLA match are both critical and further study is required to determine how to trade off dose, especially $CD34^+$ cell dose against eight-allele HLA match. Notably, selecting units based on high-resolution typing permits the avoidance of extreme mismatch in many patients. In dCBT, unit-unit HLA match need not be considered.

4.5 Other Immunogenetic Considerations in CBT

4.5.1 HLA Antibodies

In retrospective studies, the incidence of anti-HLA antibodies in CBT recipients varies from 15% to 41% (Takanashi et al. 2010; Brunstein et al. 2011; Ruggeri et al. 2013; Dahi et al. 2014b; Takanashi et al. 2008), and they are more commonly encountered in parous female patients. However, only a minority of patients with anti-HLA antibodies have donor-specific antibodies (DSAs) with specificity against the CB graft(s). The incidence of pre-formed DSAs in sCBT is estimated 5.2–6.4% (Takanashi et al. 2010; Ruggeri et al. 2013) and 3.8–24.7% in dCBT (Brunstein et al. 2011; Ruggeri et al. 2013; Dahi et al. 2014b; Cutler et al. 2011). Variability in the reported frequency of HLA antibodies is likely explained by the different composition of study populations in terms of age, gender, parity, as well as different practices such as consideration of anti-HLA antibodies as an exclusion criterion for the selection of CB grafts, the mean fluorescence intensity (MFI) threshold used to define the presence of anti-HLA antibodies, and the number of HLA antigens that are screened for.

In a cohort of 386 patients undergoing first myeloablative sCBT for hematologic malignancies, Takanashi et al. demonstrated that the presence of anti-HLA antibodies, and especially DSA antibodies, is an independent negative prognostic factor for neutrophil and platelet engraftment, with day 60 cumulative incidence of neutrophil recovery in the latter group as low as 32% (Takanashi et al. 2010), especially in patients who received a low $CD34^+$ dose. Furthermore, the presence of DSAs was associated with higher TRM and worse OS. A retrospective Eurocord study including sCBT and dCBT recipients after RIC also showed an association between DSA antibodies and a higher risk of graft failure and decreased survival (Ruggeri et al. 2013). The presence of DSA against one or both units after ATG-based dCBT has been associated with increased incidence of graft failure, delayed neutrophil engraftment, and high 100-day TRM (Cutler et al. 2011). Moreover, the MFI of the DSAs correlated with the risk of graft failure. In contrast to the above, two other large centers have shown no discernible effect of anti-HLA antibodies or DSAs against one or both units on engraftment, unit dominance, or clinical outcomes after dCBT,

with no MFI threshold effect (Brunstein et al. 2011; Dahi et al. 2014b). These conflicting findings may be explained by differences in transplantation practices between centers including conditioning regimens and immunosuppression, including the use of *in vivo* T-cell depletion. In support of this hypothesis, a recent study suggested that the presence of DSAs correlates with the presence of cytotoxic T lymphocytes (CTLs) with specificity against the same HLA antigens as the DSAs that might mediate graft rejection (Hanajiri et al. 2015).

To complicate the assessment, the effect of DSA may be influenced by the dose and HLA-match characteristics of the CB grafts, the nature of the conditioning and immunosuppression, as well as the number, titer, specificity, and complement fixation of the antibodies (Ciurea et al. 2015). Therefore, while avoiding CB units with high DSAs is prudent if multiple units of adequate size are available, the presence of DSAs does not necessarily preclude CBT. How to best incorporate the information about the presence and intensity of DSAs in the CB graft selection process requires further study, but should not compromise the cell dose or HLA match of the CB graft.

4.5.2 NIMA and IPA

The fetus receives one HLA haplotype from the father and one from the mother, termed inherited maternal antigens (IMA) and inherited paternal antigens (IPA), respectively. As a result of transplacental trafficking of cells and molecules between the fetus and mother, the fetus is exposed in utero to non-IMA (NIMA) that might result in the development of immunologic response and/or tolerance to these antigens. In addition, the mother gets exposed to cells of fetal origin and might develop immunologic response against the IPA; in turn, the IPA-sensitized maternal origin T-cells may cross the placenta and circulate in the CB. Recent studies have tried to elucidate the effect of NIMA and IPA on CB graft responses to recipient HLA, as well as on CBT outcomes.

The impact of NIMA matching was first described in an NYBC study including 1121 patients with hematologic malignancies transplanted with 4–6/6 HLA-matched single-unit CB grafts with available HLA typing of the respective mothers (van Rood et al. 2009). Maternal HLA typing was obtained retrospectively, and therefore, any NIMA matching occurred solely by chance. The total number of patients who received CB grafts with one HLA mismatch that was NIMA matched, or two HLA mismatches with at least one being NIMA matched, was small (25 and 54, respectively). Patients who received NIMA-matched CB units had faster neutrophil engraftment compared to HLA-mismatched but not NIMA-matched CBT recipients especially in the setting of a low TNC dose ($<2.5 \times 10^7$/kg). NIMA-matched grafts were also associated with lower TRM and superior PFS and OS. Survival of patients who received CB units with one HLA mismatch that was NIMA matched approached that of patients who received 6/6 HLA-matched grafts. A subsequent matched-pair analysis on behalf of the Eurocord and CIBMTR, in

which NIMA match was also retrospectively assigned, similarly showed lower TRM and improved OS in recipients of NIMA-matched CB units compared to HLA-mismatched, non-NIMA-matched units; however there was no discernible effect of NIMA matching on engraftment or relapse risk (Rocha et al. 2012).

These studies introduced the notion that graft-recipient mismatches that are NIMA matched are "permissive," likely due to their reduced immunogenicity. Therefore, substitution of a NIMA for one to three HLA loci of the CB phenotype can markedly increase the number of new "virtual" CB phenotypes (Van der Zanden et al. 2014). This virtually expands the CB inventory, increasing the likelihood of patients finding virtually matched CB units, which is affected by the number of CB units with known NIMA, size, and patient ancestry (Van der Zanden et al. 2014; Powley et al. 2016). Although some CB banks are now collecting maternal HLA typing and can provide NIMA information in a minority of the grafts, the added cost of maternal HLA is a limiting factor, and this strategy is not likely to be more effective than enriching the CB inventory with adequately sized units of diverse ancestry. Additional questions that remain unanswered include the effect of NIMA matching in the setting of allele-level HLA typing and in the setting of dCBT and whether there is a differential NIMA effect in specific HLA loci. Overall, NIMA considerations should not trump selection of CB units according to cell dose and are not feasible in the majority of patients.

An additional benefit of maternal HLA typing is the ability to deduce the IPA from the HLA genotype of the CB unit. In a follow-up NYBC study (van Rood et al. 2012), patients with acute leukemia transplanted with CB units that shared one or more IPAs at the HLA-A, HLA-B, or HLA-DRB1 loci had a decreased risk of relapse compared to patients who shared no IPA targets with their CB grafts. That reduction in relapse risk was independent of number and direction of HLA mismatches and was not associated with increased risk of GvHD. These findings provided indirect evidence that maternal microchimerism in CB (i.e., cells of maternal origin that were sensitized against IPAs during pregnancy and subsequently transferred to the patient with the graft) might confer a potent GvL effect in CBT recipients, although the exact mechanism of relapse reduction has not been elucidated. Based on the above, it has been recommended that CB units without shared IPAs should be avoided due to excessive risk or relapse. However, as is the case with the NIMA effect, this practice is hampered by the low number of CB units with available maternal typing and the small number of patients that this might be applicable to.

4.5.3 KIR and KIR-Ligand Mismatch

Natural killer (NK) cell alloreactivity is determined by the interaction of activating and inhibitory KIRs with their cognate ligands. Because inhibitory KIRs recognize (typically self) major histocompatibility complex (MHC)-I molecules, CBT with class I HLA mismatches can lead to the development of alloreactive NK cells if

their KIRs lack specificity for recipient MHC-I molecules (Mehta et al. 2016). Several studies have investigated the effect of KIR-L mismatch on clinical outcomes of CBT with variable and conflicting results. In an analysis of 218 recipients of sCBT for acute leukemia, KIR-L incompatibility was associated with decreased incidence of relapse and improved PFS and OS (Willemze et al. 2009). In contrast, a study from the University of Minnesota including 257 recipients of either sCBT or dCBT found no beneficial effect of KIR-L mismatch in the myeloablative setting and that it may be worse after reduced intensity conditioning CBT (Brunstein et al. 2009). More recently, a Japanese registry study (Tanaka et al. 2013) found no effect of KIR-L mismatch in the GvH direction on risk of relapse, GvHD, or survival after ATG-free sCBT, regardless of conditioning intensity. Furthermore, a Eurocord/CIBMTR analysis also found no association between KIR-L mismatch and clinical outcomes after 6–7/8 HLA allele-matched myeloablative sCBT, although a deleterious effect of KIR-L mismatch in the HvG direction on TRM was suggested in recipients of 3–5/8 HLA-matched CBT (Rocha et al. 2016). In the setting of dCBT, KIR-L mismatch has not been shown to impact unit dominance (Tarek et al. 2015) or relapse risk (Garfall et al. 2013).

The discordant findings of the above studies may be related to differences in patient populations and definition and assignment of KIR-L mismatch. The use of T-cell depletion may be critical as previous studies suggest T-cell alloreactivity dominates NK cell alloreactivity when T-cells are present, and therefore KIR-L mismatch may only be clinically relevant in the setting of T-cell depletion (Mehta et al. 2016; Cooley et al. 2005; Lowe et al. 2003). Furthermore, all of the above studies have used the donor-recipient KIR-ligand mismatch model of NK cell alloreactivity (Mehta et al. 2016; Ruggeri et al. 1999) which does not require KIR typing of the recipient and does not take into account the activating receptors and NK cell licensing. However, two recent studies that obtained KIR typing and evaluated the impact of KIR/HLA combinations (receptor-ligand model of NK cell alloreactivity) after dCBT also found equivocal results (Sekine et al. 2016; Rettman et al. 2016). Taken together, the available data do not support the use of KIR typing or KIR-L matching in the CB graft selection process.

4.6 Expert Point of View

CBT outcomes have improved due to advancements in CB banking and transplantation practices and are comparable to outcomes of transplantation from other graft sources, especially in experienced centers. The study of single- and double-unit CBT has revealed complex biology at play, and yet day-to-day CBT practice should observe the basic principles that unit cryopreserved TNC and $CD34^+$ cell dose and donor-recipient HLA match are the major determinants of CBT success. Banking practices that determine unit quality are an extension of cell dose in that they determine post-thaw potency and hence unit engraftment potential. Conflicting or

inadequate data exists to support the importance of other immunogenetic factors, and their incorporation in the unit selection process remains controversial. The criteria used for unit selection at MSKCC are summarized in Table 4.1. Standard practice at this time is to administer double-unit grafts in most adults as: (1) many adults cannot be assured to have an adequate single unit and (2) intermediate intensity conditioning regimens are utilized in most patients (to avoid the toxicity of high dose myeloablation) and graft-*versus*-graft interactions may be beneficial in this setting.

Table 4.1 MSKCC criteria for cord blood graft selection

CB graft characteristics	MSKCC criteria	Comments
Resolution of HLA-typing	8-allele HLA-A, HLA-B, HLA-C, HLA-DRB1	May permit avoidance of extreme mismatch
Donor-recipient HLA-match	≥4/6 HLA-A, HLA-B antigens, HLA-DRB1 alleles and ≥ 3/8 HLA-A, HLA-B, HLA-C, HLA-DRB1 alleles	Lower limit of high-resolution HLA-matched grade not yet determined
Dose/kg: *single* unit	TNC ≥ 2.5×10^7/kg $CD34^+$ cells ≥1.5×10^5/kg	Definition of adequate single unit not fully established
Dose/kg/unit: *double* unit	TNC ≥ 1.5×10^7/kg per unit $CD34^+$ cells ≥1.0×10^5/kg per unit	Double unit transplants (*vs.* single-unit graft) standard in adults but not supported in children provided good quality adequately dosed single units are available
Avoidance of units against which recipient has DSA	Usually not if malignancy	Avoid if high titer DSA if possible although should not greatly compromise cell dose and donor-recipient HLA match
Bank major criteria in selection	Yes	Determines banking practices that influence quality
Netcord-FACT accreditation considered	Yes	Associated with banking practices that influence quality
Use of RBC replete units	Avoid	Use of such units greatly increases the risk of serious infusion reactions
Mandatory testing of attached segment	Yes	Ensures unit identity
Viability testing on thawed product	Yes: % viable $CD34^+$ cells by flow (7AAD)	Ensures delivery of unit(s) with high engraftment potential on transplant day (critical if single-unit grafts used)
Back-up unit policy	1–2 domestic units	Unit is reserved but not shipped and then released to the inventory upon patient engraftment

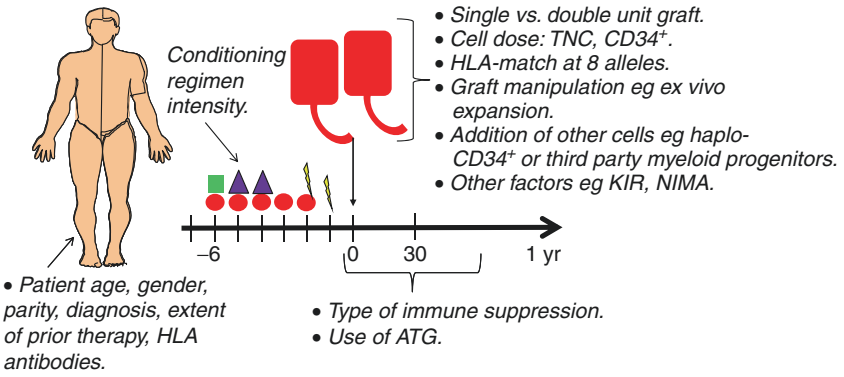

Fig. 4.4 Factors that influence CBT outcome. Recipient characteristics and transplant practices (e.g., conditioning and immunosuppression) influence CBT outcomes as well as graft characteristics. Therefore, these factors must also be carefully considered when studying the effects of cell dose and immunogenetics in CBT

4.7 Future Directions

Despite recent progress in CBT, many questions remain that require future study. Key is to determine the definition of the minimum characteristics of an "adequate" single unit and to clarify the role of double-unit grafts, especially in adults undergoing CBT with less than high intensity conditioning. How to "trade off" cell dose (including $CD34^+$) against high-resolution donor-recipient HLA match is also not fully established, and the quality of the units in a search can also further compound the complexity of unit selection decision making. Importantly, because recipient characteristics (e.g., age, comorbidities, and extent of prior therapy) and transplantation practices (e.g., conditioning intensity, nature of immunosuppression, and any *in vivo* T-cell depletion) will greatly influence CBT outcomes, it is critical that such factors are taken into account in future studies addressing the effect of cell dose and immunogenetics in CBT (Fig. 4.4). Finally, new technologies such as ex vivo expansion, strategies to enhance homing, or co-infusion of third-party progenitors could potentially speed engraftment but will also be yet another factor to consider in the analysis of the influence of cell dose and immunogenetics on CBT outcomes.

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Ex Vivo Cord Blood Manipulation: Methods, Data, and Challenges

5

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5.1 Introduction

Despite numerous advantages of umbilical cord blood (UCB) transplantation (UCBT), one of its major limitations is delayed engraftment of neutrophils and platelets as compared with peripheral blood progenitor cell (PBPC) or bone marrow (BM) grafts. After myeloablative conditioning, neutrophil engraftment is achieved in about 20–30 days with unmanipulated UCBT compared with 10–20 days after PBPC and 15–25 days after BM grafts. Similarly, platelet engraftment (>20 × 10^9/L) that occurs in about 10–20 days after PBPC and 15–30 days after BM HCT takes about 50–70 days after unmanipulated UCBT (Barker et al. 2015; Liu et al. 2014; Ruggeri et al. 2014; Sanz et al. 2012; Brunstein et al. 2010; Verneris et al. 2009; Barker et al. 2005; Takahashi et al. 2007; Eapen et al. 2010). This is explained by about tenfold fewer total nucleated cells (TNCs) in UCB graft compared with other grafts (Eapen et al. 2010; Laughlin et al. 2004; Rocha et al. 2004; Atsuta et al. 2009).

One of the ways to tackle the barrier of low cell dose is the use of two partially matched UCB grafts (Barker et al. 2005). Although the use of double-unit UCBT (dUCBT) increases the total cell content of the graft, it still does not hasten engraftment compared with adequately dosed single-unit UCBT (Ruggeri et al. 2014; Verneris et al. 2009; Wagner et al. 2014; Kindwall-Keller et al. 2012; Scaradavou et al. 2013). Instead, there is a suggestion that the use of dUCBT may in fact be associated with delayed and inferior platelet engraftment compared to that of single-unit UCBT (Wagner et al. 2014). Alternative strategies to enhance engraftment include ex vivo manipulation of UCB graft either to augment total cell dose or improve BM homing capacity of UCB progenitor cells (summarized in Table 5.1).

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_5

Table 5.1 Ex vivo graft manipulation studies to augment engraftment

Study (sample size)	Post thaw/infused TNC, median (range), $\times 10^7$/kg			Post thaw/infused $CD34^+$ median (range), $\times 10^5$/kg			Median age (range), in year	Conditioning regimen	GvHD prophylaxis	Neutrophil engraftment time, in days, median (range)		Platelet engraftment time, in days, median (range)	
	Untreated cord	Treated cord	Total	Untreated cord	Treated cord	Total				Study group	Controls	Study group	Controls
Expansion studies													
Notch (Delaney 2010) $n = 10$	3.3 (1.5–6.3)	4.6 (0.6–9.1)		2.4 (0.6–5.4)	60 (9.2–133)		27.5 (3–43)	MA: Flu/Cy/TBI	CsA/MMF	16 (7–34).	26 (16–48); $p = 0.002$	*N.R.*	*N.R.*
MSC (De Lima 2012) $n = 24$	2.28 (1.57–4.82)	5.84 (0.03–14.37)	8.34 (1.66–19.19)	3.8 (0.7–17.4)	9.5 (16–93.4)	18.1 (0.9–988)	39 (18–61)	MA: Flu/MelThio	Tac/MMF + Rabbit ATG	15 (9–42)	24 (12–52); $p < 0.001$	42 (15–62)	49 (18–264); $p = 0.03$
StemEx (Stiff 2013) $n = 101$			2.2 *(N.R.)*			9.7 *(N.R.)*	37 (12.6–55.8)			21 *(N.R.)*	28 *(N.R.)*; $p < 0.0001$	54 (*N.R.*)	105(*N.R.*); $p = 0.008$
NAM (Horwitz 2014) $n = 11$			3.1	0.7 (0.3–4.8)	35 (9–183)		45 (21–61)	MA: Flu/TBI +/− Cy	Tac/ MMF	13 (7–26)	25 (13–38) $p < 0.001$	33 (26–49)	37 (20–66); $p = 0.085$
SR1 (Wagner 2016) $n = 17$	2 (2–5)	5 (1–12)	7 (3–17)	4 (2–9)	175 (14–483)	182 (23–485)	29.9 (12–54)	MA: Flu/Cy/TBI	CsA/MMF	15 (6–30)	24 (*N.R.*)	49 (28–136)	89 (*N.R.*); $p = 0.001$

Homing studies												
dmPGE2 (Cutler 2013) Cohort1: $n = 9$	1.8	2.17		0.68	0.7		43.0 (29–64)	Flu/Mel	Tac/Siro ATG	24 *(N.R.)*	21 *(N.R.)*; $p = 0.045$	72.5 *(N.R.)*	N.R.
Cohort 2: $n = 12$	1.7	1.8		0.56	0.74		57.5 (19–66)			17.5 (14–31)		43 (26–60)	
Fucosylation (Popat 2015) $n = 22$	2.59 (1.54–4.75)	1.75 (0.64–2.5)	4.26 (2.73–6.3)	–	–	–	42 (20–68)	MA: Bu/Flu/ Clo/ TBI RIC: Flu/Mel	Tac/ MMF + Rabbit ATG	17 (12–34)	26 (11–48); $p = 0.0023$	35 (18–100)	45 (27–120); $p = 0.05$

Abbreviations: *ATG* antithymocyte globulin, *Bu* busulfan, *Cy* cyclophosphamide, *CsA* cyclosporine A, *Clo* clofarabine, *dmPGE2* 16,16-dimethyl prostaglandin E2, *Flu* fludarabine, *GvHD* graft-*versus*-host disease, *MA* myeloablative, *Mel* melphalan, *MMF* mycophenolate mofetil, *MSC* mesenchymal stromal cells, *NAM* nicotinamide, *RIC* reduced intensity conditioning, *SR1* StemRegenin-1, *Tac* tacrolimus, *Thio* thiotepa, *TBI* total body irradiation, *TNC* total nucleated cells

5.2 Historic Methods of UCB Expansion Using Cytokine-Supported Culture Media Failed to Demonstrate Clinical Benefit

One of the earliest methods of ex vivo expansion was the use of "static culture," whereby purified UCB $CD34^+$ cells were cultured in medium containing stem cell factor (SCF), granulocyte-colony stimulating factor (G-CSF), and megakaryocyte growth and differentiation factor for 10 days (Shpall et al. 2002). Despite fourfold median expansion of $CD34^+$ cells, the median infused $CD34^+$ dose in adult patients was only 0.89×10^5/kg (median TNC, 0.79×10^7/kg). This was due to significant upfront cell loss from $CD34^+$ selection with the Isolex 300-i device (Nexell, Irvine, CA) and anti-CD34 antibody used in this method. The median time to neutrophil engraftment ranged from 26 to 31 days and 73–126 days for platelet engraftment, which was not different from results of unmanipulated UCBT trials.

Investigators from the Duke University Medical Center explored ex vivo UCB expansion using an automated device that perfused cultures with cytokines and maintained optimal culture conditions based on computerized monitoring of biological and physiological environment of the culture (Jaroscak et al. 2003). In a clinical trial using this system, CB samples were thawed on day 0 and a majority of cells were infused unmanipulated on the same day (median TNC count, 2.05×10^7/kg). A small fraction (TNC count, $1–2 \times 10^8$/kg) was expanded ex vivo and infused on day 12. The culture was supported by PIXY321 (a fusion protein of interleukin (IL)-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF)), Fms-like tyrosine kinase 3 ligand (FLT3L), and erythropoietin, which resulted in median TNC expansion of 2.4-fold, but the expansion of $CD34^+$ was rather feeble (median, 0.5-fold; range, 0.09- to 2.45-fold). The median time to neutrophil engraftment was 22 and that of or platelet was 71 days. Out of 22, 3 patients failed to achieve engraftment, and 2 died prior to engraftment.

5.3 Methods to Block *In Vitro* Differentiation of $CD34^+$ Cells to Expand Early Progenitor Cells (EPCs) Demonstrated Encouraging Results

One of the concerns with cytokine-supported culture system is potential loss of early progenitor cells (EPCs) as the cytokines stimulate $CD34^+$ cells to differentiate into mature cells (Koller et al. 1995). This provides rationale for exploring methods that block *in vitro* differentiation of $CD34^+$ cells by means of nicotinamide analogs (Horwitz et al. 2014), copper chelators such as tetraethylenepentamine (TEPA) (Stiff et al. 2013), or targeting *Notch* signaling pathway (Delaney et al. 2010).

In a study by Delaney et al. (2010), human UCB $CD34^+$ cells were transduced with an engineered Notch ligand ($Delta1^{ext\text{-}IgG}$) and then cultured for about 2 weeks in serum-free medium with IL-3, IL-6, thrombopoietin (TPO), SCF, and FLT3L. This led to an average 222-fold expansion of $CD34^+$ cells and rapid engraftment in non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mice infused

with these cells as compared with control mice (Delaney et al. 2010). This method is now under investigation in a phase I clinical trial in patients with acute leukemia, where recipients receive myeloablative conditioning followed by infusion of one unmanipulated UCB unit followed 4 h later by the infusion of the UCB unit that has been expanded ex vivo for 2 weeks. In preliminary analysis, neutrophil engraftment occurred at a median of 16 days in ten patients and graft rejection occurred in one. At a median follow-up 354 days, 70% of the patients were in complete remission with sustained engraftment. Two patients had long-term engraftment from expanded cells for up to 240 days post UCBT, but it did not persist beyond 1 year after which the unexpanded CB unit contributed to engraftment (Delaney et al. 2010).

The use of a polyamine copper chelator, TEPA (StemEx®), was explored in a multicenter international trial, including 101 adult patients (median age 37 years, median weight 68 kg) with hematological malignancies (Stiff et al. 2013). In this study, $CD133^+$ cells were enriched from a fraction (20–50%) of single-unit cryopreserved UCB sample that was thawed on day −20. These were cultured in bags with TEPA, FLT3L, IL-6, TPO, and SCF. Three weeks of culture resulted in an impressive expansion of TNCs to 400×10^7/kg (baseline median 3.06×10^7/kg) and $CD34^+$ cells to 9.7×10^5/kg (baseline median 1.64×10^5/kg). Total median infused doses were 2.2×10^7 TNC/kg and 9.7×10^5 $CD34^+$/kg. The median times to neutrophil engraftment (21 *vs.* 28 days, $p < 0.0001$) and platelet engraftment (54 *vs.* 105 days, $p = 0.008$) were significantly faster in the study group compared to a control group of dUCBT recipients ($n = 295$) from the Center for International Blood and Marrow Transplant Research (CIBMTR) and the Eurocord registries. Even more interestingly, survival at day 100 was significantly superior in the study group compared with controls (84.2 *vs.* 74.6%; $p = 0.035$).

Another blocker of EPC differentiation is nicotinamide (NAM), which when cultured with UCB cells along SCF, TPO, IL-6, and FLT3L results in expansion not only of $CD34^+$ cells but also of $CD34^+Lin^-$ EPCs (Peled et al. 2012). These expanded cells also have enhanced BM homing potential compared with untreated cells (Peled et al. 2006). A phase I clinical trial using this strategy enrolled 11 adult patients (median age 45 years, median weight 83 kg) with hematological malignancies. For expansion, one UCB unit was thawed on day −21, selected for $CD133^+$ cells, and expanded ex vivo. The negative fraction was cryopreserved and later infused along with the expanded fraction and a second unmanipulated unit after myeloablative conditioning. The median doses of unmanipulated (2.6×10^7 TNC/kg and 0.12×10^6 CD34/kg) and expanded (2.5×10^7 TNC/kg and 0.17×10^6 CD34/kg) units were similar. Compared with a historical cohort of dUCBT, the time to neutrophil engraftment (13 *vs.* 25 days, $p < 0.001$), but not the platelet engraftment (33 *vs.* 37 days, $p = 0.085$), was significantly shorter in the study population. Engraftment was attained from the expanded cord in 50% of patients, while 25% engrafted with unexpanded cord and 25% had dual chimerism from both the cords. Further, the expanded cord provided long-term engraftment in eight of ten evaluable patients, which remained stable for up to 36 months (Horwitz et al. 2014).

Most recently, Wagner et al. reported the results of a phase I–II trial of UCB expansion using StemRegenin-1 (SR-1), which is an inhibitor of aryl hydrocarbon

receptor, which is crucial for differentiation of EPCs (Wagner et al. 2016). In this study, 17 patients (median age 29.9 years years) received myeloablative conditioning with fludarabine, cyclophosphamide, and total body irradiation (TBI), followed by dUCBT with one unmanipulated and one ex vivo expanded unit. For expansion, $CD34^+$ cells were enriched from the smaller of two UCB units on day −15 and cultured with SR1, SCF, FLT3L, TPO, and IL-6. The negative fraction was re-cryopreserved. After 15 days of culture, a median 330-fold expansion of a number of $CD34^+$ cells and an 854-fold expansion of TNCs were noted. On day 0, the unmanipulated unit was infused first followed 4 h later by the expanded unit, followed by infusion of the $CD34^-$negative fraction 4–24 h later. No graft failure was observed. Median times to neutrophil engraftment (15 *vs.* 24 days) and platelet engraftment (49 *vs.* 89 days, $p = 0.001$) were significantly faster than historical institutional controls. The expanded cord provided engraftment in 65% (11/17) of patients and the rest engrafted from the unmanipulated cord. Patients who engrafted from the expanded cord had durable myeloid engraftment (median follow-up, 272 days) with neutrophil engraftment occurring at a median of 11 days compared to 23 days (range, 14–30 days) in those who engrafted from the unmanipulated cord. There were no differences in other outcomes including acute or chronic GvHD, transplant-related mortality, and overall survival compared with controls. However, study population had significantly shorter duration of hospitalization (median 30 days) compared with controls (median 46 days), $p < 0.001$. The authors are evaluating the safety and efficacy of this approach as a "stand-alone" graft, in the setting of single-unit UCBT.

5.4 Coculture of UCB Cells with Mesenchymal Stromal Cells Simulates BM Niche and Provides Molecular Signals Crucial for UCB Expansion

A distinct technique of ex vivo expansion focuses on simulating the *in vivo* environment of EPC growth by coculturing UCB cells with mesenchymal stromal cells (MSCs) that form a BM "niche" and produce cytokines that regulate cell proliferation and homing (Robinson et al. 2011). Investigators from the M. D. Anderson Cancer Center tested this technique in 31 adult patients. Most common diagnoses were acute myeloid leukemia or myelodysplasia (68%) and acute lymphoblastic leukemia (16%), and about 60% of the patients had active disease at the time of transplantation. All patients underwent myeloablative conditioning with fludarabine (160 mg/m^2), melphalan (140 mg/m^2), thiotepa (10 mg/kg), and rabbit antithymocyte globulin (ATG 3 mg/kg), followed by dUCBT with one expanded and one unmanipulated UCB unit. The source of MSC was haploidentical donor in the first seven patients, while the rest of the patients received "off-the-shelf" MSC precursor cells (Mesoblast Limited, Melbourne, Australia) to circumvent the obvious logistic difficulties associated with obtaining haploidentical MSC. Smaller of the two UCB units was thawed on 14 days prior to transplantation and cocultured with MSCs along with SCF, FLT-3L, TPO, and G-CSF for 2 weeks. The culture resulted in

12.2-, 30.1-, and 17.5-fold expansions in TNC, $CD34^+$, and colony-forming unit-C populations, respectively, producing final median doses of 5.84×10^7/kg, 0.97×10^6/kg, and 3×10^6/kg, respectively. The unmanipulated unit was thawed on day 0, washed, and infused followed by infusion of the expanded UCB unit. Compared with 80 control patients from the CIBMTR, neutrophil engraftment was significantly faster (median 15 *vs.* 24 days, $p < 0.001$) and improved in the study group compared with the controls. The cumulative incidence of engraftment at day 42 was 96% (95% confidence interval (C.I.) 74–99%) in the study group compared with 78% (95% C.I. 67–86%) in controls, $p = 0.005$. The median time to platelet engraftment was a week shorter (42 *vs.* 49 days, $p = 0.03$) in the study group. Long-term engraftment beyond 1 year was provided primarily by the unmanipulated unit, and the expanded unit was present in only 13% of the patients at 6 months post UCBT (de Lima et al. 2012).

5.5 Ex Vivo Manipulation Can Also Improve BM Homing Capacity of UCB Cells Without the Need for Long-Term Cultures

Ex vivo treatment of EPCs with prostaglandin E2 (PGE2) up-regulates apoptosis-inhibiting protein survivin, proliferation genes such as cyclin D1 (leading to selective self-renewal capabilities of EPCs), and chemokine receptor CXCR4, collectively stimulating their growth and homing potential (Hoggatt et al. 2009). A study from the Dana-Farber Cancer Institute tested this hypothesis in a phase I clinical trial with a PGE2 derivative (dmPGE2) in adult patients (median age 57.5 years) undergoing dUCBT after a conditioning regimen of melphalan (100 mg/m^2), fludarabine (180 mg/m^2), and ATG (4 mg/kg). Both UCB units were thawed on day 0 – one of which was treated. Larger unit was infused first followed 4 h later by the smaller unit. In the first cohort of this study, UCB unit (smaller unit in six patients and larger unit in three patients) was incubated with dmPGE2 for 1 h at 4 °C. The median times to neutrophil (24 days) and platelet engraftment (72.5 days) were not improved compared to their historical controls. Plus, two patients experienced graft failure. The authors then optimized their culture conditions and enrolled additional 12 patients where the larger UCB unit was incubated with dmPGE2 for 2 h at 37 °C. With this modification, they observed significantly improved median time to neutrophil engraftment compared to historical controls (17.5 *vs.* 21 days, respectively, $p = 0.045$). No patient in this cohort experienced graft failure. Further, 10 of the 12 patients had sustained engraftment from the dmPGE2-treated cord, which could be seen for up to 27 months post UCBT for some patients. A randomized phase II trial is ongoing (Cutler et al. 2013).

Another method of enhancing BM homing potential of UCB cells was tested by researchers from the M. D. Anderson Cancer Center. This method was based on our understanding that successful homing of transplanted cells requires interactions between adhesion molecule receptors (E- or P-selectins) on vascular endothelial cells and selectin ligands on hematopoietic cells. It is known that UCB cells have

poor fucosylation of selectin ligands, such as P-selectin glycoprotein ligand 1, which likely contributes to their deficient BM homing (Xia et al. 2004). In mouse models, human CB $CD34^+$ cells treated with fucosyltransferase (FT)-VI and GDP-fucose demonstrated improved homing capability compared to untreated human CB $CD34^+$ cells, leading to faster and significantly higher rates of engraftment (Robinson et al. 2012). A phase I clinical trial included 22 adult patients (median age 42 years) who received dUCBT after either myeloablative conditioning with fludarabine (40 mg/m^2), clofarabine (120 mg/m^2), and busulfan (area under the curve of 16,000 μmol/min) and 2 Gy of TBI or reduced intensity conditioning with fludarabine (160 mg/m^2) and melphalan (140 mg/m^2). All patients received rabbit ATG (3 mg/kg), tacrolimus, and mycophenolate mofetil for GvHD prophylaxis. On the day of transplantation, the larger UCB unit was infused unmanipulated, while the smaller unit was treated ex vivo with FT-VI and GDP β-fucose for 30 min at room temperature, washed, and then infused. In the study population, median times to neutrophil engraftment (17 *vs.* 26 days, $p = 0.0023$) and platelet engraftment (35 *vs.* 45 days, $p = 0.05$) were significantly shorter than their historical controls. One patient had secondary graft failure. All evaluable patients had 100% chimerism on day 30 post UCBT – 40% from fucosylated cord, 40% from unmanipulated cord, and 20% having dual chimerism from both cords. Moreover, there were no differences in neutrophil and platelet engraftment among those who engrafted from treated *versus* untreated cord (Popat et al. 2015).

Investigators from Indiana University School of Medicine are investigating another method of improving homing of UCB cells without any ex vivo manipulation at all. In a phase II trial, patients with hematological malignancies undergoing UCBT are given sitagliptin 600 mg orally 1–3 times a day for 4–12 doses, starting day 1 [Clinicaltrials.gov, NCT00862719]. The drug works by inhibition of dipeptidyl peptidase-IV (DPPIV)/CD26, which is a membrane-bound peptidase that inhibits the migratory potential of $CD34^+$ cells by cleaving CXCL12/SDF-1α (stromal cell-derived factor 1) (Christopherson et al. 2002, 2004).

5.6 Graft Manipulation Techniques Advanced the Field of Adoptive Immunotherapy Tremendously

Disease relapse, GvHD, and infections are the leading causes of mortality after allogeneic hematopoietic cell transplantation (HCT) (Brunstein et al. 2010; Eapen et al. 2010; Scaradavou et al. 2013; Wagner et al. 2016; Popat et al. 2015; Pasquini and Zhu 2015). Relapse after UCBT is especially devastating as it excludes one of the potential therapeutic strategies, which is the use of donor lymphocyte infusion (DLI). Now with ex vivo expansion techniques, large-scale production of UCB T-cells and NK cells is feasible. Culturing UCB cells with anti-CD3/CD28 magnetic beads and IL-2 can expand UCB T-cells by a median of 100-fold while maintaining their polyclonal TCR repertoire (Parmar et al. 2006). Using this strategy, a phase I clinical trial is evaluating the role of UCB DLI in patients with relapse after UCBT [ClinicalTrials.gov:NCT01630564].

Several different methods exist for expansion of UCB NK cells. One of such methods is a two-step process where enriched UCB $CD34^+$ cells are first expanded in serum-free media with cytokine cocktail for 2 weeks, followed by differentiation and expansion of NK cells using a separate NK-cell differentiation medium for 5 weeks. This method resulted in 3- to 4-log expansion and generation of *functional* human NK cells that demonstrated activity against various leukemia and melanoma cell lines (Spanholtz et al. 2010). As it is technically tedious and a lengthy procedure, a different approach is to enrich $CD56^+$ $CD3^-$ NK cells from UCB mononuclear cells (MNCs) upfront and culture them in IL-2-containing media for 2 weeks. This method led to a median of 92-fold expansion of NK cells with enhanced *in vivo* antileukemia activity in NOD-SCID-IL2Rγ^{null} mouse (Xing et al. 2010). Another innovative technique includes culturing of MNCs with IL-2 with a feeder layer of irradiated artificial antigen-presenting cells (aAPC) that express membrane-bound IL-21, 4-1BBL, CD64, and CD86, which provide necessary signals for NK cell activation, maturation, and proliferation (Denman et al. 2012). After 2 weeks of culture, $CD3^+$ cells are depleted and the remaining cells are re-cultured for another week. This method resulted in an enormous expansion of NK cells from either fresh (mean 1848-fold) or cryopreserved (2389-fold) UCB samples, and the expanded NK cells demonstrated significant *in vivo* cytotoxicity against multiple myeloma target in mouse model (Shah et al. 2013). Two early phase clinical trials are evaluating the safety and efficacy of prophylactic UCB NK cell infusion (a) in the setting of UCBT in patients with chronic lymphocytic leukemia [ClinicalTrials.gov: NCT01619761] and (b) in conjunction with autologous HCT in patients with multiple myeloma [ClinicalTrials.gov:NCT01729091].

Similarly, antigen-specific chimeric antigen receptor (CAR) T-cells are being created from UCB directed against various antigens like CD19 (Kebriaei et al. 2013; Huls et al. 2013; Pegram et al. 2015) or carcinoembryonic antigen (CEA) (Yasmine van et al. 2015). What is more, CD19 CARs have been generated that not only have antitumor effect but also activity against multiple viruses including Epstein-Barr virus (EBV), cytomegalovirus (CMV), and adenovirus (Micklethwaite et al. 2010), which are the most common viral infections encountered after UCBT.

5.7 Use of Ex Vivo Expanded Lymphocytes to Treat Infections

Generation of virus-specific cytotoxic T lymphocytes (CTLs) from UCB is challenging given not only the finite numbers of but also the naivety of UCB T-cells. These barriers are overcome with ex vivo expansion and priming of UCB T-cells with specific pathogens (Hanley et al. 2009; Park et al. 2006; Sun et al. 1999). Several studies demonstrated successful generation of virus-specific CTL against EBV (Sun et al. 1999; Leen et al. 2013), CMV (Park et al. 2006; Leen et al. 2013), or adenovirus (Leen et al. 2013). More recently, CTLs specific against multiple viruses (EBV, CMV, and adenovirus) have been created and are currently under investigation in a clinical trial (Hanley et al. 2009; Hanley et al. 2013). In this study,

the ex vivo expansion was performed from only 20% fraction of UCB graft in the setting of single UCBT. Preliminary results ($n = 8$) showed that CTL infusion was able to clear reactivated CMV infections within a week of infusion in a majority of patients without the use of conventional treatment. Similarly, all patients with high EBV loads and almost everybody with adenovirus infection were able to clear the viruses (Hanley et al. 2013). No toxicities occurred in any of the patients treated so far (Barrett and Bollard 2015) [NCT00880789].

5.8 Risk of GvHD Can Be Reduced with Prophylactic Use of Ex Vivo Expanded Regulatory T-Cells

Although the risk of GvHD is lower after UCBT compared with mismatched PBPC or BM grafts, it still contributes to significant morbidity and mortality after transplantation (Brunstein et al. 2010; Takahashi et al. 2007; Eapen et al. 2010; Laughlin et al. 2004; Rocha et al. 2004; Atsuta et al. 2009; Brunstein et al. 2012; Chen et al. 2012; Le Bourgeois et al. 2013; Majhail et al. 2012; Majhail et al. 2008; Weisdorf et al. 2014). Regulatory T-cells (Tregs) are a subset of $CD4^+$ T-cells that modulate immune response and play crucial role in self-tolerance. A landmark dose escalation trial from the University of Minnesota (Brunstein et al. 2011) showed that UCB-derived ex vivo expanded Tregs could reduce the incidence of grade II–IV acute GvHD. In that study, $CD25^+$ cells were positively enriched from a 4 to 6/6 HLA-matched third UCB unit using anti-CD25 magnetic microbeads and then cultured with anti-CD3/CD28 monoclonal antibody-coated Dynabeads for about 18 days in the presence of 300 IU/mL IL-2. The culture method resulted in a median 211-fold (range, 13–1796-fold) expansion of Tregs. Twenty-three patients (median age 52 years) received nonmyeloablative regimen of fludarabine, cyclophosphamide, and TBI followed by dUCBT. Prophylaxis against GvHD was provided with mycophenolate mofetil plus either cyclosporine or sirolimus. All patients received expanded Tregs on day +1 at dose levels ranging from 1 to 30×10^5 Tregs/kg, and a second cohort received an extra dose of 30×10^5 Tregs/kg on day +15. No dose-limiting toxicities were observed. Although infused Tregs did not persist beyond 2 weeks, the risk of grade II–IV acute GvHD by day 100 (43%, 95% CI, 23%–64%) was reduced compared with historical controls (61%, 95% CI, 51%–72%, $p = 0.05$). The risks of grade III–IV acute GvHD (17% *vs.* 23%, p = NS), infection, relapse, nonrelapse mortality, and disease-free survival were similar in both the groups. With a median follow-up of 368 days (range, 226–388 days), no chronic GvHD was observed in the study group, compared with 26% (95% CI, 17%–35%) in the controls.

In their subsequent trial (Brunstein et al. 2016), instead of using anti-CD3/CD28 immunomagnetic beads, the authors modified their protocol by expanding positively selected $CD25^+$ cells with anti-CD3 monoclonal antibody plus K562 aAPC that expressed high-affinity Fc receptor (CD64) and CD86. This method resulted in impressive 13,000-fold (range, 1352 to 27,183-fold) expansion of Tregs allowing doses up to 100×10^6 Treg/kg. None of the 11 study

patients experienced dose-limiting toxicity. In contrast to their previous study where grade II–IV acute was seen in 43% of the study patients, cumulative incidence of grade II–IV acute GvHD in the current study was reduced to 9% (95% CI, 0–25), which was considerably lower compared with 45% (95% CI, 24–67, $p = 0.05$) in historical controls. One study patient developed grade III–IV acute GvHD compared with 27% (95% CI, 9–46, $p = 0.06$) in controls. With a median follow-up of 20 months, no study patient developed chronic GvHD compared with 14% in the controls. The density of bacterial, viral, and fungal infections (infections per 1000 patient days) was similar in study and control groups. Similar to the previous study, no Tregs persisted beyond 14 days, despite massive doses of products infused.

5.9 Expert Point of View

Graft engineering methods have evolved significantly over time – starting from culturing UCB cells with cytokines alone to the addition of supporting layer of MSCs to simulate the BM "hematopoietic niche" ex-vivo (de Lima et al. 2012) or blocking differentiation of EPCs leading to expansion of hematopoietic stem cells using nicotinamide analogs (Horwitz et al. 2014), copper chelators (Stiff et al. 2013), or targeting the *Notch* signaling pathway (Delaney et al. 2010). Alternative approach is to improve homing capacity of UCB cells with an aim to accelerate engraftment. This is achieved by treating UCB cells with prostaglandin E2 derivatives (Cutler et al. 2013), enforcing fucosylation of UCB progenitor cells (Popat et al. 2015; Robinson et al. 2014), or using dipeptidyl peptidase-IV (DPPIV) inhibitors (Christopherson et al. 2002, 2004). Graft manipulation has also permitted the generation and clinical use of antivirus and antitumor adoptive immunotherapies as well as cellular therapies for GvHD prevention.

With these novel strategies, the traditional challenge of UCBT – low graft dose – has now been ameliorated. This is expected to increase the available pool of potentially better human leukocyte antigen (HLA)-matched UCB grafts which would otherwise be deemed ineffectual due to inadequate cell content. All of these methods are currently performed in the setting of dUCBT. However with maturing experience, they are now being explored in the setting of single-unit UCBT also, which can potentially reduce the cost of transplantation.

5.10 Future Directions

Novel graft manipulation strategies have remarkably improved time to neutrophil engraftment after UCBT, which now approaches that of other grafts. Yet, time to platelet engraftment, although superior to unmanipulated UBCT, still remains prolonged as compared with PBPC or BM HCT. Therefore, studies focusing on manipulating megakaryocytes or their precursors to enhance platelet engraftment would be of interest. Further, almost all of the currently available expansion strategies

require extended period of incubation for about 2–3 weeks before the final product can be used. This not only delays treatment but also adds to the manufacturing cost and poses extra risk of graft contamination. Studies to shorten the expansion duration are warranted. Moreover, these techniques can currently be utilized only at specialized centers. Attempts to generate "off-the-shelf" products are ongoing which will extend the usability of these products to remote centers as well. Last but not least, the impact on these methods on immune reconstitution is yet to be determined, as none of the expansion techniques have shown reduction in risk of infections compared with unmanipulated UCBT.

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Conditioning Regimens for Cord Blood Transplantation

6

Kelly Ross and Jonathan Gutman

6.1 Introduction

The field of umbilical cord blood transplantation (UCBT) is rapidly evolving. Initially used as a third-line hematopoietic graft choice for patients without HLA-matched sibling donor (MSD) or HLA-matched unrelated donor (MUD), umbilical cord blood (UCB) is increasingly being used as a second-line graft source (Rubinstein et al. 1998; Zhang et al. 2012; Eapen et al. 2007; Wall and Chan 2008; Laughlin et al. 2004).

The purpose of the conditioning regimen prior to hematopoietic cell transplantation (HCT) for malignant disease is twofold: to reduce the remaining burden of disease prior to transplant through direct tumor cytotoxicity and to suppress the immune system enough to allow the infused HSCs to engraft in the bone marrow. For nonmalignant conditions, the conditioning regimen aims only for the latter.

Allogeneic transplantation was originally developed as a strategy to provide stem cell support to overcome dose-limiting hematopoietic stem cell toxicity of chemoradiation used to eradicate malignancy. The discovery of the graft-*versus*-tumor (GvT) effect following allogeneic Hematopoietic cell transplant (HCT) allowed for the development of less toxic regimens while still achieving a cure. This has allowed for the expansion of HCT into a greater population of patients due to the decrease in regimen-related mortality. There is, however, a trade-off between the risk of toxicity from a higher-intensity regimen and the risk of relapse

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_6

or graft failure from a lower-intensity regimen. The selection of conditioning regimen for transplantation must account for each of these risks. The Sorror comorbidity index (Sorror et al. 2005, 2015) has been developed as a pretransplant risk assessment tool that may be used to help determine the appropriate conditioning intensity for a given patient. In UCBT, given the relatively fewer T-cells and $CD34^+$ cells infused as compared to other donor transplants, graft failure is a particular concern, and optimizing conditioning regimens to ensure engraftment is an ongoing issue (Wagner et al. 2002).

This chapter will review preparative regimens for UCBT to treat hematologic malignancies, including myeloablative conditioning (MAC), reduced-intensity conditioning (RIC), and nonmyeloablative (NMA) regimens, as defined by the Center for International Blood and Marrow Transplant Research (Bacigalupo et al. 2009). It will also review the controversial inclusion of antithymocyte globulin (ATG) in UCBT regimens and discuss conditioning regimens for nonmalignant diseases. Our institutional algorithm for the selection of regimen intensity is provided in Fig. 6.1, and a summary table of selected conditioning regimens is provided in Table 6.1.

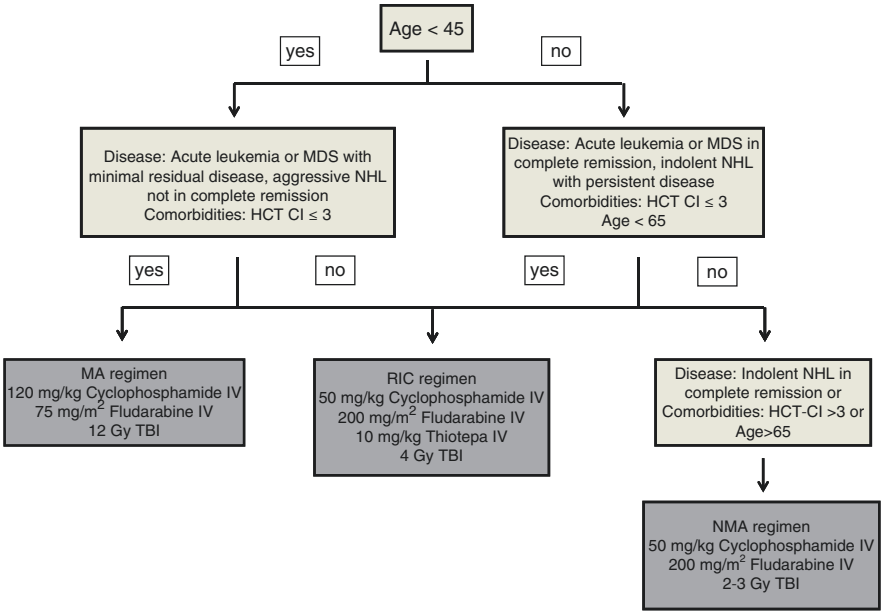

Fig. 6.1 An algorithm for conditioning regimen selection

Table 6.1 A selection of studies of myeloablative and nonmyeloablative conditioning regimens

	Study	Year	Regimen	No. of pts	Engraftment rate	Med. time to engraftment (days)	Treatment-related mortality	Disease-free survival	Relapse rate	Overall survival
Myeloablative										
	Wagner et al. (2002)	2002	TBI 13.2–13.75 Gy, CY 120 mg/kg, ATG 90 mg/kg	102	88%	23	30% at 1 year		37% at 2 years	58% at 1 year, 47% at 2 years
	Barker et al. (2005)	2005	TBI 13.2 Gy, Flu 75 mg/m^2, CY 120 mg/kg	22	100%	23	22% at 6 months	57% at 1 year		
	Brunstein et al. (2010)	2010	TBI 12–13.2 Gy, Flu 75 mg/m^2, CY 120 mg/kg	128		26	34% at 5 years	51% at 5 years	15% at 5 years	
	Sanz et al. (2013)	2013	TBI 13.2 Gy, Flu 75 mg/m^2, CY 120 mg/kg	91	86%	24	24% at day 100, 35% at 5 years	52% at 5 years	19% at 5 years	
	Sanz et al. (2013)	2013	BU 9.6 mg/kg, TT 10 mg/kg, CY 120 mg/kg or Flu 150 mg/m^2, ATG	102	93%	20	13% at day 100, 34% at 5 years	34% at 5 years	42% at 5 years	
	Barker et al. (2015)	2015	TBI 13.2 Gy, Flu 75 mg/m^2, CY 120 mg/kg	56	89%	22	39% at 3 years	50% at 3 years	11% at 3 years	52% at 3 years

(continued)

Table 6.1 (continued)

	Study	Year	Regimen	No. of pts	Engraftment rate	Med. time to engraftment (days)	Treatment-related mortality	Disease-free survival	Relapse rate	Overall survival
	Kanda et al. (2011)	2011	TBI 13.5 Gy, Flu 160 mg/m^2	27	80%	24	28% at 2 years	52% at 2 years	20% at 2 years	58% at 2 years
	Kurtzberg et al. (2008)	2008	TBI 13.2–13.75 Gy, CY 120 mg/kg, ATG 90 mg/kg	191	80%	27	26% at 6 months		20% at 2 years	57% at 1 year, 50% at 2 years
	Yamada et al. (2008)	2008	TBI 12 Gy, CY 120 mg/kg, cytarabine 8–12 g/m^2, G-CSF	25	83%	23	16% at 1 year	60% at 2 years	22% at 2 years	68% at 2 years
	Ooi et al. (2008)	2008	TBI 12 Gy, CY 120 mg/kg, cytarabine 8–12 g/m^2, G-CSF	77	95%	21	10% at 5 years	63% at 5 years	26% at 5 years	66% at 5 years
	Okada et al. (2008)	2008	TBI 12 Gy, Flu 150 mg/m^2, Ara-C 10 g/m^2, G-CSF	38	97%	23	24% at day 100	34% at 3 years	29% at 4 years	37% at 4 years
	Liu et al. (2014)	2014	TBI 12 Gy, CY 120 mg/kg, Ara-C 8 g/m^2	70	97%	19	34% at 6 months, 36% at 2 years	55% at 3 years	12% at 3 years	54% at 3 years
	Horwitz et al. (2008)	2008	BU 520 mg/m^2, Flu 160 mg/m^2	10	20%					

	Mehta et al. (2014)	2014	Flu 160 mg/m^2, Mel 140 mg/m^2, TT 10 mg/kg, ATG	107	92%	19	21% at day 100, 49% at 1 year		64%	46% at 1 year
			Targeted BU, Flu 160 mg/m^2, ATG	24	63%		12% at day 100, 33% at 1 year			29% at 1 year
			Targeted BU, Flu 160 mg/m^2, Clo 120 mg/m^2, TBI 2 Gy, ATG	17	100%	23	6% at day 100, 6% at 1 year			88% at 1 year
	Sanz et al. (2012)	2012	BU 9.6 mg/kg, Flu 150 mg/m^2, TT 10 mg/kg, ATG	88	99%	19	44% at 5 years	37% at 5 years	18% at 5 years	
Nonmyeloablative/ RIC										
	Barker et al. (2003)	2003	BU 8 mg/kg, Flu 200 mg/m^2, TBI 2 Gy	21	76%	26	48% at day 100	24% at 1 year		
			Flu 200 mg/m^2, CY 50 mg/kg, TBI 2 Gy	22	94%	10	28% at day 100	41% at 1 year		
	Komatsu et al. (2007)	2007	BU 8 mg/kg, Flu 180 mg/m^2	17	69%	18	17%	33% at 1 year	39%	39%
	Brunstein et al. (2007)	2007	Flu 200 mg/m^2, CY 50 mg/kg, TBI 2 Gy	110	92%	12	19% at 6 months, 26% at 3 years	38% at 3 years	31% at 3 years	45% at 3 years

(continued)

Table 6.1 (continued)

	Study	Year	Regimen	No. of pts	Engraftment rate	Med. time to engraftment (days)	Treatment-related mortality	Disease-free survival	Relapse rate	Overall survival
	Rio et al. (2015)	2015	Flu 200 mg/m^2, CY 50 mg/kg, TBI 2 Gy	79	89%	15	20% at 2 years	35% at 2 years	46% at 2 years	44% at 2 years
	Ostronoff et al. (2013)	2013	Flu 200 mg/m^2, CY 50 mg/kg, TBI 2–3 Gy	30	97%	17	29% at 1 year	45% at 1 year	27% at 1 year	53% at 1 year
	Somers et al. (2014)	2014	Flu 200 mg/m^2, CY 50 mg/kg, TBI 4 Gy	53	92%	36	19% at 2 years	42% at 2 years	39% at 2 years	57% at 2 years
	Abedin et al. (2014)	2014	BU 12.8 mg/kg, Flu 160 mg/m^2, TLI 4 Gy	20	89%	16	35% at 1 year	35% at 2.5 years	30% at 2.6 years	40% at 1 year
	Ponce et al. (2013)	2013	Flu 150 mg/m^2, CY 50 mg/kg, TT 10 mg/kg, TBI 4 Gy	30	97%	26	20% at 6 months, 28% at 2 years	60% at 2 years	11% at 2 years	60% at 2 years
	Milano et al. (2012)	2012	Flu 150 mg/m^2, treosulfan 42 g/m^2, and TBI 2 Gy	25	92%	23	18% at 2 years	54% at 2 years	21% at 2 years	62% at 2 years

TBI total body irradiation, *CY* cyclophosphamide, *ATG* antithymocyte globulin, *Flu* fludarabine, *BU* busulfan, *Ara-C* cytarabine, *Mel* melphalan, *TT* thiotepa, *Clo* clofarabine, *TLI* total lymphoid irradiation

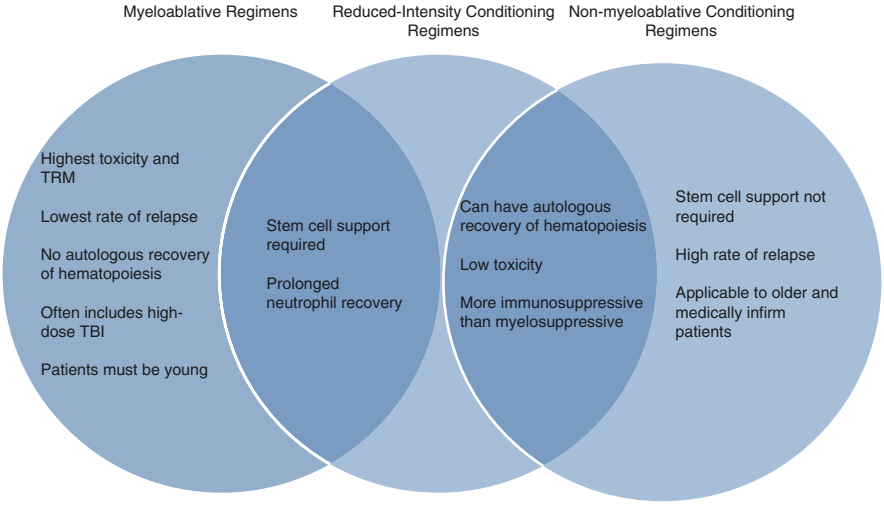

Fig. 6.2 A comparison of types of conditioning regimens

6.2 Myeloablative Conditioning Regimens

The CIBMTR defines MAC regimen as a regimen from which there is no expected hematopoietic recovery without stem cell support (Fig. 6.2) (Bacigalupo et al. 2009). MAC regimens more aggressively deplete the body of any remaining malignant cells and thus have lower rates of disease relapse with higher disease-free survival (Oran et al. 2011), with the trade-off of higher regimen-related toxicity and 30-day mortality. Thus, myeloablative conditioning regimens should be used for young patients with poor-risk aggressive diseases and limited comorbidities.

6.2.1 TBI-Based Regimens

Early UCBT were done with high-dose total body irradiation (TBI)-based MAC regimens, with radiation doses from 12 to 13.75 Gy. Many trials reporting on MAC regimens do not use identical regimens, either in dose of TBI or in adjunctive chemotherapy (Hill-Kayser et al. 2011; Barker et al. 2005, 2015; Brunstein et al. 2010; Sanz et al. 2013; Kanda et al. 2011; Ooi et al. 2008; Liu et al. 2014; Okada et al. 2008; Yamada et al. 2008; Kurtzberg et al. 2008).

The most commonly used MAC regimen is fludarabine (Flu) 75 mg/m^2, cyclophosphamide (Cy) 120 mg/kg, and TBI 13.2 Gy. This regimen has been assessed primarily in acute leukemia patients. In one of the first studies of this regimen, Barker et al. (2005) evaluated 21 patients with a median age of 24. The patients demonstrated engraftment at a median of 23 days, and all had complete donor

chimerism with no graft failure. At a median follow-up of 10 months, the 1-year disease-free survival was 57%.

Subsequent studies of this regimen have yielded similar results. Brunstein et al. (2010) evaluated 128 AML patients. Median time to neutrophil engraftment was 26 days. NRM was 34% at 5 years, and LFS was 51% at 5 years, with 15% rate of relapse. Sanz et al. (2013) evaluated 91 patients. Seventy-eight of 91 acute leukemia patients had stable engraftment at a median of 24 days, and time to engraftment was correlated with cell dose of the graft. Thirty-two patients died at a median of 62 days after transplant, and NRM at 100 days was 24%. At 5 years, the overall LFS was 52%, and the overall rate of relapse was 19%. Barker et al. (2015) evaluated 56 patients, all with high-risk acute leukemia or myelodysplastic syndrome (MDS). Rate of engraftment was 89% by day +100, with a median time to engraftment of 22 days. In patients who engrafted, 100% had full chimerism from a single unit by day +60. The 3-year TRM was 39%, relapse was 11%, and OS was 52%.

Another regimen of Flu 160 mg/m^2 and TBI 13.5 Gy was evaluated in 27 patients by Kanda et al. (2011) from Duke. The cumulative incidence of neutrophil engraftment was 80% by day +50. The 2-year OS and DFS were 58% and 52%, and the 2-year incidence of TRM was 28%.

Several studies out of Asia have incorporated cytarabine into MAC conditioning regimens. Ooi et al. (2008) evaluated 77 AML patients using Cy 120 mg/kg, cytarabine 8–12 g/m^2, and TBI 12 Gy, along with G-CSF. Engraftment occurred in 95% of patients at a median time of 21 days. At 5 years, TRM was 10%, DFS was 63%, relapse was 26%, and OS was 66%. Liu et al. (2014) compared UCBT with sibling transplantation for leukemia using the same regimen, and while the outcomes were not as positive, they were in line with those from other MAC conditioning regimens, with 97% engraftment, 36% TRM at 2 years, relapse rate of 12% at 3 years, and OS of 54% at 3 years. Okada et al. (2008) evaluated 38 patients with a variety of hematologic malignancies with a TBI 12 Gy/FLAG (Flu 150 mg/m^2, cytarabine 8 g/m^2, and GCSF) conditioning regimen. The engraftment rate was 97%, with engraftment occurring at a median of 23 days. TRM was 24% at day 100, with 34% DFS at 3 years, relapse of 29% at 4 years, and OS of 37% at 4 years.

6.2.2 Chemotherapy-Based Regimens

As TBI remains a mainstay of MAC UCBT, there is limited data on regimens based on chemotherapy alone. Much of the literature on chemotherapy-based conditioning regimens centers around busulfan (Bu), a bifunctional DNA alkylating agent. Bu is used frequently in MAC regimens for peripheral blood or bone marrow transplants, and while it is myeloablative, it is not potently immunosuppressive, nor does it kill mature lymphocytes (Ciurea and Andersson 2009; Santos and Tutschka 1974). Horwitz et al. (2008) evaluated the use of sequential *versus* concurrent Bu 130 mg/m^2 daily × 4 days and Flu 40 mg/m^2 daily × 4 days, but only ten patients were enrolled, as the study was stopped early due to only two out of ten patients

engrafting. Many MAC UCBT chemotherapy regimens have used antithymocyte globulin (ATG), the use of which will be discussed below. Mehta et al. (2014) evaluated three separate regimens, all of which included ATG. The first regimen was Flu 160 mg/m^2, melphalan (MEL) 140 mg/m^2, and thiotepa (TTP) 10 mg/kg. This regimen demonstrated a high rate of engraftment, 92% at a median of day 19, but demonstrated a 21% 100 day NRM, with 46% 1-year survival. This prompted Mehta et al. to evaluate another regimen, targeted Bu and Flu 160 mg/m^2. This demonstrated only a 12% NRM but had a 38% rate of graft failure, with 64% of patients dying from their primary disease. Clofarabine and TBI 2 Gy were added to this regimen, which showed 8% NRM, 94% engraftment, and 83% 1-year overall survival. Sanz et al. (2012) evaluated Flu 150 mg/m^2, Bu 9.6 mg/kg, TTP 10 mg/kg, and ATG in 88 hematologic malignancy patients undergoing single UCBT in Spain. Of the 84 evaluable patients, 83 had engraftment at a median of day +19 with full donor chimerism. The 5-year NRM was 44%, and the 5-year relapse rate was 18%, with 5-year DFS of 37%.

6.3 Nonmyeloablative/Reduced-Intensity Conditioning Regimens

NMA conditioning has opened up transplant as a viable option for an increasingly wide patient population, including older and more medically infirm patients who might previously have been denied transplant. The use of NMA conditioning allows for a more tolerable conditioning regimen, relying heavily on graft-*versus*-tumor effect for cure. Data supports the efficacy and safety of NMA transplants for patients well into their 70s (Sandhu et al. 2016). As defined by the CIBMTR, patients receiving NMA conditioning regimens experience minimal cytopenias and can have autologous recovery of hematopoiesis without stem cell support (Bacigalupo et al. 2009). The function of the conditioning regimen is to provide immunosuppression, rather than myelosuppression, to allow cells to engraft (Fig. 6.2). However, these regimens carry the highest risk of graft failure, due in large part to the lack of marrow ablation. Relapse rates also are typically higher following NMA regimens.

RIC regimens fall into neither the MAC nor the NMA category – as defined by the CIBMTR, they are regimens with variable duration of cytopenias, which do require stem cell support. Autologous recovery of hematopoiesis can occur, but generally after a prolonged period of cytopenias associated with increased morbidity and mortality (Bacigalupo et al. 2009). These regimens walk the fine line between more intensive regimens to prevent relapse, with less regimen-related toxicity. There is variable recovery of hematopoiesis without stem cell support, and the definition of what exactly constitutes a RIC regimen *versus* a NMA regimen remains in flux (Fig. 6.2).

Barker et al. (2003) initially investigated a Flu 200 mg/m^2, Bu 8 mg/kg, and TBI 2 Gy as an NMA regimen following UCBT. While Flu/Bu/TBI 2 Gy demonstrated 100% donor chimerism, it was associated with only 76% engraftment as well as

prolonged neutropenia. Komatsu et al. (2007) evaluated 17 patients with a regimen of Flu 180 mg/m^2 and Bu 8 mg/kg. Of 13 evaluable patients, nine (69%) achieved neutrophil engraftment at a median of day 18, and six of those nine patients had 100% donor chimerism by day 100. At 13.1 month follow-up, six of the 13 patients remained in remission. Because of low rates of engraftment associated with its use, Bu is generally no longer used in NMA regimens. The Minnesota group went on to replace Bu in its regimen with 50 mg/kg Cy, and Flu 200 mg/m^2, Cy 50 mg/kg, and TBI 2 Gy have become the most commonly used NMA conditioning regimen. Barker et al. (2003) evaluated 22 patients with this regimen and demonstrated 94% engraftment at a median of 10 days, but with only 74% donor chimerism by day +21, although all patients achieved total donor chimerism by day +100. TRM was 28% at day +100, with 41% DFS at 1 year. In a larger series, Brunstein et al. (2007) evaluated 110 patients with the same regimen and found 92% engraftment at a median of 12 days, again with a delay in full donor chimerism at with 89% at day +21, and again with all patients eventually achieving full chimerism. At 3 years, TRM was 26%, DFS was 38%, rate of relapse was 31%, and OS was 45%. Rio et al. (2015) also evaluated the same regimen and found 89% neutrophil engraftment by day +60, with a median time to recovery of 15 days. Full donor chimerism was seen by day +30 and in 92% by day +60. At 2 years, the NRM was 20%, and the incidence of relapse was 46%.

Although the toxicities of this regimen were acceptable, graft failure rates remained high among select patients. Brunstein et al. (2007) defined this population as patients who had never had an autologous Hematopoietic cell transplant (HCT) or who had received <2 cycles of chemotherapy within 3 months prior to their transplant. In this high-risk cohort of patients, ATG was used to help promote engraftment. However, as discussed below, ATG carries several risks as related to UCBT. Ostronoff et al. (2013) used the same Flu/Cy/TBI regimen evaluated at Minnesota, although they allowed for dose escalation of TBI to improve engraftment in this high-risk cohort. Patients deemed to be high risk received TBI 3 Gy, whereas those with heavily pretreated marrows received TBI 2 Gy. Thirty patients were evaluated, and eight were considered high risk and received TBI 3 Gy. Median time to engraftment was 17 days. There were no cases of primary graft failure and one case of secondary graft failure. The 1-year OS was 53%, with 45% PFS. Somers et al. (2014) further increased the TBI dose and investigated Flu/Cy/TBI 4 Gy. Fifty-three patients were evaluated, with 92% engraftment and a median time to neutrophil recovery of 36 days. At 2 years, relapse and nonrelapse mortality were 39% and 19%, respectively, with PFS of 42% and OS of 57%.

Abedin et al. (2014) evaluated a regimen of Flu 160 mg/m^2, BU 12.8 mg/kg, and total lymphoid irradiation (TLI) at 4 Gy in 20 patients undergoing UCBT. Neutrophil engraftment was 89% with median time to recovery of 16 days, and all patients engrafting had complete donor chimerism by day +30. The incidence of TRM at 1 year was 35%, and the incidence of relapse was 30% at 2.6 years, with a 1-year OS of 40%.

In addition to graft failure issues, relapse remains a significant concern following NMA transplant. The development of more intensive “middle intensity” regimens

has generated promising early data regarding relapse rates. Building upon the NMA backbone of Flu/Cy/TBI 2 Gy reported by the University of Minnesota group, Ponce et al. (2013) reported on 30 patients using a regimen of Flu 150 mg/m^2, Cy 50 mg/kg, TTP 10 mg/kg, and TBI 4 Gy. Patient factors considered when choosing this RIC over a MAC or NMA included age $\geq$ 50, extensive prior therapy, and/or significant comorbidities. The regimen did have the delayed engraftment seen in MAC regimens, without brief autologous recovery of cells seen in NMA transplant. Additionally, at a median day +21, donor chimerism was 100%, as is seen in more myeloablative regimens, rather than the mixed chimerism often seen in NMA regimens. The median time to engraftment was 26 days, with one case of graft failure. TRM was 20% at day 180, and relapse was 11% at 2 years, with both DFS and OS 60% at 2 years. Milano et al. (2012) reported 92% cumulative incidence of engraftment and median time of neutrophil recovery 23 days following Flu 150 mg/m^2, treosulfan (Treo) 42 g/m^2, and TBI 2 Gy. NRM at 2 years was 18%, relapse incidence was 21%, and overall survival was 61.7% in a high-risk patient cohort.

In addition to a wider population of patients that are able to be transplanted, a wider variety of diseases are also being successfully transplanted using NMA or RIC regimens (Chao et al. 2004; Robin et al. 2015). Lymphoid malignancies in particular have increasing data on the use of UCBT. Rodrigues et al. (2009) evaluated patients with lymphoid malignancies (non-Hodgkin lymphoma, Hodgkin lymphoma, and chronic lymphocytic leukemia) and found that lower-intensity conditioning regimens that incorporated low-dose TBI and did not use ATG had improved NRM, PFS, and OS. Rodrigues et al. (2014) then compared the use of a variety of RIC regimens between UCBT and MUD and found no difference in NRM, relapse, PFS, or OS. Majhail et al. (2006) compared the use of UCBT with MSD PBHCT in Hodgkin lymphoma using Flu/Bu/TBI 2 Gy or Flu/Cy/TBI 2 Gy and found comparable outcomes between the two groups, with a PFS at 2 years of 25% in UCBT and 20% in HLA-MSD.

6.4 ATG in Preparative Regimens

ATG, which can be either horse or rabbit derived, is a polyclonal antibody used in allogeneic transplant for its T-cell-depleting properties. For decades, ATG has been used in bone marrow and PBHCT to prevent graft failure and graft-*versus*-host disease (GvHD) and to improve rates of engraftment. The depletion of T-cells, however, can slow immune reconstitution and potentially blunt the graft-*versus*-tumor effect seen in a T-cell replete graft.

6.4.1 Delay in Immune Reconstitution

One of the challenges of UCB as a graft source over other sources of HSCs is prolonged delay in immune reconstitution, which increases the risk of opportunistic infection in the early period following transplantation. The use of ATG in UCBT has

been increasingly scrutinized, as the use of ATG may be a contributing factor in the delayed immune reconstitution. Initial studies done for both single- and dual-cord blood transplantation used MA regimens that incorporated ATG. Thomson et al. (2000) evaluated 30 children undergoing a myeloablative single UCBT using either Cy/TBI 14.4 Gy/ATG or Bu/Mel/ATG and found that CD4, CD8, B-cells, and NK cells recovered posttransplant at a median time of 12 months, 9 months, 6 months, and 2 months, respectively. This time to reconstitution, particularly in the T-cell population, is longer than either bone marrow or peripheral blood stem cell sources (Mohty 2007; Thomson et al. 2000; Ruggeri et al. 2011; Danby and Rocha 2014; Cantó et al. 2005; Talvensaari et al. 2002). Jacobson et al. (2012) compared 102 adult unrelated donor recipients with 42 adult double UCBT recipients, all of whom had received rabbit ATG. Reconstitution of T-cell subsets was significantly lower at 6 months post-HCT in the UCBT patients as compared to the matched unrelated donors.

6.4.2 Infection/Posttransplant Lymphoproliferative Disorder (PTLD)

The major consequence of delay in immune reconstitution is opportunistic infection, particularly viral infections. Reactivation of CMV, EBV and PTLD, HHV-6, adenovirus, and HSV can all be deadly for the cord blood recipient. Conditioning regimens without ATG have been shown to have significantly fewer viral reactivations and a lower risk of death from viral reactivation (Lindemans et al. 2014; Sauter et al. 2011). EBV reactivation also carries with it an increased risk of PTLD. Brunstein et al. (2006) evaluated 335 patients undergoing UCB transplant and found comparable rates of complications of EBV, mainly PTLD, between those patients receiving a MAC regimen (3.3%) and those receiving a nonmyeloablative regimen (7.4%). However, those patients who received a nonmyeloablative ATG-containing regimen had significantly higher incidence of PTLD over those that did not (21% *vs.* 2%, $p < 0.01$). Ballen et al. (2010) also found a trend toward increased risk of PTLD in patients receiving an ATG-containing regimen.

6.4.3 Graft-*Versus*-Host Disease (GvHD)

In transplant for nonmalignant disorders, the avoidance of GvHD is paramount; however, for malignant disorders, measures to avoid GvHD can often contribute to relapse. Serum levels of ATG are detectable weeks to months following infusion, and higher serum levels of ATG are correlated with lower rates of acute GvHD (Remberger et al. 2012; Remberger and Sundberg 2009). Pascal et al. (2015a) evaluated 661 patients receiving a RIC cord blood transplant conditioning regimen with Cy (50 mg/kg), Flu (200 mg/m^2), and a single fraction of low-dose TBI ± ATG and found a cumulative incidence of grade II–IV acute GvHD of 15% and 41% in the ATG and non-ATG groups, respectively, and a cumulative incidence of grade III–IV

acute GvHD of 1.3% and 16% in the ATG and non-ATG groups, respectively. Lindemans et al. (2014) evaluated 127 children receiving UCBT with either a MAC or RIC regimen and early (days −9 to −5), late (days −5 to 0), or no ATG and found that while patients receiving late ATG had significantly lower rates of acute GvHD than either early or no ATG, there was no difference among rates of chronic GvHD. Ponce et al. (2015), however, did find a lower rate of chronic GvHD in children and adolescents with ALL treated with a regimen that included ATG than those without ATG, 22% *versus* 43%.

6.4.4 Engraftment

One of the disadvantages of the use of UCBT is the lower rate of engraftment compared to other stem cell sources, and arguments for the incorporation of ATG into the conditioning regimen in UCBT include improvement in engraftment. However, recent studies have demonstrated no difference in engraftment rates between those patients that are receiving ATG during UCBT for malignant disease and those that are not (Lindemans et al. 2014; Pascal et al. 2015a, b; Ponce et al. 2015; Hagen et al. 2014; Zheng et al. 2015). During the nascent days of UCBT, ATG may have had a role in aiding engraftment, but with the use of two cord blood units to augment cell dose, the use of low-dose TBI, and the addition of Flu to conditioning regimens, the effect of ATG on engraftment appears to be blunted by these other effective mechanisms of aiding stem cell engraftment.

6.4.5 Outcome

Although ATG does improve rates of acute GvHD in cord blood transplantation, Pascal et al. (2015a) found the rate of nonrelapse mortality to be 46% in the ATG group and 26% in the non-ATG group (HR 1.68, $p = 0.0009$) and the 3-year probability of overall survival to be 30% in the ATG group and 48% in the non-ATG group ($p < 0.0001$). Hagen et al. (2014) found no difference in engraftment, TRM, or GvHD between patients treated with and without ATG. Zheng et al. (2015) showed a trend toward inferior OS (64.1% *vs.* 54.1%) and significantly inferior LFS (56.6% *vs.* 37.7%) in children receiving ATG. Given this recent outcome data, ATG should be used judiciously in UCBT conditioning regimens, balancing the risk of graft failure, relapse, and GvHD with that of infection, delayed immune reconstitution, and mortality.

6.5 Conditioning Regimens for Nonmalignant Disease

The goal of transplantation for nonmalignant disease is to replace the abnormal host hematopoietic system with a normal donor hematopoietic system with a minimally toxic conditioning regimen without any need for the graft-*versus*-tumor

effect. There are a variety of nonmalignant diseases for which allogeneic transplantation is used, which include bone marrow failure syndromes (idiopathic severe aplastic anemia, Fanconi anemia, Diamond-Blackfan anemia, Shwachman-Diamond syndrome), hemoglobinopathies, inborn errors in metabolism, congenital immunodeficiencies, and sickle cell anemia. Because no GvT is needed, no amount of GvHD is desirable, so regimens for nonmalignant disease often include a T-cell-depleting agent such as ATG. Because patients with nonmalignant disease have often not been heavily pretreated with chemotherapy, engraftment is the primary challenge in using UCB as the donor source. Patients with marrow failure syndromes have often been heavily pretransfused and are at a high risk of alloimmunization and donor-specific antibodies. The use of Flu in preparative regimens and a higher TNC dose have both been shown to be effective techniques in improving engraftment (Peffault de Latour et al. 2011; Gluckman et al. 2007). RIC conditioning regimens are often associated with the emergence of a mixed chimera, and while full donor chimerism is often not necessary in transplants performed for nonmalignant disease, the presence of a mixed chimera is also a risk factor for eventual graft failure.

Studies evaluating the use of UCBT in bone marrow failure syndromes have been sparse compared to UCBT for malignant disease, given the concern for graft failure. MacMillan et al. (2015) used Flu 140 mg/m^2, Cy 40 mg/kg, ATG, and TBI 3 Gy as a preparative regimen for patients with Fanconi anemia. Fifteen out of 17 patients had neutrophil engraftment, and all those who engrafted had 100% donor chimerism by day 100. Ruggeri et al. (2008) also used Flu-based regimens in evaluating 14 patients with bone marrow failure syndromes and found the median time to engraftment was 28 days, and the median incidence of neutrophil recovery at day +60 was 57%. Six patients experienced graft failure. Of nine evaluable patients at day +100, seven had full donor chimerism. By 6 months, seven of the 14 patients had died, four of infection, two of acute GvHD, and one of thrombotic microangiopathy. Parikh et al. (2014) evaluated 22 children undergoing single UCBT with a regimen consisting of alemtuzumab 3 mg/kg, hydroxyurea 30 mg/kg/day, Flu 150 mg/m^2, Mel 140 mg/m^2, and TTP 200 mg/m^2. Most patients had primary immunodeficiencies or inborn errors of metabolism. Three patients experienced graft failure (one primary, two secondary). Median time to neutrophil engraftment was 20 days. Of 18 evaluable patients at 6 months posttransplant, 15 had >90% donor chimerism. At 1-year posttransplant, OS and EFS were 77.3% and 68.2%, respectively. Bizzetto et al. (2011) evaluated 64 children undergoing UCBT for hereditary bone marrow failure syndromes other than Fanconi anemia. Twenty of these children received UCB units from related donors, and outcomes were excellent, with 95% 3-year survival. In the patients receiving unrelated donor UCBT, the incidence of neutrophil recovery by day +60 was only 55%, with a 61% 3-year survival.

A more fully MAC regimen of targeted Bu, Cy 50 mg/kg, and ATG 90 mg/kg was evaluated by Prasad et al. (2008) in 159 patients with inherited metabolic disorders. Neutrophil engraftment was achieved in 87% of patients at a median of 22 days. Of those, 97% achieved >90% donor chimerism. TRM was 28.3%, with

1- and 5-year OS of 71.8% and 58.2%, respectively, although the subset of children with a higher-performance status did better, with 1-year and 5-year OS of 84.5% and 75.7%, respectively.

Regimens relying on alemtuzumab, a CD52 antibody, as a T-cell-depleting agent have not been as successful. Marsh et al. (2015) evaluated a large series of patients with metabolic disorders, primary immune deficiencies, or non-Fanconi anemia, with many donor sources, only ten of which had a cord blood transplant. The conditioning regimen used consisted of Flu 150 mg/m^2, MEL 140 mg/m^2, and alemtuzumab. The UCBT patients had the highest likelihood of mixed chimerism at 78%. Kamani et al. (2012) evaluated the same regimen in children with severe sickle cell disease and again found that only three out of eight patients enrolled had sustained donor engraftment. Withdrawal of immunosuppression and donor lymphocyte infusion (DLI) are two possible treatment options to resolve a mixed chimera and prevent eventual graft failure, but in UCBT a DLI is not an option, and so this regimen is not recommended in cord blood transplant. Radhakrishnan et al. (2013) reported similarly high rates of graft failure in a regimen containing Flu 180 mg/m^2, Bu 12.8 mg/kg/day, and alemtuzumab 54 mg/m^2.

For patients with primary or relapsed/refractory hemophagocytic lymphohistiocytosis, limited data is available on UCBT. Sawada et al. (2013) reviewed 53 cases with a variety of conditioning regimens and found an OS rate of 65.4% with a RIC regimen.

6.6 Expert Point of View

The selection of a conditioning for UCBT affects all of the relative risks of post-transplantation complications: graft failure, regimen-related toxicities, GvHD, infections, and relapse rates. In UCBT, graft failure is a particular concern given the unique characteristics of the graft. While modern intensive conditioning regimens and modern cord blood selection criteria have reduced graft failure rates in this population, patients unable to tolerate intensive conditioning and who have had minimal pretreatment and/or have fibrotic diseases of the marrow as well as patients with nonmalignant diseases remain at ongoing high risk for graft failure. At our center, we are strong advocates for the use of modest doses of TBI rather than T-cell-depleting agents to help overcome immune barriers to engraftment as we feel that the infectious complications associated with T-cell depletion in the UCBT setting are unacceptable and that GvHD following T-cell replete UCBT is manageable. With regard to regimen-related toxicity, we believe that the most intensive high-dose TBI-based regimens carry such extreme risks in terms of acute toxicities as well as exacerbating aGvHD and profoundly delaying neutrophil recovery that we have greatly decreased our use of these regimens and we have limited TBI to 12 Gy rather than 13.2 Gy. We reserve this regimen for extremely high-risk disease in young patients (Fig. 6.1). With regard to disease relapse, we have significant concerns about relapse rates following NMA regimens, and we advocate strongly for ongoing investigation of middle-intensity regimens designed to optimize the

balance of acute toxicities against the risk of relapse. We are particularly encouraged by preliminary data associated with Ponce et al. (2013) Flu 150 mg/m^2, Cy 50 mg/kg, TTP 10 mg/kg, and TBI 4 Gy regimen and have had excellent outcomes with this regimen in our experience.

6.7 Future Directions (Conclusion)

Against the backdrop of other developments in the field of UCBT, including use of double-unit transplants to decrease graft failure, ex vivo expansion and other strategies to decrease time to engraftment, and accumulation of an increasing inventory of CB units allowing for selection of larger and better matched units, continued optimization of conditioning regimens should continue to improve outcomes. Ongoing investigations of the utility of the Sorror comorbidity index to help stratify patients to appropriate conditioning regimens may provide better guidance in the future. Optimization of disease-specific regimens continues, and increasing incorporation of targeted therapies into conditioning regimens and as maintenance therapies will hopefully reduce relapse rates. Continued development of middle-intensity regimens providing a more broad profile of risks of toxicity should allow better matching of the conditioning regimen to the patient's pretransplant disease status-specific risk of relapse and hopefully reduce relapse without significantly increasing toxicity. Ongoing investigation of minimally toxic regimens sufficiently immunosuppressive to promote engraftment in nonmalignant diseases also remains an important goal.

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Cord Blood Transplants for Nonmalignant Disorders: Data, Consensus, and Challenges

7

Vinod K. Prasad

7.1 Introduction

Since the first cord blood transplantation (CBT) in a child with Fanconi anemia in 1988 (Gluckman et al. 1989), a wide spectrum of nonmalignant diseases (NMD) has been successfully treated with CBT. Broadly, NMD could be subgrouped as primary immunodeficiency diseases (PID) like severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), and Wiskott-Aldrich syndrome (WAS); hemoglobinopathies (Hb-pathies) like thalassemia and sickle cell disease (SCD); bone marrow failure (BMF) syndromes like Fanconi anemia (FA), other inherited BMF syndromes, acquired severe aplastic anemia (SAA), and osteopetrosis; and inherited metabolic disorders (IMD) like leukodystrophies and mucopolysaccharidoses.

Following a successful transplant, the donor-derived cells are able to the correct the underlying defect either by direct repopulation of the hematopoietic and immune systems or by indirect delivery of the missing enzymes or other critical building blocks across the cellular membranes. In addition to the aforementioned clinically accepted indications, cellular therapy utilizing cord blood units (CBU) or cells derived from the CBU are being explored for regenerative medicine. A number of review articles and book chapters from our group have detailed the status of CBT in NMD in the last few years and make the basis of this chapter (Page et al. 2014; Prasad and Kurtzberg 2008, 2009, 2010a, b). Current chapter will review the

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_7

indications, conditioning regimen, unit selection, as well as outcomes of CBT for nonmalignant disorders and will summarize findings from the published literature and our own institution's significant experience in the field.

7.2 General Comments of CBU and CBT

Increasing utilization of unrelated CBT (UCBT) in children, particularly for NMD, reflects a number of specific advantages of CBT in this setting. CBT offers unique advantages including broader donor access, lower risk of graft-*versus*-host disease (GvHD), and rapid availability of ≥4/6 matched cord blood CBU containing suitable cell dose (Eapen et al. 2007; Kurtzberg et al. 2008; Martin et al. 2006; Prasad et al. 2008a; Rocha et al. 2000, 2001, 2004; Rocha and Gluckman 2006; Rubinstein et al. 1998; Wagner et al. 2002). In the last 29 years since the first CBT in 1988 (Gluckman et al. 1989) and 24 years since the first unrelated donor CBT (UCBT) in 1993 (Kurtzberg et al. 1994), many studies have demonstrated that hematopoietic progenitor cells derived from related or unrelated umbilical CB units are at least as effective as those derived from the bone marrow or growth factor mobilized peripheral blood (Eapen et al. 2007; Kurtzberg et al. 2008; Rocha et al. 2001; Barker et al. 2001). Most of NMD in children that can be cured by HCT are genetic in nature and may affect more than one child in a family, and therefore the probability of finding a suitable HLA-matched sibling or other HLA-matched related donor for NMD patients is even lower compared to other HCT candidates. For patients without a MRD, CB offers an attractive option because of ready availability and less stringent HLA matching requirement. Biologically, transplantation in patients with nonmalignant diseases facilitates the study of the impact of graft characteristics on transplant outcomes in an environment where graft-*versus*-malignancy effect is not a competing risk factor. Currently, HLA typing by intermediate resolution for class I (A and B) loci and high resolution for HLA class II (DRB1) is considered acceptable, and CB units matching at ≥4/6 loci are considered adequate. However, if available, high-resolution typing of HLA-A, −B, −C and −DRB1 is utilized for donor selection. CB units with pre-cryopreservation cell counts ≥2.5−3 × 10^7 nucleated cells/kg are considered adequate for better matching grafts, but doses above 5 × 10^7/kg yield superior results and can be achieved in most pediatric patients (Eapen et al. 2007; Kurtzberg et al. 2008; Wagner et al. 2002; Prasad et al. 2008b; Barker and Wagner 2003).

7.3 CBT for Patients with Primary Immunodeficiency Disorders (PID)

7.3.1 Overview

The PIDs comprise a clinically heterogeneous group of disorders associated with frequent life-threatening infections and early mortality. HCT is curative in most children with PID, and the best outcomes are achieved when HCT is performed

early in life (Pai et al. 2014). However, patients who have had multiple infections and related comorbidities are at a high risk for posttransplant morbidity and mortality. In general, bone marrow transplant (BMT) from a HLA-matched related donor (MRD) offers the best outcomes in patients with PID. As described earlier, most PID patients are unlikely to find HLA-MRD. However, these patients tend to be younger, and therefore CBU with adequate to high cell doses are often available. Low risk of GvHD following CBT is additionally useful as many patients with PID like Wiskott-Aldrich syndrome (WAS), Omen syndrome, and on occasion SCID have inherent skin, gut, and liver problems. Another immediately available graft source is a haploidentical or mismatched related donor (MMRD), usually from one of the parents or relatives. There is significant literature on the use of T-cell-depleted grafts from haploidentical parents in patients with severe combined immunodeficiency (SCID); however, many patients will fail to reconstitute B-cell function (Buckley et al. 1993). Furthermore, anecdotal reports of late graft failure are emerging as longer follow-up is available.

Due to rarity and heterogeneity of PID, there are no direct or randomized studies to evaluate the outcomes of these approaches, but recent registry-based analyses have been helpful in defining the relative merits of these two graft sources. A number of small reports and registry-based studies in the last 15–20 years have reported successful outcomes following both related (Bhattacharya et al. 2005; Rocha et al. 2000; Soncini et al. 2009; Tewari et al. 2012) and unrelated donor CBTs (Gennery and Cant 2007) in patients with PID (Bhattacharya et al. 2005; Fernandes et al. 2012; Chan et al. 2013; Frangoul et al. 2010; Diaz de Heredia et al. 2008; Knutsen and Wall 2000). In the original report of 562 unrelated cord blood transplants performed between 1992 and 1997, at least 31 patients were treated for PID (SCID, 24; WAS, 7). Their outcomes were similar to the rest of the group (Rubinstein et al. 1998). In another large study, Gluckman et al. reported 91% overall survival following RCBT in more than 300 recipients with nonmalignant diseases, 36 of whom had SCID (Gluckman et al. 2011). The 5-year OS for PID patients who received myeloablative conditioning followed by UCBT was approximately 70% in two retrospective reports (Diaz de Heredia et al. 2008; Morio et al. 2011). As opposed to T-cell-depleted haploidentical transplant, normal immunologic reconstitution including intravenous immunoglobulin independence was seen in all of the survivors of CBT (Diaz de Heredia et al. 2008). On behalf of Eurocord, Cairo et al. reported the outcomes of UCBT in 93 children with severe PID in 2007 (Cairo et al. 2008). Subjects were transplanted at 40 different centers, had a median age of 0.9 years (range: 0–26), and a median weight of 8 kg (3–39). The diagnoses included SCID ($n = 61$), WAS ($n = 20$), and others ($n = 12$). Almost 60% received a 5/6 or 6/6 HLA-matched CBU. Bu/Cy was the most common regimen (46%) followed by fludarabine-containing regimen (26%). Radiation was included in the regimen in 8%, while 12% did not receive any conditioning. The median number of infused TNC of 8.3×10^7/kg (0.1–94) and $CD34^+$ of 3.4×10^5/kg (0.4–33) was high. Seventy-four patients (80%) received cyclosporine/steroids as GvHD prophylaxis. The cumulative incidences (CI) for neutrophil and platelet recoveries were 85% and 77%, respectively. CIs for acute II–IV and cGvHD were 41% and 23%,

respectively. TRM at 2 years was 31%, and the overall survival (OS) at 2 years was 68% for all patients. OS was higher in those receiving 5/6 or 6/6 matched compared to the lesser matched CBU (78% *vs.* 58%; multivariate analysis, $p = 0.04$).

7.3.2 Severe Combined Immunodeficiency (SCID)

Heterogeneity of the clinical phenotype and molecular genotype makes it challenging to group all SCIDs together (Murray et al. 2013). In general, the immunologic profile, infection history, and comorbidities in individual SCID patient must be taken in to account before deciding on the preparative regimen and donor selection. Fernandes et al. recently described the HCT outcomes of SCID patients who received either UCBT ($n = 74$; as reported to Eurocord) or MMRD HCT ($n = 175$, as reported to EBMT) (Fernandes et al. 2012). The engraftment rates between the cohorts were similar, but a higher proportion of UCBT recipients achieved full-donor myeloid chimerism and B-cell engraftment. While the 5-year OS was similar between the two cohorts (57% and 62% for UCBT and MMRD, respectively), UCBT patients were less likely to need a repeat transplant due to poor graft function. Chronic GvHD was higher in the UCBT group. HLA match of the CBU had a significant impact on survival. HLA-matched CBU recipients had better survival (76%) than the recipients of 5/6 (62%) or 4/6 (35%) grafts (Fernandes et al. 2012). In a very interesting report involving two half-siblings with X-linked SCID, one treated with maternal haploidentical BMT and the other with UCBT matched at 7 of 8 loci after the same myeloablative preparative regimen, the sibling with UCBT achieved >95% donor T-cell chimerism and B-cell engraftment, but the sibling undergoing haploidentical BMT did not achieve B-cell engraftment and had mixed T-cell chimerism (29% donor) (Chan et al. 2013). These studies suggest that while both UCBT and MMRD are viable options, immune reconstitution appears to be more robust after UCBT.

7.3.3 Wiskott-Aldrich Syndrome (WAS)

The clinical phenotype of this X-linked primary immunodeficiency caused by loss-of-function mutations in the WAS gene manifests a wide spectrum of severity. Many patients with severe phenotype have thrombocytopenia and risk of bleeding, persistent eczema, susceptibility to severe opportunistic bacterial and viral infections, various autoimmune and inflammatory complications, and an elevated risk of lymphoid malignancies. Allogeneic HCT is curative for WAS patients. A comprehensive joint European and American study of the long-term HCT outcomes of 194 WAS patients, most receiving myeloablative conditioning, with a subset receiving UCBT ($n = 24$, 12.4%) showed excellent outcomes (Moratto et al. 2011). The 5-year OS in patients transplanted more recently (including all UCBT recipients) was 89%. Full-donor chimerism was seen in 72.3% of patients who survived at least a year posttransplant. In patients with mixed chimerism, higher rates of posttransplant complications were

noted. The best outcomes were seen in recipients of MRD BMT. However, the outcomes following unrelated BMT and CBT were similar. Given their unique clinical features, described above, it was no surprise that higher rates of autoimmunity and neurologic sequelae post-HCT were seen in WAS patients compared to other PIDs. In another study of 88 PID patients, an excellent 5-year OS of 82% was reported in 23 WAS patients who received UCBT. Again, compared to the rest of PID patients, the rates of GvHD and infection were higher in WAS patients (Morio et al. 2011). A report from our center described the outcomes of 15 boys (median age 12 months, range 6–51 months) with WAS undergoing unrelated CBT following myeloablative conditioning with Bu/Cy/ATG +/− Flu (Sun et al. 2009). The CBU were HLA matched at 4/6 (n = 10), 5/6 (n = 3), or 6/6 (n = 2) and contained a median-infused TNC of 8.31×10^7/kg (range, 4.87–16.40). All patients engrafted with a median time to ANC 500 and platelet 50 k of 21 (10–38) and 67 (46–139) days, respectively. All but one patient maintained complete donor chimerism posttransplant. Four patients experienced grade II–IV acute GvHD, and one developed chronic GvHD. Nine patients were surviving with a median follow-up of 89 months (range 9–127). These studies suggest that in the absence of HLA-MRD, both UCBT and MUD are viable options. CBU can be procured at short notice and is more likely to be available for patients with less common HLA types. A search for unrelated CBU should be undertaken as soon as a decision is made to use an alternate donor. Additionally, recipients are more likely to have better outcomes following a myeloablative UCBT if transplanted early in the course of the disease.

7.3.4 Hemophagocytic Lymphohistiocytosis (HLH)

While the success of HCT utilizing bone marrow graft from related and unrelated donor is well documented, the CBT experience in HLH is limited. Myeloablative conditioning in some HLH patients can pose a higher risk of TRM due to preexistent organ dysfunction resulting from prior chemotherapy and immunosuppressive treatments (Baker et al. 2008). Therefore, it is noteworthy that in the context of BMT, good outcomes following Flu/Mel-based RIC regimens have been reported from London and Cincinnati (Cooper et al. 2006; Marsh et al. 2010). However, RIC for CBT in HLH is very challenging. In a recent report f 38 UCBT patients from Japan, a very high graft failure rate of 60% was noted following RIC regimens (Sawada et al. 2013). In contrast, following myeloablative conditioning (MAC) and UCBT in nine HLH, a CIBMTR study reported engraftment in all and survival in six of nine patients (Baker et al. 2008). At our center, OS of 70% was seen in 13 HLH patients who received UCBT. Of these, ten 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 an otherwise suitable donor. Novel RIC or reduced-toxicity approaches utilizing higher immunosuppression should be further explored to optimize the outcomes in these patients.

7.3.5 Chronic Granulomatous Disease (CGD)

Gungor et al. recently reported the results of an international prospective study of HCT in 56 patients with CGD who received a RIC regimen followed by either HLA-MRD ($n = 21$) or HLA-MUD ($n = 35$) BMT or PBHCT (Gungor et al. 2014). Conditioning was 30 mg/m^2/day × 6 of fludarabine, low-dose targeted busulfan (cumulative area under the curve 45–65 mg/L × h) given between days −5 and −3, and serotherapy (antithymocyte globulin, thymoglobuline, or alemtuzumab). Overall survival of 96% and low incidence of GvHD (4% with acute GvHD of grade III–V and 7% with chronic GvHD) are excellent. Similarly, the 2-year EFS of 95% (95%CI 72–99) and 89% (95%CI 72–96) for HLA-MRD or HLA-MUD BMT recipients, respectively, with a low TRM (7%) are also excellent. Graft failure rate was very low (5%) and myeloid chimerism (≥90% in 93% of surviving patients) very high. However, longer follow-up will provide further information about the trajectory of donor chimerism, particularly in patients who have lower levels of lymphoid chimerism and in patients who were still receiving posttransplant immunosuppression. This should be the preferred approach for patients with a fully matched related or unrelated bone marrow donor.

For patients lacking a fully matched adult donor, the use of CBT with myeloablative conditioning is recommended on the basis of following reports including the largest pediatric series of CBT for CGD to date that was published from our center (Bhattacharya et al. 2005; Suzuki et al. 2007; Mochizuki et al. 2009; Shigemura et al. 2013; Ayas et al. 2013a; Tewari et al. 2012). In our study, eight patients underwent UCBT (seven UCBT, one RCBT) after myeloablative conditioning using Bu/Cy/ATG + fludarabine (Tewari et al. 2013). All are alive and disease-free at a median of 5.2 year posttransplant. Two of these patients experienced graft rejection and were successfully re-transplanted, after additional RIC conditioning, with second UCBTs. In a large study of UCBT in PID ($n = 88$), seven CGD patients were included (Morio et al. 2011). Following RIC conditioning, the CI of neutrophil engraftment was low at 43% and only three of seven patients surviving. Thus, RIC for UCBT poses unique challenges, particularly in relation to graft failure which is more common in chemotherapy-naive patients with nonmalignant diseases (Prasad 2014).

7.3.6 Summary PID

CBT as a good therapeutic option in patients with PID is clearly underlined by the studies reviewed in this section, and it should be considered in every patient lacking a matched related donor. The specific donor choice and conditioning regimen decision must be made on the basis of the diagnosis, clinical phenotype, molecular defect, radiation or chemosensitivity, immune profile, infection history, and other comorbidities. Recently, newborn screening for SCID has been initiated in several US states which will likely increase the number of PID patients needing timely access to HCT (Griffith et al. 2014). In that context, CBT provides several advantages as a donor source including low risk of GvHD, high probability of finding a match, and rapid availability.

7.4 CBT for Patients with Hemoglobinopathies

7.4.1 Overview

Thalassemia major, sickle cell disease (SCD), and other diseases arising from the defects in hemoglobin structure and function cause significant morbidity and mortality. Collective experience in the last 3 decades has conclusively demonstrated not only the curative role of allogenic HCT in the treatment of hemoglobinopathies but also the efficacy of early transplantation in preventing and reversing many of the problems that arise from ineffective erythropoiesis, hemolysis, and iron toxicity (Page et al. 2014). While these diseases are similar at a fundamental level, their clinical phenotypes and natural histories differ significantly. For example, in thalassemia, the major problems are caused by iron overload from red blood cell transfusions, while those in SCD are caused by vasculopathy and resulting tissue ischemia. Therefore, the specific questions regarding the time of transplantation, criteria for patient selection, and supportive care guidelines differ between these two diseases even though the overall concept and design of transplantation is similar.

Since the first successful HLA-MRD BMT for thalassemia and SCD in the early 1980s, a few thousand patients with hemoglobinopathies have received allogeneic HCT. Thalassemia accounts for the majority of these transplants which were performed predominantly using MRD following Bu/Cy-based myeloablative regimens. In general, the outcomes of BMT from HLA-MRD in thalassemia have been excellent with high disease-free survival (DFS, 80–90%) (Lucarelli et al. 1990, 2002; Chandy et al. 2005). The outcome of HLA-MUD BMT for thalassemia has also improved, but they are not as good as those of MRD transplants (La Nasa et al. 2005a, b). The Pesaro group from Italy developed a risk classification system to predict the outcomes of HCT in children (<18 years) with thalassemia (Lucarelli et al. 1993). The system uses three adverse risk factors: inadequacy of prior iron chelation, hepatomegaly, and portal fibrosis. Pesaro class 1 includes patients with no risk factors, Pesaro class 2 patients have one to two risk factors, and Pesaro class 3 patients have all three risk factors. In earlier reports, the DFS for class 1–2 patients was 80–90%, whereas that for class 3 was inferior at 50–60% (Lucarelli et al. 1998). With modifications in the conditioning approach to increase immunosuppression but reduce regimen-related dose intensity for class 3 patients, improved outcomes comparable to lower-risk groups were demonstrated (Wagner et al. 2002; Gluckman et al. 1989; Kurtzberg et al. 1994; Barker et al. 2001; Sodani et al. 2004).

The cumulative experience of BMT in SCD is less than that in thalassemia. Nonetheless, SCD patients undergoing HLA-MRD BMT have comparably good outcomes with OS and DFS between 92–94% and 82–86%, respectively (Bernaudin et al. 2007; Vermylen et al. 1998; Walters et al. 1996, 2000a). Indications for HCT in SCD include moderate to severe SCD manifesting as history of stroke, increased risk of stroke based on elevated transcranial Doppler velocities, multiple acute chest syndromes, and multiple vaso-occlusive crises. Long-term outcomes after BMT have demonstrated stabilization of lung disease and vasculopathy (Walters et al. 2000). Subsequent subsections will review related and unrelated CBT in

hemoglobinopathies from published reports and provide evidence of growing acceptance of CBT in the treatment of hemoglobinopathies. Some information about ongoing clinical trial will also be provided.

7.4.2 Related Donor CBT (RCBT) for Hemoglobinopathies

The first report of sibling CBT for a hemoglobinopathy was published in 1995 and documented successful treatment of a 2½-year-old girl with hemoglobin E–β- thalassemia with Bu/Cy myeloablative conditioning (Issaragrisil et al. 1995). In 2005, Walters et al. reported the outcomes of 14 thalassemia and eight SCD patients who received related CBT from CBU that were stored under an NIH funded Sibling Donor Cord Blood (SDCB) program (Walters et al. 2005). At a median follow-up of 124 months (05–77 months), 12 of 14 thalassemia and six of eight SCD patients were alive and disease-free. Similar results were reported by Locatelli et al. in thalassemia ($n = 33$) or SCD ($n = 11$) patients transplanted at various centers around the world (Locatelli et al. 2003). Some of SDCB program patients were also included in this analysis. All cord blood donors were siblings and all but three were fully HLA-matched. The median age was 5 years (1–20 years). All thalassemia patients were Pesaro class 1 ($n = 20$) or 2 ($n = 13$). Conditioning regimens were myeloablative in all cases: Bu/Cy with ATG/ALG in 73% of SCD and 30% of thalassemia, Bu/Cy without ATG/ALG in 18% of SCD as well as thalassemia, Bu/Fludarabine (Flu)/Thiotepa (TT) in 9% of SCD and 21% of thalassemia, and Bu/Cy/TT in 27% of thalassemia patients. Sixty-eight percentage of patients received cyclosporine A alone as GvHD prophylaxis. The median TNC infused was 4×10^7/kg. The actuarial OS in both thalassemia and SCD was 100%. The probability of EFS in thalassemia and SCD was 79% and 90%, respectively. Graft failure represented the major reason for failure. Among the thalassemia patients, higher-intensity conditioning regimen utilizing busulfan plus thiotepa with either fludarabine or Cytoxan (Bu/Flu/TT and Bu/Cy/TT) was associated with a significantly higher probability of EFS compared to Bu/Cy or Bu/Cy/ATG/ALG (94% *vs.* 62%, respectively, $p = 0.03$) due to lower risk of graft failure. The EFS in thalassemia patients who had classes 1 and 2 features was 89 and 62%, respectively. EFS was significantly higher ($p = 0.005$) if MTX was not used. The K-M probability of acute and chronic GvHD was 6% and 11%, respectively. In a smaller study of nine thalassemia patients with advanced disease (six, Pesaro 2; and 3, Pesaro 3), EFS was not as good, particularly in patients receiving mismatched CB units (Fang et al. 2004). A recent Eurocord study of the outcomes of 485 patients with hemoglobinopathies undergoing either HLA-MRD CBT or MRD BMT showed excellent survival in both groups (Locatelli et al. 2013). In thalassemia, the 6-year DFS were 86% and 80% with HLA-MRD BMT and HLA-MRD CBT, respectively. In SCD, the 6-year DFS were 92% and 90% with HLA-MRD BMT and HLA-MRD CBT, respectively. HLA-MRD CBT group had slower neutrophil engraftment and lower GvHD rates confirming previous reports (Rocha et al. 2000; Locatelli et al. 2003). Extensive chronic GvHD was not seen in the CBT group compared to 5% in MRD BMT recipients (Locatelli et al. 2013).

Thus, RCBT offers low risk of GvHD and high probability of engraftment and EFS in most patients with hemoglobinopathies. Since graft-*versus*-leukemia effect is not required in these patients, the decreased risk of GvHD seen with HLA-MRD CB donors can be a very important factor when selecting the optimal donor for transplantation. In addition, survival outcomes following RCBT are comparable to HLA-MRD BMT.

7.4.3 Unrelated Donor CBT (UCBT) for Hemoglobinopathies

In comparison to RCBT, the experience in the use of UCBT in hemoglobinopathies is limited and their outcomes are worse. In that context, it is important to note that while most RCBT data comes from 6/6 matched CBU, the chances of finding a 6/6 matched unrelated CBU is less than 15% (Kurtzberg et al. 2008; Prasad et al. 2008b). Additionally, UCBT is more challenging in hemoglobinopathies due to increased risk of graft rejection from marrow hyperactivity to compensate for chronic anemia, alloimmunization resulting from multiple transfusions, and a competent immune system due to lack of prior chemotherapy exposure (Page et al. 2014). Nonetheless, it is encouraging to see a few case reports of UCBT in hemoglobinopathies in the last 10 years (Hall et al. 2004; Fang et al. 2003; Tan et al. 2004). The youngest patient was a 2-month-old boy with beta-thalassemia major who received a high cell dose, 4/6 HLA-matched unrelated CB units following Bu/Cy/ATG conditioning (Hall et al. 2004). He promptly engrafted with donor cells and is currently alive and well with 100% donor chimerism almost 16 years after transplant. Until 2007, a total of 16 patients with thalassemia and seven patients with SCD had been reported to have undergone unrelated CBT (Fang et al. 2003; Hall et al. 2004; Adamkiewicz et al. 2007; Jaing et al. 2005, 2007; Mazur et al. 2006; Vanichsetakul et al. 2004; Tan et al. 2004). More recent reports have been encouraging, but they also highlight the challenges of using UCBT for the treatment of hemoglobinopathies. In a Taiwanese study of 35 children with thalassemia undergoing UCBT after myeloablative conditioning (Bu/Cy/ATG), the CI of engraftment was 70% with six patients experiencing graft failure despite a high median TNCC of 7.8×10^7/kg (Jaing et al. 2012). Most had received a single CB graft (68%) that was HLA mismatched at 1–2 loci (83%). A high incidence of grades II–III acute GvHD was also reported (80%). The 5-year OS and DFS were 88.3% and 73.9%, respectively. While these results are encouraging, the rates of GvHD and graft failure are higher and survival lower when compared with HLA-MRD grafts. In another report, the same group reported data on five older children (median age 11·1 years, range 10–13·1 years) with beta-thalassemia who received double-CBT from unrelated mismatched donors following same cytoreduction (Jaing et al. 2007). One patient developed secondary graft failure, and the other four developed transient corticosteroid-responsive grades I–III acute GvHD and later limited skin GvHD. At 18·5 months (range 11–32 months), four were alive of whom three were transfusion independent. 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 ($n = 16$) (Ruggeri et al. 2011). Most (76%) received myeloablative conditioning and a mismatched CBU. The CI of graft failure was very high (27 of 51 patients) and was primarily associated with low 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. While this report reviews retrospective registry data, it raises concerns about the overall feasibility of UCBT as currently practiced by the general transplant community (Page et al. 2014).

Outcomes of RIC UCBT in hemoglobinopathies have shown major problems of engraftment. 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 a received RIC regimen (alemtuzumab/Flu/Melphalan) followed by UCBT (Kamani et al. 2012). The EFS for these patients was only 37.5% (Kamani et al. 2012). Similarly, DFS of 50 % were reported with alemtuzumab/Flu/Bu RIC regimen (Radhakrishnan et al. 2013). 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 (Kamani et al. 2012; Shenoy et al. 2004) with thiotepa and hydroxyurea (Parikh et al. 2013). 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 =one, 1-locus mismatch =eight), with graft failure in one patient (median follow-up of 1 year) (Murray et al. 2013).

7.4.4 Summary Hemoglobinopathies

Despite convincing evidence of efficacy, allogenic HCT is underutilized in the treatment of hemoglobinopathies. An important reason could be a lower probability of finding a HLA-MRD due to the genetic nature of hemoglobinopathies. Additionally, many patients, particularly those of ethnic and racial minorities, precisely the demographic with higher incidence of these diseases, are unable to find a suitable HLA-MUD in a timely manner from large registries of unrelated donors. Many wait for a suitable bone marrow donor instead of using a CBT, while the disease and transfusion-related organ dysfunction continues to worsen. Additionally, difficulties in defining the criteria for patient selection and real and perceived risks of potentially serious toxicities have prevented UCBT from becoming more common. Myeloablative conditioning leads to significant mortality and long-term morbidity including gonadal dysfunction and infertility, and RIC leads to high risks of graft failure. Critical assessment of the balance between the risks of early TRM and long-term toxicity and continuing damage from disease process is important as decision to proceed with allogeneic HCT in made for any patient (Prasad and Kurtzberg 2009).

7.5 CBT for Patients with Bone Marrow Failure (BMF) Syndromes

7.5.1 Overview

Inherited BMF syndromes comprise many diseases caused by gene defects that lead to decreased production of single or multiple hematopoietic cell lineages resulting in anemia and/or cytopenia. Many inherited BMF syndrome patients are predisposed to somatic abnormalities, chemotherapy, radiation sensitivity, and an increased risk of malignancies, all of which lead to serious challenges relating to choice of cytoreduction, risks of posttransplant complications, and the long-term prognosis. Increasing experience in the use of CBT for the treatment of various inherited and acquired BMF syndromes points to a greater acceptance of CBT in these diseases. Interestingly, the longest surviving recipient of CBT who is also the world's first patient to undergo CBT was treated for Fanconi anemia. He is alive and well more than 25 years after transplant (Gluckman et al. 1989).

7.5.2 Fanconi Anemia

Fanconi anemia (FA) is characterized by congenital abnormalities, progressive BMF, and increased susceptibility to cancers (Rosenberg et al. 2005). Their tissues are very sensitive to alkylating chemotherapy (Berger et al. 1980) and irradiation (Pasquini et al. 2008) resulting in DNA damage and tissue and organ injury. Additionally, they have difficulties repairing tissue damage caused by GvHD (Guardiola et al. 2004). Early attempts to transplant FA patients were made prior to the recognition of chemotherapy sensitivity and utilized standard dose cytoreduction and therefore resulted in prohibitively high TRM and very low OS (Gluckman et al. 1980, 1983). However, once the intensity of conditioning regimens was reduced by significantly decreasing the doses of alkylating agents like cyclophosphamide, minimizing radiation, and incorporating fludarabine (Flu), the TRM decreased dramatically and the survivals improved (Pasquini et al. 2008; Gluckman et al. 1984; Wagner et al. 2007; Tan et al. 2006; Peffault de Latour et al. 2013). Recently, the European Group for Blood and Marrow Transplantation (EBMT) reported the 40-year experience on the outcomes of FA patients (n = 795) transplanted with related or unrelated bone marrow and/or mobilized peripheral blood hematopoietic cells (PBHCs) (Peffault de Latour et al. 2013). Improved outcomes after HCT were seen in younger (<10 years) patients and those without evidence of leukemic transformation or abnormalities in the bone marrow (Wagner et al. 2007; Peffault de Latour et al. 2013; Ayas et al. 2013a; Mitchell et al. 2013; Alter et al. 2000). Independently, the development of GvHD was associated with increased rates of second malignancies (Guardiola et al. 2004; Deeg et al. 1996), which in turn negatively impacted the long-term OS. Due to the genetic nature of the disease, most FA patients are unlikely to have a nonaffected HLA-MRD. In those patients, unrelated CBU is an attractive option compared to HLA-MUD given the lower incidence of GvHD.

7.5.3 Related CBT Experience in FA

A recent Eurocord registry study of 596 patients undergoing RCBT included 36 FA patients. The data was not specifically analyzed for FA, but in the whole group, the CI of neutrophil engraftment was 91% (±2). The 4-year OS for non-malignant patients, including FA, was 91% (±2) (Gluckman et al. 2011). These results are similar to outcomes following related BMT where the engraftment rates and OS for FA patients in recent reports exceeded 85% and 75% at 5 years, respectively (Pasquini et al. 2008; Peffault de Latour et al. 2013; Locatelli et al. 2007; Bonfim et al. 2007). These comparable outcomes justify the use of either marrow or CB from a MRD for transplantation in FA patients. If a CBU is banked from a MRD, the decision to use CBU alone or in combination with some donor bone marrow can be made on the basis of per kg TNCC of the banked unit.

7.5.4 Unrelated CBT Experience in FA

Early experience with UCBT in FA patients, as described by Rubinstein et al., demonstrated difficulties with engraftment and higher TRM (Rubinstein et al. 1998). A Eurocord retrospective study of 93 FA patients receiving UCBT between 1994 and 2005 not only defined the outcomes but also highlighted many of the challenges in FA patients (Gluckman et al. 2007). The median age was 8.6 years, most CBU were mismatched (6/6, 14%; 5/6, 38%; and 4/6 or lower, 48%), and the median-infused TNC and $CD34^+$ doses were 4.9×10^7/kg and 1.9×10^5/kg, respectively. A majority (61%) received fludarabine-containing regimen, the most common being Flu (25 mg/m^2/day × 4), Cy (10 mg/kg/day × 4) and TBI (200 cGy × 1). Most patients received cyclosporine with prednisone for GvHD prophylaxis. The CI of neutrophil recovery was 60 ± 5% at day +60. CI of grades II–IV acute and chronic GvHD was 32% ± 5% and 16% ± 4%, respectively. With a median follow-up of 22 months (range, 3–121 months), overall survival (OS) was 40% ± 5. However, improved engraftment was seen in patients receiving Flu-based conditioning (72% *vs.* 42%) and receiving TNCC ≥4.9×10^7/kg (50% *vs.* 25%). These two factors along with negative cytomegalovirus (CMV) recipient status were also associated with significantly improved survival (Gluckman et al. 2007). The benefits of fludarabine in the conditioning have been described in various reports including an analysis by Wagner et al. of 98 unrelated donor BMT cases (Wagner et al. 2007). 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) (Ruggeri et al. 2008). 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.

7.5.5 Other Inherited Bone Marrow Failure Syndromes

It is difficult to put such diverse diseases as dyskeratosis congenital and Diamond-Blackfan anemia or Kostmann syndrome and congenital amegakaryocytic thrombocytopenia (CAMT) under the same group, but individually these diseases are exceedingly rare, and thus their transplant experience is very limited. The timing of allogeneic HCT is an important consideration for most IBMF syndromes. In severe congenital neutropenia (SCN), HCT prior to malignant transformation leads to better outcome (Vlachos et al. 2001; Roy et al. 2005; Donadieu et al. 2012; Choi et al. 2005). In Diamond-Blackfan anemia (DBA), as in hemoglobinopathies, HCT is recommended before multiple transfusions have caused iron toxicity and should be considered when medical management fails (Roy et al. 2005; Vlachos et al. 2008). Higher graft failure rates have been seen in infants with malignant infantile osteopetrosis (Peters and Steward 2003; Fischer et al. 1986; Steward 2010; Driessen et al. 2003; Kulpiya et al. 2012), which may be important when selecting donors. Conditioning regimens for these diseases must take into consideration the disease-specific toxicities. For example, post-HCT, pulmonary, and liver problems are high in dyskeratosis congenita (DC) (Yabe et al. 1997; Brazzola et al. 2005; Rocha et al. 1998; Gadalla et al. 2013); cardiac issues in Shwachman-Diamond syndrome (SDS) (Burroughs et al. 2009), pulmonary HT, sinusoidal obstructive syndrome (SOS), and hypercalcemia are high in osteopetrosis (Peters and Steward 2003; Fischer et al. 1986; Steward 2010; Driessen et al. 2003; Kulpiya et al. 2012). Using RIC approaches in DC has shown success, but the role of CBT in this disease has yet to be defined (Ayas et al. 2013b; Nishio et al. 2011).

7.5.6 Related CBT in Other Inherited BMF Syndromes

RCBT experience in non-FA-inherited BMF syndromes (including DBA, DC, CAMT, SCN, and SDS) is limited to individual case reports and small series (Carlsson et al. 2011; Mahadeo et al. 2011; Rocha et al. 2000; Smythe et al. 2007; Gluckman et al. 1997; Cesaro et al. 2005; de Wreede et al. 2013). In a Eurocord study ($n = 64$) of non-FA-inherited BMF syndromes undergoing HCT, 20 patients (DBA, $n = 13$) received HLA-MRD CBT grafts. In the overall cohort, myeloablative conditioning was given to 80% of the patients (Bizzetto et al. 2011). The cumulative incidence of ANC500 by day 60 was 95% (95%CI, 85–100%) and an estimated 3-year OS of 95% (95%CI, 85–100%). These results are comparable to the outcomes after HLA-MRD BMT (Roy et al. 2005; Gadalla et al. 2013; de Wreede et al. 2013; Lipton et al. 2006). In view of the chronic nature of these diseases and superior outcomes using HLA-MRDs, it has been recommended that families may want to consider preimplantation diagnosis and IVF. If a suitable pregnancy is achieved, then the availability of matched CB donors for their affected child could be beneficial (Zierhut et al. 2013).

7.5.7 Unrelated CBT in Other Inherited BMF Syndromes

A study of five CAMT patients treated with CBT at our center was recently published in which all received myeloablative conditioning and partially HLA-mismatched UCBT ($n = 4$) and RCBT ($n = 1$) (Mahadeo et al. 2015). Median times to ANC500 and platelet 20K engraftment were 19 and 57 days, respectively. Acute GvHD, grade II, developed in one patient, who subsequently developed limited chronic GvHD. At median follow-up of 14 years, all patients were alive with sustained donor cell engraftment concluding that CBT is a successful curative option for patients with CAMT. In the Eurocord registry study of 44 inherited BMF recipients of UCBT, a CI of neutrophil engraftment of 55% by day 60 with a high rate of primary graft failure (39%) was seen (Bizzetto et al. 2011). 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). All patients with DC, receiving UCBT after myeloablative conditioning, died (seven transplant-related causes, one unknown). Subsequently, Dietz et al. described a RIC approach in 6 DC patients receiving unrelated HCT (UCBT $n = 3$) (Dietz et al. 2011). Two of the 3 UCBT patients were alive at 12 months and one died secondary to infection. In the eight patients with DBA, three deaths due to graft rejection and two toxicity-related deaths were reported. Others have also seen challenges to engraftment in DBA (Vlachos et al. 2001; Vettenranta and Saarinen 1997; Shaw et al. 1999; Mugishima et al. 2007). At our institution from 1996 to 2011, we have transplanted six children with DBA using unrelated CB grafts after myeloablative busulfan (Bu)-based conditioning (McFarren et al. 2014). All engrafted with full-donor chimerism. Four are alive, well, and transfusion-free ranging from 3 to 16 years post-transplant. These children all received high TNCC grafts which may have overcome engraftment barriers seen by others. Recently, Ruggeri et al. described the outcomes of 45 children with osteopetrosis who received UCBT from 1995 to 2012 as reported to the Eurocord registry (Ruggeri et al. 2013). Almost all received myeloablative conditioning (primarily Bu-based regimens) followed by infusion of CB grafts delivering high TNCC doses, but 18 of the 45 patients experienced graft failure with only three of 18 surviving. At 3 years, the OS for these children was 45 (±8%). Sinusoidal obstructive syndrome (SOS) was seen in six patients (Ruggeri et al. 2013). Using RIC regimens followed by UCBT has largely been unsuccessful in this disease with only one survivor reported (Gonzalez Llano et al. 2008; Tolar et al. 2006; Tsuji et al. 2005).

7.5.8 Acquired BMF Syndromes

Outcomes of unrelated donor CBT for acquired severe aplastic anemia have historically been poor mainly due to primary graft failure (Rubinstein et al. 1998; Neudorf et al. 1995). The high risks of graft rejection could be attributed to a combination of inadequate donor hematopoietic cell number in low cell dose CB units, relative

immune competence of the host, and prior exposure to multiple blood and platelet transfusions. In an early report of CBT 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 (Rubinstein et al. 1998). 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 to 2009 (Peffault de Latour et al. 2011). The CI of neutrophil engraftment by day 60 was only 51% (±6%) with a 3-year OS of 38% (±6%). Improved engraftment (58% *vs.* 33%) and 3-year OS (59% *vs.* 49%) was seen in patients receiving higher pre-cryopreservation TNCC doses ($>3.9 \times 10^7$/kg) as compared to those receiving lower doses, respectively. A trend toward 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. In a Japanese study of 31 patients with a median age of 28 years (range, 0.9–72) followed for a median of 33.7 months (range, 6–77), the probability of OS in the entire group at 2 years was 41.1% (Yoshimi et al. 2008). However, improved outcomes were seen in a subgroup of five patients who received TBI (2–5 cGy), fludarabine, and cyclophosphamide, where the probability of overall survival was 80% ($p = 0.02$). The risk of acute and chronic GvHD was low. In a recent report in pediatric patients undergoing unrelated donor CBT following failure of immunosuppressive therapy, durable engraftment was seen following fludarabine 35 mg/m^2/day × 5, Cytoxan >120 mg/kg divided over 2 or 3 days, rabbit ATG 3 mg/kg/day × 3, and 200 cGy TBI. The overall survival was seen in seven of nine (78%) patients (Chan et al. 2008). Co-infusion of a single CBU along with CD34$^+$-selected HSCs collected from a haploidentical relative was investigated in eight treatment-refractory pediatric SAA patients (Gormley et al. 2011). All patients promptly engrafted (seven with CBU cells, one with haploidentical cells). Early T-cell chimerism predominately indicated engraftment of the CB cells. Conversely, myeloid engraftment was initially from the haploidentical cells, but later transitioned to CBU. Seven of the eight patients are alive and transfusion independent. One died 14 months after transplantation 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. 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. Furthermore, it is possible that selection of CB units with higher cell dose, use of double CB units, and escalation of cytoreductive regimen intensity will likely improve outcomes.

7.6 CBT for Patients with Inherited Metabolic Disorders

The biochemical and clinical consequences of IMD, a disease group which in most cases does not have any direct or indirect hematopoietic problems, are corrected by allogeneic HCT because of two important factors. The first is the ability of

hematopoietic cells to differentiate and become an integral part of non-hematological organs like microglial cells in the brain, alveolar macrophages in the lungs, and Kupffer cells in the liver (Prasad and Kurtzberg 2010a). The second is the ability of donor-derived cells to induce "cross-correction," a phenomenon by which the close proximity of normal cells can correct the biochemical consequences of enzymatic deficiency within the neighboring cells (Muenzer and Fisher 2004; Fratantoni et al. 1968; Neufeld and Fratantoni 1970). Our group had extensively published a number of studies in the field (Escolar et al. 2005; Beam et al. 2007; Staba et al. 2004) including the largest series of UCBT in 159 patients with IMD (Prasad et al. 2008b). A number of review articles and a separate chapter in this book address the subject comprehensively.

7.6.1 General Guidelines: RCBT and UCBT for Nonmalignant Diseases (Modified from Prasad and Kurtzberg (2009))

1. Transplantation in early stages of disease – prior to development of comorbidities from organ and tissue damage, exposure to multiple transfusions, and/or infective complications – leads to less graft failure, lower transplant-related mortality, and better survival.
2. Inquire if a sibling cord blood unit was stored and what is the degree of HLA matching? HLA-matched and partially mismatched (≥4/6 match) cord blood units should be used alone if their cell dose is adequate (≥2.5 × 10e7/kg). If the cell dose is inadequate and the donor is 9/10 or 10/10 match, then supplemental BM from the same donor may be used to augment the graft.
3. If transplant is indicated and a HLA-matched related bone marrow donor is unavailable, then start a search for alternative donors including cord blood units as soon as possible. Do ask the family if CBU was saved from any of the siblings. If an adequate cord blood unit is available (≥4/6match, >3 × 10e7cells/kg but preferably >5 × 10e7 cells/kg), proceed to transplant. Do not wait for an unrelated adult match to become available on the registry.
4. Consider 9/10 or 10/10 HLA-matched (high resolution for A, B, C, DRB1, and DQB1) unrelated BM donors for all patients except those with inherited metabolic diseases. If no such donor is available, or if the patient is diagnosed with an IMD, unrelated cord blood should be used.
5. For patients considering CBT:
   (a) Refer to a transplant center experienced in CBT and the particular disease group.
   (b) Identify unrelated cord blood donors and screen them appropriately (e.g., carrier state for IMD transplants or autologous donation from the patient).
6. Table 7.1 provides summary of disease-specific outcomes, comments, and recommendations.

Table 7.1 Unrelated cord blood transplantation (UCBT) outcome summary and recommendations

Disease	Estimated number of UCBT in literature	Engraftment cumulative incidence or % engrafted	DF survival cumulative incidence or % alive	Comments and recommendations	Bibliography
Primary immunodeficiency disorders (PID)					
SCID	139	74–100%	64–100%	Early transplantation with the best available cord or BM donor	Bhattacharya et al. (2005); Fernandes et al. (2012); Frangoul et al. (2010); Diaz de Heredia et al. (2008); Knutsen and Wall (2000); Morio et al. (2011); Gluckman et al. (1997)
WAS	53	60–91%	60–82%	Early transplantation with the best available cord or BM donor, less GvHD with UCBT	Frangoul et al. (2010); Morio et al. (2011); Moratto et al. (2011); Diaz de Heredia et al. (2007)
CGD	17	43–83%	43–83%	Early transplantation with the best available cord or BM donor, prefer mismatched cord over mismatched unrelated BM donor, high-donor chimerism with UCBT	Tewari et al. (2012); Morio et al. (2011); Suzuki et al. (2007); Mochizuki et al. (2009); Shigemura et al. (2013); Goussetis et al. (2010); Bhattacharya et al. (2003)
HLH	65	Unk-100%	40–70%	Early transplantation with the best available cord or BM donor	Frangoul et al. (2010); Baker et al. (2008); Sawada et al. (2013); Patel et al. (2016)

(continued)

Table 7.1 (continued)

Disease	Estimated number of UCBT in literature	Engraftment cumulative incidence or % engrafted	DF survival cumulative incidence or % alive	Comments and recommendations	Bibliography
Hemoglobinopathies					
Thalassemia	86	43–100%	21–100%	Early transplantation for best results, UCBT data limited, need for increasing exploration of cord blood expansion protocols like NiCord	Murray et al. (2013); Issaragrisil et al. (1995); Fang et al. (2003); Hall et al. (2004); Tan et al. (2004); Jaing et al. (2012); Ruggeri et al. (2011); Parikh et al. (2014)
Sickle cell disease	39	38–91%	38–91%	Early transplantation for best results; UCBT data limited, need for increasing exploration of cord blood expansion protocols like NiCord	Adamkiewicz et al. (2007); Ruggeri et al. (2011); Kamani et al. (2012); Radhakrishnan et al. (2013); Brichard et al. (1996)
Bone marrow failure (BMF) syndromes					
Fanconi anemia	147	60–64%; 63%	40%; 43%	Transplant early with best available donor before malignant transformation; UCBT is a good option; outcomes better with Flu-based conditioning, TNCC $\geq 4.9 \times 10^7$/kg and negative CMV status	Rubinstein et al. (1998); Gluckman et al. (1997, 2007); Ruggeri et al. (2008); Smythe et al. (2007); Shaw et al. (1999); Wagner et al. (1995); Del Toro et al. (2004)
Dyskeratosis congenita	11	66%	66%	Transplant early with best available donor before malignant transformation; very limited UCBT data	Ruggeri et al. (2008); Gluckman et al. (1997); Bizzetto et al. (2011); Dietz et al. (2011); Nobili et al. (2002)

Table 7.1 (continued)

Disease	Estimated number of UCBT in literature	Engraftment cumulative incidence or % engrafted	DF survival cumulative incidence or % alive	Comments and recommendations	Bibliography
Diamond-Blackfan anemia	20	Unk-100%	50%	Transplant early with best available donor, very limited UCBT data	Rubinstein et al. (1998); Smythe et al. (2007); Gluckman et al. (1997); Bizzetto et al. (2011); Shaw et al. (1999); McFarren et al. (2014)
Shwachman-Diamond	4	100%	100%	Transplant early with best available donor, very limited UCBT data	Bizzetto et al. (2011); Vibhakar et al. (2005); Sauer et al. (2007); Fleitz et al. (2002)
CAMT	20	Unk-100%	69–100%	Transplant early with best available donor, good outcomes with UCBT	Rocha et al. (2000); Bizzetto et al. (2011); Mahadeo et al. (2015); Passos-Coelho et al. (2007); Macmillan et al. (1998); Savoia et al. (2007)
Severe congenital neutropenia	28	Unk-100%	67–100%	Transplant early with best available donor, good outcomes with UCBT	Morio et al. (2011); Carlsson et al. (2011); Bizzetto et al. (2011); Oshima et al. (2010); Mino et al. (2004); Nakazawa et al. (2004); Yesilipek et al. (2009)

(continued)

Table 7.1 (continued)

Disease	Estimated number of UCBT in literature	Engraftment cumulative incidence or % engrafted	DF survival cumulative incidence or % alive	Comments and recommendations	Bibliography
Acquired severe aplastic anemia	101	Unk-63%	41–77%; Unk-88%	Data with matched unrelated BMT is much better than UCBT; if HLA-MUD is not available, then consider double cord blood or haplo-cord transplant using Flu-containing regimen	Rubinstein et al. (1998); Ruggeri et al. (2008); Yoshimi et al. (2008); Chan et al. (2008); Wagner et al. (1995); Liu et al. (2012); Ohga et al. (2006)
Osteopetrosis	64	60%	45%	Transplant early with best available donor; poor UCBT outcomes but data is limited	Rubinstein et al. (1998); Gluckman et al. (1997); Shaw et al. (1999); Ruggeri et al. (2013); Tsuji et al. (2005); Jaing et al. (2008); Buchbinder et al. (2013)

Data adapted from Page et al. (2014)
Unk data is unavailable, *SCID* severe combined immunodeficiencies, *WAS* Wiskott-Aldrich syndrome, *CGD* chronic granulomatous disease, *HLH* hemophagocytic lymphohistiocytosis, *CAMT* congenital amegakaryocytic thrombocytopenia, *MUD* matched unrelated BM donor, *BMT* bone marrow transplant

Conclusion

CBT from related and unrelated donors offers promising and effective therapy for many patients with nonmalignant disorders. The use of CB increases access to transplantation for almost all patients in need and allows for quicker donor identification and selection. Future strategies to facilitate earlier diagnosis and to decrease transplant-related risks should further improve short- and long-term outcomes. Other approaches like improved cellular criteria for CBU selection, use of preimplantation genetic diagnosis and embryo selection, and fertility preservation prior to chemotherapy in patients who have reached puberty have been undertaken to improve the outcomes and to make transplants available to larger numbers of eligible patients. Several experimental approaches to increase the available TNCC for transplantation involving ex vivo manipulation to improve engraftment of CBUs are ongoing. At Duke, early results using nicotinamide-based expansion

(NiCord®) in SCD (https://clinicaltrials.gov/ct2/show/NCT01590628?term=NICORD&rank=2) are showing promise and have been expanded to include thalassemia. Pilot trials are ongoing with other expansion technologies in patients with hematologic malignancies, and, if successful, these approaches may ultimately be applied to UCBT, thus broadening the utility of UCBT in these diseases (Horwitz et al. 2013; Robinson et al. 2013; Peled et al. 2012; Delaney et al. 2010; Cutler et al. 2013). Additionally, standardized approaches for conditioning and supportive care should be developed and validated in specialized centers and then exported for widespread applications. 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 a transplantable diseases. Finally, every effort should be made to perform transplantation early in the course of disease before extensive damage to various tissues and organs ensues.

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Part II

Cord Blood Transplants for Neoplastic Diseases in Children: Data, Consensus and Challenges

Cord Blood Transplants for Myeloid Malignancies in Children

8

Andrew C. Dietz and Michael R. Verneris

8.1 Introduction

Childhood cancer is rare, with myeloid malignancies representing a relatively uncommon subset of hematologic malignant diseases. Pediatric myeloid malignancies consist of groups that include (Vardiman et al. 2009):

- Acute myeloid leukemia (AML)
- Myelodysplastic syndromes (MDS)
- Myeloproliferative neoplasms including:
  - Chronic myelogenous leukemia (CML)
  - Juvenile myelomonocytic leukemia (JMML)
  - Even more rare entities in pediatrics not discussed here

AML is the most common of these diagnoses in children, accounting for only about 5% of annual pediatric cancer diagnoses in the United States (Ward et al. 2014). Only about 50% of children diagnosed with AML can be cured with standard chemotherapy. Thus, allogeneic transplantation (allo-HCT) is required for a considerable proportion of these patients. In contrast to AML, allo-HCT is the only curative option for patients with CML, MDS, and JMML. Many hematopoietic graft sources are currently being used, including umbilical cord blood (UCB), bone marrow (BM), or peripheral blood hematopoietic cells (PBHC), all of which can be

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_8

isolated from HLA-matched related donors (MRD), HLA-matched unrelated donors (MUD), mismatched unrelated donors (MMUD), and HLA-haploidentical donors. Previously considered an alternative donor source, UCB use has become more mainstream due to the high rates of disease-free survival (DFS), with the low incidence of chronic graft-*versus*-host disease (GvHD). This chapter reviews current outcome data including engraftment, relapse, acute and chronic GvHD, transplant-related mortality (TRM), DFS, and overall survival (OS) when UCB is used as a donor source for allo-HCT in pediatric clonal (neoplastic) myeloid disorders.

8.2 Why We Do/Don't Like UCB as a Donor Source in Myeloid Malignancy

As UCB transplantation has grown, there have been major efforts in the field of cord blood banking to make this hematopoietic graft source available to everybody in need of transplantation, which is reviewed in more detail in chapter "Current Cord Blood Banking Concepts and Practices." While the current inventory of UCB units is less than 10% of adult volunteer donors in the various registries, the ability to use cord blood with less well-matched human leukocyte antigen (HLA) generally means a higher likelihood of finding an appropriate donor within banked UCB units (Barker et al. 2010). Additional advantages and disadvantages are listed in Table 8.1 (Appelbaum and Thomas 2009; Anasetti 2015).

A number of modifiers influence the above items including presence of donor-specific anti-HLA antibodies that increase the risk of graft failure (Cutler et al. 2011) or total nucleated cell/kg (TNC dose), degree of HLA mismatch, occurrence of GvHD, use of total body irradiation (TBI), and use of serotherapy that changes the rate of hematopoietic and immune reconstitution (Appelbaum and Thomas 2009). While reviewed in more detail in chapter "Immune Reconstitution After Cord Blood Transplantation," generally natural killer (NK) cells can recover about 1 month after transplantation with B-cell recovery around 6 months, thymopoiesis around 6–12 months, mitogen stimulation response around 6–9 months, $CD8^+$ T-cell recovery around 9 months, and $CD4^+$ T-cell recovery around 6–12 months. During the process of reconstitution, especially the first 3–6 months, patients remain

Table 8.1 Advantages and disadvantages of UCB

Advantages	Disadvantages
Complete DNA typing frequently available	Higher rates of graft failure
Search and acquisition extremely fast	Slower neutrophil and platelet recovery
No donor-associated risks	Slower immune reconstitution
Rare haplotypes (minority) represented with the ability to find a donor in most searches, including diverse populations	No donor lymphocyte infusions available
	No current immunotherapy potential
	Theoretical concern of congenital disease
Less chronic GvHD	Cell dose requirements may limit use
No concern for viral transmission	Acquisition costs

Table 8.2 Answers to questions posed above

Question	Answer
Is UCB a valid donor source in pediatric AML HCT?	Yes
Is UCB a preferred donor source in pediatric AML HCT?	In some settings
Is UCB a valid donor source in pediatric CML HCT?	Yes
Is UCB a valid donor source in pediatric MDS HCT?	Yes
Is UCB a valid donor source in pediatric JMML HCT?	Yes

at very high risk for infections. This can ultimately translate into higher TRM and decreased survival (Appelbaum and Thomas 2009). As will be outlined below, OS ends up being similar to other donor sources owing to decreased rates of relapse, particularly in myeloid diseases. Further optimization of UCB use can start to mitigate many of the disadvantages and tip the balance toward the many advantages with this donor source. The next five sections of this chapter address questions related to individual myeloid malignancies, followed by Table 8.2 that summarizes answers to the questions posed.

8.3 Is UCB a Valid Donor Source in Pediatric AML?

Due to the rarity of AML in pediatrics, studies that examine the role of HCT for pediatric AML generally include either adult AML patients or pediatric patients with other hematologic malignancies such as acute lymphoblastic leukemia (ALL), lymphoma, CML, MDS, JMML, and/or a combination of all the above. Additionally, data on the use of UCB are often mixed in with reports on other donor sources. However, overall safety and efficacy of UCB HCT for AML have now been firmly established (Appelbaum and Thomas 2009).

From Europe, an initial study examining 95 patients transplanted with UCB revealed neutrophil recovery of 78% ± 4%, grades II–IV acute GvHD of 35% ± 5%, chronic GvHD of 15% ± 5% with mostly limited disease, TRM of 20% ± 4%, relapse of 29% ± 5%, and 2-year DFS of 42% ± 5%. Notably TRM was worse with a lower TNC (HR 4.16, $p = 0.01$), and relapse was lower with smaller patients (HR 2.77, $p = 0.02$) and those in first complete remission (HR 3.84, $p = 0.001$). Interestingly, patients with unfavorable cytogenetic characteristics had similar DFS to those without these poor-risk features. Thus, UCB was assessed to be a good therapeutic option for those lacking a HLA-MRD (Michel et al. 2003). A follow-up from Europe of 290 children confirmed similar 2-year DFS of 65% (no CI given) for those in first complete remission, 43% (no CI given) for those in second complete remission, and 22% (no CI given) for those with advanced phase disease.

In the United States, an initial study of patients with hematologic malignancies included 191 patients with about one-third having myeloid malignancies. Neutrophil recovery by day 42 was 79.9% (95% CI 75.1–85.2%), acute grades III–IV GvHD by day 100 was 19.5% (95% CI 13.9–25.5%), chronic GvHD at 2 years was 20.8% (95% CI 14.8–27.7%), relapse was 19.9% (95% CI 14.8–25.7%), and 2-year

survival was 49.5% (95% CI 42.3–57.0%). TNC >2.5 x10^7 NC per kg was significantly associated with improved survival (HR 1.97, $p = 0.04$) (Kurtzberg et al. 2008). A subsequent comparison study including 503 children was performed looking at the difference between UCB and BM as donor sources. Approximately one-third of the patients had AML, with the remainder having ALL. At 5 years, DFS was similar between all sources (Eapen et al. 2007). In another study that examined the role of a single-UCB unit *versus* a double UCB in children with hematologic malignancies, again approximately one-third of the patients had AML. At 1 year, the OS rates were similar at 65% (95% CI 56–74%) for double and 73% (95% CI 63–80) for single, with no observed differences in DFS, neutrophil recovery, TRM, relapse, infections, immune reconstitution, and grades II–IV acute GvHD (Wagner et al. 2014). The single- *versus* double-UCB concept is reviewed more completely in chapter "Single or Double Cord Blood Unit for Transplant: What Have We Learned?" In each of these studies, no separate analyses were done for AML as opposed to the other hematologic malignancies. However, each of these studies helped to further demonstrate that UCB represents a good therapeutic option in AML.

8.4 Is UCB a Preferred Donor Source in Pediatric AML HCT?

There have been reports that consistently show UCB to have lower rates of chronic GvHD as well as lower rates of relapse. This is at the cost of slower hematopoietic and immune reconstitution resulting in more infections and TRM, which results in similar survival (Appelbaum and Thomas 2009; Rocha et al. 2009; Ballen and Lazarus 2016). Overall the advantage in relapse compared to HLA-MUD HCT appears to be most pronounced when there are two HLA-antigen mismatches (RR 0.54, 95% CI 0.36–0.83, $p = 0.0045$) (Eapen et al. 2007).

Ultimately, specific patients may benefit from individualized approaches. Patients more at risk of infection may be less likely to get an UCB graft, whereas patients more at risk of relapse might preferentially be treated with a UCB graft (Ballen 2015). As infectious complications and TRM are being decreased, the potential to improve long-term survival can be realized. One example of this, reported from the Italian BMT working group, showed relapse after UCB HCT for AML was very low (7.7% ± 5.2%) and, similarly, TRM was very low (7.4% ± 2.5%) which translated into a much higher 8-year EFS of 92.3% ± 5.2% (Locatelli et al. 2015).

It is important to remember that most of these data do not take into account more contemporary treatment information including minimal residual disease testing and the use of posttransplant consolidative therapy including kinase inhibitors, checkpoint inhibitors, cytokine therapy, or cellular therapy. Current and future studies will likely incorporate these approaches as we learn about myeloid disease that may be more chemoresistant *versus* myeloid disease that may be more resistant to the graft-*versus*-leukemia (GvL) effect.

8.5 Is UCB a Valid Donor Source in Pediatric CML?

Due to the rarity of the disease, particularly in pediatrics, studies that examine the role of HCT for pediatric CML are few, with limited patients. More data are available when examining reports that include treatment of adult CML patients or when examining reports that include treatment of pediatric patients with CML, AML, MDS, JMML, and/or a combination of all the above.

The Japanese examined the use of UCB in 86 CML patients of all ages. This report only included nine children under the age of 16, but the next age range of 16–50 also included an unknown number of patients that would fit into an adolescent and young adult patient population often treated by pediatric HCT providers. They found high rates of engraftment in patients with a TNC over 3.0 x10^7 NC per kg, which were naturally easier to achieve in children. While 47% (95% CI 36–58%) experienced grades II–IV acute GvHD, there were only two patients in the entire group that experienced chronic extensive GvHD. TRM was 25% (95% CI 16–34%) for the entire cohort but only 11% (95% CI 0–33%) in children less than 16 years old, which was significantly lower than any other age category ($p = 0.026$). While there was 37% (95% CI 26–48%) relapse at 2 years for the entire cohort, those with a TNC over 3.0 x10^7 NC per kg had only 9% (no CI given) relapse, significantly less than 44% (no CI given) with those patients under this limit ($p = 0.01$). Finally, the observed EFS was highest in the youngest age group at 74% (95% CI 48–100%, $p = 0.049$). They concluded that UCB was a reasonable alternative when a HLA-MRD or HLA-MUD is not available. Their data show this is especially true for pediatric patients where advanced disease is less likely and where large cell doses can easily be achieved (Nagamura-Inoue et al. 2008).

The Chinese, in a smaller but comparative study, examined 16 patients that received UCB *versus* 16 patients that received HLA-MRD BM or PBHC. All patients included had advanced disease, which is known to fare worse with HCT and be rarer in pediatrics, though the study age range included those down to 12 years of age. They saw a similar delay in neutrophil and platelet engraftment observed in many UCB studies. There was no difference in the occurrence of any form of GvHD. TRM at day 180 was significantly higher with UCB (37.5% *vs.* 12.5%, $p = 0.013$), but relapse was significantly lower (14.2% *vs.* 42.7%, $p = 0.03$) with a trend for better 5-year OS (62.5% *vs.* 48.6%, $p = 0.10$) (Zheng et al. 2013).

Many other reports that incorporate CML along with other diseases generally note the acceptability of UCB as a donor source today. This is further confirmed in a review published in the United States (Venepalli et al. 2010). An important issue for this disease includes the use of tyrosine kinase inhibitors (TKI) to achieve response prior to HCT, which allows for less acute toxicity, less TRM, improved DFS, and improved OS. TKI posttransplantation may also help prevent or treat relapse. Another issue that arises with UCB in CML is that donor lymphocyte infusion (DLI) has proven useful in this disease (Simula et al. 2007) and is not generally available with UCB. Perhaps this is mitigated some by the suggestions of decreased relapse after the use of UCB (Venepalli et al. 2010).

8.6 Is UCB a Valid Donor Source in Pediatric MDS HCT?

Due to the rarity of the disease, particularly in pediatrics, studies that examine the role of HCT for pediatric MDS are few with limited patients. More data are available when examining reports that include treatment of adult MDS patients or when examining reports that include treatment of pediatric patients with MDS, AML, CML, JMML, and/or a combination of all the above.

The most comprehensive study to date was a joint effort on behalf of the Eurocord-European Blood and Marrow Transplantation Group (Eurocord-EBMT), the European Working Group on Childhood MDS (EWOG-MDS), and the Center for International Blood and Marrow Transplantation Registry (CIBMTR). There were 70 children with MDS identified. Neutrophil recovery occurred in 76% (95% CI 64–84%) by 60 days and was faster in patients with more closely matched UCB ($p = 0.024$), TNC of greater than or equal to 6.0 x10^7 NC per kg ($p = 0.024$), TBI regimens ($p = 0.014$), and those with monosomy 7 ($p = 0.045$) on multivariate analysis. Grades II–IV acute GvHD was 30% (95% CI 20–41%) at 100 days, and 3-year chronic GvHD was 23% (95% CI 14–33%). While TRM was 53% prior to 2001, it was only 31% after 2001 (HR 0.41, 95% CI 0.20–0.84, $p = 0.015$). Also, while DFS was 27% before 2001, it was up to 50% after 2001 ($p = 0.018$) (Madureira et al. 2011). Notably, DFS was also higher in patients transplanted within 6 months from diagnosis (66% *vs.* 20%, no p-value or HR given) and in patients with a diagnosis that included monosomy 7 (61%, HR 2.04, $p = 0.017$). These results compare favorably with the historical control of approximately 60% DFS after HLA-MRD HCT (Madureira et al. 2011).

Two additional smaller studies have confirmed some of these observations. At Duke, 23 patients were given HCT for MDS using UCB as a donor source. There were three patients with graft failure, acute GvHD occurred by 100 days in 13% (95% CI 0–27%); there was no chronic extensive GvHD, relapse at 3 years occurred in 13% (95% CI 0–27%), and TRM was 27% (95% CI 8–46%) at 1 year. EFS, similar to historical reports, at 3 years was 60.9% (95% CI 38.3–77.4%), with those under age 11 and/or under 38 kg faring better (Parikh et al. 2009). At the University of Minnesota, all HCT for MDS in children were examined, with nine of them using UCB as the donor source. They also observed that earlier to HCT was beneficial and reported the highest DFS for patients who received an UCB graft (65% *versus* 53% for HLA-MRD, 43% for HLA-MUD, and 17% for HLA-MMUD, $p = 0.03$) (Smith et al. 2013).

8.7 Is UCB a Valid Donor Source in Pediatric JMML?

Due to the rarity of the disease, particularly in pediatrics, studies that examine the role of HCT for JMML are few with limited patients. More data are available when examining reports that include treatment of pediatric patients with JMML, MDS, AML, CML, and/or a combination of all the above.

The most comprehensive study to date was a joint effort on behalf of the Eurocord-European Blood and Marrow Transplantation Group (Eurocord-EBMT), the European Working Group on Childhood MDS (EWOG-MDS), and the Center for International Blood and Marrow Transplantation Registry (CIBMTR). There were 110 children with JMML identified. Neutrophil recovery by 60 days was 82% ± 4%. Grades II–IV acute GvHD occurred in 41% ± 4% by 100 days and was slightly less if serotherapy was used ($p = 0.03$). Chronic GvHD occurred in only 15% ± 4% by 5 years, with only 6 of 16 patients having extensive disease. At 5 years, TRM was 22% ± 4%, while relapse was 33% ± 5%. Factors associated with less relapse included age under 1.4 years ($p = 0.01$) and occurrence of grades II–IV acute GvHD ($p = 0.02$). The 5-year DFS was 44% with age under 1.4 years (HR 0.4, $p = 0.005$), closer HLA matching (HR 0.4, $p = 0.009$), and a karyotype other than monosomy 7 (HR 0.5, $p = 0.02$) all associated with improved DFS. Historic rates of relapse are 30–50% from other donor sources, and DFS is 55% after MRD HCT and 49% after MUD HCT. They concluded that UCB is a suitable option but that disease recurrence remains the major cause of treatment failure, as with other donor sources (Locatelli et al. 2013). Given the potential utility of DLI in these patients, UCB has the same limitations as described for CML.

8.8 What's Going on with UCB Manipulation?

As mentioned earlier in this chapter, there may be some problematic issues related to the use of UCB, including concerns about hematopoietic engraftment and immune reconstitution. These issues can result in infections and may increase TRM. Given lower rates of relapse and similar rates of survival compared to other donor sources, strategies to accelerate the probability and speed of neutrophil engraftment and lymphocyte immune reconstitution could elevate UCB into a preferred donor status in certain cases.

While briefly reviewed here, this is discussed in greater detail in chapter "Ex-vivo Cord Blood Manipulation: Methods, Data and Challenges." Table 8.3 shows a few of the UCB manipulation strategies currently under investigation. In some instances, the goal is to expand the number of stem/progenitor ($CD34^+$) cells or to home the stem cells into the bone marrow niche or to redirect the T-cell component to directly fight viral infections or leukemia. Additionally, there are strategies aimed at isolating certain elements of the UCB including T regulatory cells that can help protect against GvHD or NK cells to help protect against disease relapse (Brunstein et al. 2016). Finally, there are strategies aimed at improving engraftment by combination with human placental-derived stem cells or bridging the neutropenic period by combination with haploidentical cells (Ballen 2015; Cairo et al. 2016; Thompson et al. 2015).

The ultimate test will be to see if any of these methods actually improve survival. Clinical efficacy and feasibility will need to be demonstrated in larger studies, and likely a combination of approaches will ultimately lead to improved

Table 8.3 Cord blood manipulation strategies

Stem cell expansion
Notch (Delaney et al. 2010)
Mesenchymal stromal cells (de Lima et al. 2012)
Nicotinamide (Horwitz et al. 2014)
StemRegenin 1 (Wagner et al. 2016)
Stem cell homing
Prostaglandin E2 (Cutler et al. 2013)
Intra-marrow injection (Carrancio et al. 2013)
Fucosyltransferase (Robinson et al. 2012)
Guanosine diphosphate fucose (Popat et al. 2015)
Complement fragment 3a (Brunstein et al. 2013)
Sitagliptin (Farag et al. 2013)
Specifying T-cell targets
Chimeric antigen receptor T-cells (Micklethwaite et al. 2010)
Virus-specific cytotoxic T lymphocytes (Hanley et al. 2009)

outcomes. A great futuristic view is stated in a review by Thompson et al., where they imagine a future with two cords used. The first cord is split into two parts with about 50–60% being ex vivo expanded to improve engraftment and the remainder being used to generate T regulatory (Tregs) cells and NK cells to be used peri-transplant. The second cord could have 20% taken out to be utilized for virus-specific T-cells and/or chimeric antigen receptor T-cells with the remaining 80% infused unmanipulated to provide long-term hematopoiesis and immune reconstitution (Thompson et al. 2015).

8.9 Expert Point of View and Future Directions (Conclusion)

UCB is an excellent donor source for HCT in myeloid malignancies that include AML, CML, MDS, and JMML. This is particularly true for centers with experience and comfort monitoring immune reconstitution and dealing with infections, especially opportunistic viruses. UCB, when used by those able to mitigate factors associated with TRM, may ultimately be the best donor source due to decreased rates of relapse. With a variety of cord blood manipulation strategies under investigation, the landscape of UCB use in myeloid malignancies will continue to evolve for the betterment of our patients.

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Cord Blood Transplantation for Lymphoid Malignancies in Children

9

Hisham Abdel-Azim and Michael A. Pulsipher

9.1 Introduction

Cord blood (CB) is commonly used for transplanting children with hematological malignancies. CB is a readily available alternative graft source of hematopoietic stem cells (HSCs) that is capable of reconstituting hematopoiesis after hematopoietic cell transplantation (HCT). Broxmeyer et al. demonstrated that CB progenitors had higher proliferative capacity as compared with bone marrow (BM) cells (Broxmeyer et al. 1989; Gluckman et al. 1989). In 1998, Gluckman et al. reported the first clinical use of CB in HCT for a child diagnosed with Fanconi anemia (Gluckman et al. 1989).

Unlike adult CB HCT studies which included Hodgkin and non-lymphoma in addition to acute lymphoblastic leukemia (ALL), most of the pediatric studies for CB HCT included ALL and myeloid malignancies with fewer studies confined solely to ALL.

An important development in HCT that has significantly impacted the treatment of children with ALL has been the expansion in number and capacity of CB banks over the past two decades (Lazzari et al. 1996; Gluckman et al. 1993; Saccardi et al. 2016). More than 400,000 CB units have been collected and stored, and more than 20,000 CB HCT have been performed worldwide (Gluckman 2009). Recent surveys estimate that since 1998, 20% of HCT performed worldwide in patients under 20 years old have utilized CB grafts. In Japan, approximately 50% of HCT from unrelated donors have been performed with CB grafts (Gluckman 2009).

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_9

Advantages of CB grafts include rapid availability (Lown and Shaw 2013) (especially for high-risk patients in whom remissions are likely to be short) and reduced graft-*versus*-host disease (GvHD) allowing an ability to perform HCT with multiple HLA-mismatched units. Increasing CB graft cell dose abrogates many effects of HLA mismatching, but the best outcomes have been noted in patients given unrelated CB grafts from donors with 0, 1, or 2 HLA incompatibilities (6/8 HLA matches) (Eapen et al. 2007; Gluckman et al. 2004; Rocha and Gluckman 2009). CB recipients receive a much lower T-cell dose, with engraftment and immune reconstitution generally delayed, resulting in early infectious morbidity. However, the reduced T-cell dose probably contributes to less chronic graft-*versus*-host disease (cGvHD), despite the frequent use of HLA-mismatched grafts (Ruggeri et al. 2016).

9.2 Clinical Outcomes After Allogeneic HCT for Pediatric Acute Lymphoblastic Leukemia by Stem Cell and Donor Source

9.2.1 CB Transplantation from HLA-Identical Siblings

The use of CB from matched sibling donors (MSD) has led to acceptable outcomes when sufficient cell dose is infused (Gluckman et al. 1989). A retrospective study from the Eurocord-EBMT registry reported HCT outcomes of pediatric patients ($n = 147$ including 82 patients with ALL, median age = 5 years, median follow-up = 6.7 years, range = 7 months-18 years) with hematological malignancies who received HLA-identical CB grafts. The cumulative incidence of neutrophil recovery was found to be 90% at day +60, while the cumulative incidence of both acute and chronic GvHD was 12% and 10% at 2 years, respectively. At 5 years, the cumulative incidence of non-relapse mortality and disease recurrence was 9% and 47%, respectively. In multivariate analysis, remission status at time of transplant and the use of total body irradiation during the conditioning regimen remained independently associated with a lower relapse incidence in acute leukemia patients (Herr et al. 2010).

The probability of DFS at 5 years for patients with acute leukemia was affected by remission status and was 57 ± 9%, 46 ± 7%, 31 ± 13%, and 21 ± 11% for patients transplanted in CR1, CR2, CR3, and in refractory disease, respectively ($p = 0.006$). The 5-year DFS of patients who did or did not receive methotrexate (MTX) was 24 ± 10% and 48 ± 5%, respectively ($p = 0.007$). In multivariate analysis, the remission status and avoiding MTX were associated with improved DFS. These outcomes using CB grafts from sibling donors provide an impetus to encourage banking of HLA-identical sibling cord blood for potential future use when siblings are born to children with hematological malignancies (Reed et al. 2003).

A US study reported the outcomes of 44 patients who had undergone sibling donor CB HCT of whom 18 patients were transplanted for the treatment of acute leukemia. The majority of patients received a CB unit that was fully matched or had a single-antigen mismatch. The probability of event-free survival (EFS) was 46% (Wagner et al. 2007). Smythe and colleagues reported three children with ALL who

received HCT using sibling CB (Smythe et al. 2007). Two patients died from relapse, and one was alive 4 years after the procedure. Rocha and colleagues reviewed 113 children who received an UCB from a HLA-identical sibling transplanted between 1990 and 1997 and compared them to 2052 children who were transplanted using BM from HLA-identical siblings during the same period. The recipients of CB were younger than the recipients of BM (median age, 5 years *vs.* 8 years; $p < 0.001$), weighed less, and were less likely to have received methotrexate for GvHD prophylaxis. Multivariate analysis demonstrated a lower risk of acute GvHD (relative risk, 0.41; $p < 0.001$) and chronic GvHD (relative risk, 0.35; $p < 0.02$) among recipients of matched sibling CB HCT. Although overall engraftment rates were similar, the likelihood of neutrophil and platelet recovery was significantly delayed after CB transplantation compared to sibling BM. Mortality was similar in both groups. Deaths related to infection from any cause and hemorrhage were more common in the CB group, whereas deaths related to GvHD, interstitial pneumonitis, and organ failure were more common in the BM group. The number of relapse-related deaths was similar in the two groups. Overall survival (OS) was similar between the two groups (Rocha et al. 2000).

9.2.2 Unrelated Donors: BM, PBHC (Peripheral Blood Hematopoietic Cells), and CB

Since only 20–25% of children with an indication for allogeneic HCT have a MSD, the availability of HLA-matched unrelated donors (MUD) has widened the donor pool over the past decade. The chance of finding a suitable donor mainly depends on ethnic group (ranging from 60–70% for Caucasians to 20–40% for patients belonging to some ethnic minorities) and the frequency of the HLA phenotype of the patient. High-resolution DNA matching of HLA class I and II of unrelated donors and recipients has impacted outcome with reduced morbidity and mortality over the last decade (Afify et al. 2005; Cornish 2005; Green et al. 1999). Consequently, the use of matched unrelated donors has now become standard in children lacking an HLA-identical sibling. Retrospective studies over the past years have shown reduced early toxicity, especially acute and chronic GvHD, lower TRM, and similar relapse rates compared with the early reported MUD-BMT experience (Locatelli et al. 2002; Dini et al. 2003; Eapen et al. 2006). It is likely that improved outcomes have resulted not only from better HLA matching but also from the use of intensified GvHD prophylaxis by *in vivo* or *in vitro* T-cell depletion (MacMillan et al. 2008).

9.3 Unrelated BM *Versus* CB

A retrospective European registry study comparing unrelated BM (unmanipulated and T-cell depleted) and CB HCT in children with leukemia showed similar survival among the three groups. The CB group showed decreased acute GvHD compared to the unmanipulated BM group and decreased relapse compared to the T-cell-depleted BM group (Rocha et al. 2001; Gluckman and Rocha 2004).

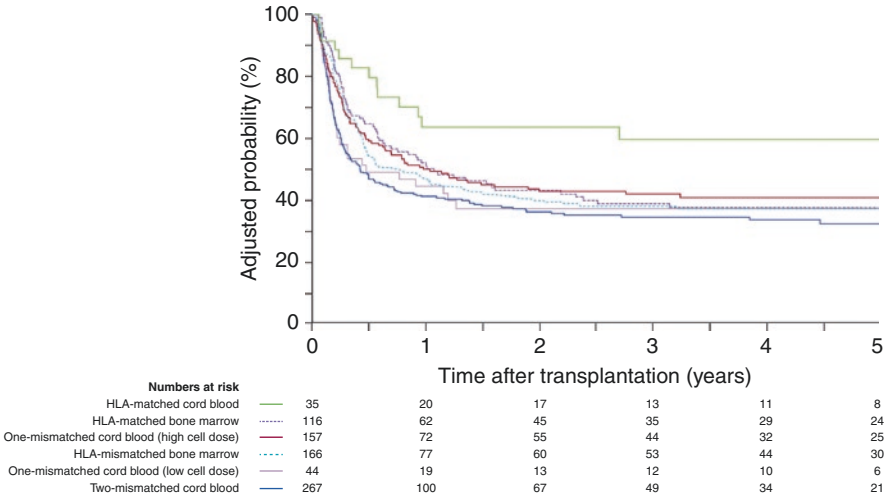

Fig. 9.1 Probability of leukemia-free survival after bone marrow and cord blood transplantation adjusted for disease status at transplantation

Another large registry study from the CIBMTR and New York Blood Center compared outcomes of 503 children (<16 years) with acute leukemia undergoing unrelated CB HCT with outcomes of 282 BM-HCT recipients between 1995 and 2003 (including 495 patients with ALL; 186 in BM group and 309 in CB group). Five-year LFS was similar in recipients of 8/8 allele-matched BM compared to unrelated CB mismatched for either one or two antigens. GvHD- and transplant-related mortality (TRM) rates were higher after transplants of two-antigen HLA-mismatched CB (RR 2.31, $p = 0.0003$) and possibly after one-antigen HLA-mismatched low-cell-dose ($<0.3 \times 10^5$/kg) CB transplants (RR 1.88, $p = 0.0455$). Relapse rates were lower after two-antigen HLA-mismatched CB transplants (54%, $p = 0.0045$) suggesting a greater graft-*versus*-leukemia (GvL) effect. In this report, cell dose and HLA match affected the risk of TRM; recipients of two-antigen and one-antigen mismatched UCB with low-cell dose had worse outcomes. Figure 9.1 illustrates the probability of leukemia-free survival after bone marrow and cord blood transplantation adjusted for disease status at transplantation (Eapen et al. 2007).

A recent prospective pediatric study compared overall survival (OS) for recipients of a single CB unit treated for hematological malignancy on the original cord blood transplantation (COBLT) study ($n = 191$) conducted from 1999 to 2004 with the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0501 study ($n = 113$) conducted from 2006 to 2012. The 1-year probability of overall survival was 57% (95% CI 50–64%) in COBLT and 71% (95% CI 62–79%) in BMT CTN 0501 ($p = 0.01$). The improved survival in the CTN study possibly reflects improved supportive care, and better methods of CB unit selection over time, but could also be related to the different methods of GvHD prophylaxis used on the studies (horse ATG and prednisone used with cyclosporine (CSP) in COBLT, while BMT CTN 0501 used CSP/mycophenolate mofetil (MMF) (Kurtzberg et al. 2013).

9.4 Unrelated CB *Versus* Related Haplo-T-Depleted PBHC

A retrospective study comparing unrelated CB with haploidentical T-cell-depleted PBHC HCT in children with ALL showed that failure of engraftment was significantly higher following unrelated CB than after haplo-HCT (23 *vs.* 11%, $p < 0.007$). Acute II–IV GvHD was higher in the unrelated CB cohort. Relapse incidence was higher in the haploidentical cohort compared with unrelated CB recipients. TRM and leukemia-free survival (LFS), however, were not significantly different (Hough et al. 2009).

Table 9.1 summarizes clinical outcomes for CB HCT from studies with the largest lymphoid malignancy cohorts.

Table 9.1 Outcomes of studies for CB HCT with the largest lymphoid malignancy cohorts

Study	Graft type	*N* total (ALL)	Results
Eurocord-EBMT (Gluckman et al. 1989)	Cord blood from matched sibling donor	147 (82)	aGvHD (12%), cGvHD (10%), NRM (9), relapse (47%) DFS %: 57 ± 9 (HCT in CR1), 46 ± 7 (HCT in CR2), 31 ± 13 (HCT in CR3), 21 ± 11 (HCT in refractory disease)
CIBMTR (Eapen et al. 2007)	Cord blood *vs.* bone Marrow	503 (495) CB = 309 BM = 186	Similar LFS for CB (1 or 2 mismatched antigens) and BM (8/8 allele matched) Higher GvHD and TRM rates with 2 antigens mismatched CB (RR 2.31, $p = 0.0003$) Lower relapse rate were with 2 antigens mismatched CB (54%, $p = 0.0045$)
Kurtzberg et al. (Kurtzberg et al. 2013)	Cord blood (COBLT *vs.* BMT CTN 0501)	COBLT ($n = 191$) BMT CTN 0501 ($n = 113$)	OS: 57% (95% CI 50–64%) in COBLT and 71% (95% CI 62–79%) in BMT CTN 0501 ($p = 0.01$) BMT CTN improved OS: improved supportive care, better selection of CB unit, and different methods of GvHD prophylaxis
Hough et al. (Hough et al. 2009)	Cord blood *vs.* haplo-T-depleted PBHC	CB = 341 Haplo = 127	non-engraftment were higher in CB *vs.* haplo-HCT (23 *vs.* 11%, $p < 0.007$) aGvHD (II-IV) with CB Higher relapse with haplo compared to CB recipients TRM and LFS were similar with CB or haplo-HCT

EBMT European Blood and Marrow Transplant, *CIBMTR* Center for International Blood and Marrow Transplantation, *PBSC* peripheral blood stem cells, *CB* cord blood, *BM* bone marrow, *NRM* non-relapse mortality, *DFS* disease-free survival, *CR* complete remission, *OS* overall survival, *aGvHD* acute graft-*versus*-host disease, *Haplo* haploidentical

Collectively, these studies show that while CB and T-depleted or unrelated BM-HCT outcomes are similar, an increased incidence of non-engraftment and/or delayed engraftment is the main drawback of CB HCT. Approaches to enhance engraftment, such as the use of double CB grafts, supplementing CB with haploidentical donor grafts or third-party mesenchymal cells, ex vivo expansion of CB, and better HLA matching, may help overcome this drawback for children with acute leukemias (Barker et al. 2009; Fernandes et al. 2007; Gonzalez-Vicent et al. 2010; Kelly et al. 2009).

Despite promising results in experienced single centers, to date, no studies have prospectively compared CB and haploidentical family donor grafts in pediatrics.

9.5 Suggested Approach to Choosing Stem Cell Source (Unrelated BM *Versus* CB) for HCT for Children with ALL

If an HLA-identical sibling is available, HCT with BM is the preferred graft source, followed by a matched-related donor CB unit with a sufficient total nucleated cell (TNC) dose (generally $\geq 3 \times 10^7$/kg). If lower CB cell doses are present, the CB unit can be used to supplement BM from the sibling (generally only recommended if the BM stem cell dose is low).

If no HLA-identical siblings are available, a search for an unrelated donor (matched unrelated BM [MUD] or unrelated CB) is indicated.

The minimum acceptable level of matching for CB graft is four out of six with antigen-level typing at HLA-A and HLA-B and allele-level typing at HLA DRβ1 compared to T-cell replete unrelated BM (or PBHC) grafts where the minimum acceptable degree of matching is seven out of eight (A, B, C, DRB1) allele-level matching.

Generally, unrelated donor selection can be prioritized as follows: (1) 10/10 MUD or 8/8 (6/6) unrelated CB would be top priority. (2) 9/10 MUD or 6–7/8 unrelated CB using one cord with a TNC dose $\geq 3 \times 10^7$/kg recipient body weight. (3) If the TNC dose in single cord is low, two cords can be used, with at least one of the cord having a 6–8/8 match. (4) If well-matched cords or MUD donors are not available, 4/6 unrelated CB unit or units with a TNC dose of $\geq 5 \times 10^7$/kg or haploidentical family donors are indicated. (5) If an unrelated donor is needed urgently, unrelated CB with sufficient TNC dose or a graft from a haploidentical family member may be an acceptable option.

HLA matching is one of the most important factors in CB unit selection, and the best HLA-matched unit is preferred. Compared with HLA-matched CB units, neutrophil recovery was lower with mismatches at 3, 4, or 5 but not 1 or 2 alleles. Nonrelapse mortality (NRM) is higher with units mismatched at 1, 2, 3, 4, or 5 alleles compared with HLA-matched units. The observed effects are independent of cell dose and patient age (Eapen et al. 2007; Ruggeri et al. 2016; Eapen et al. 2014).

These data support allele-level HLA matching in the selection of CB grafts. When selecting CB units for HCT, priority should be to choose a unit with the greatest similarity in allele-level HLA type to the recipient. If an 8 of 8 HLA-matched unit is not available, at least 4 of 8 HLA-matched unit is an acceptable

alternative. Priority is given to unidirectional mismatches in the GvHD direction, and mismatches in the host-*versus*-graft direction should be avoided, if feasible (Stevens et al. 2011).

9.6 Therapeutic Implications of Persistent MRD Pre-HCT and CB HCT

Several studies have demonstrated that patients with measurable MRD at the time of HCT have an increased risk of relapse.

A European retrospective registry study investigated the significance of persistent MRD in ALL, using either PCR or flow cytometry, in children undergoing HCT with CB ($n = 170$, median follow-up = 4 years). The presence of MRD before HCT was predictive for increased risk of relapse and decreased LFS. The probability of LFS at 4 years was 44% (56%, 44%, and 14% for HCT in CR1, CR2, and CR3, respectively ($p = 0.0001$)). LFS was improved in patients who were MRD negative *vs.* positive before CB HCT (54% *vs.* 29%; HR = 2, $p = 0.003$). The presence of detectable MRD assessed by PCR ($n = 119$) or by flow cytometry ($n = 51$) was associated with decreased LFS (PCR: $p = 0.02$, flow cytometry: $p = 0.05$) and increased relapse (PCR: $p = 0.04$, flow cytometry: $p = 0.01$). Multivariate model that showed persistent MRD before CB HCT is an adverse risk factor independent of other factors, including cytogenetics (Ruggeri et al. 2012).

A more recent retrospective study compared outcomes in 582 consecutive patients with hematological malignancy (including 185 pediatric and adult patients diagnosed with ALL) who received a first myeloablative HCT from an unrelated cord blood donor ($n = 140$), an HLA-matched unrelated donor ($n = 344$), or an HLA-mismatched unrelated donor ($n = 98$). Among patients with detectable pre-HCT MRD (by flow cytometry), the probability of overall survival after unrelated CB HCT was at least as favorable as HCT from an HLA-matched unrelated donor (BM or PBHC) and was significantly higher than the probability after HCT from an HLA-mismatched unrelated donor (BM or PBHC). Additionally, the probability of relapse was lower in CB group than in either of the other two groups (Milano et al. 2016).

9.7 Non-myeloablative Conditioning and CB HCT

In general, the data on using CB grafts in children with this approach is very limited. The Pediatric Blood and Marrow Transplant Consortium (PBMTC) ONC0313 study reported the outcomes of a reduced intensity conditioning (intravenous busulfan 6.4 mg/kg, fludarabine 180 mg/m2, and thymoglobulin 10 mg/kg). The study also included recipients of related and unrelated PBHC, BM, and unrelated CB grafts. The study included heavily pretreated group of patients, 64% of whom received a prior myeloablative HCT. Twelve patients received unrelated CB grafts. Nine of the ten evaluable patients are engrafted. Univariate analysis did not reveal CB HCT as an adverse prognostic factor (Pulsipher et al. 2009).

9.8 Impact of Killer-Cell Immunoglobulin-Like Receptors and KIR-Ligand Incompatibilities on Outcomes After Unrelated Cord Blood Hematopoietic Cell Transplant (HCT)

In general, the data on the role of KIR in CB grafts in children undergoing HCT for ALL with is very limited. A Eurocord-Netcord-EBMT retrospective registry study examined the role of KIR ligand in unrelated CB HCT for acute leukemia. The study included 218 patients (median age = 13.8 years, range = 0.5–69 years), undergoing a single-unit CB HCT for ALL ($n = 124$) or AML ($n = 94$) in CR between 1997 and 2007. Grafts were HLA-A, -B, or -DRB1 matched ($n = 21$) or mismatched ($n = 197$). Patients and donors were categorized according to their degree of KIR-ligand compatibility in the GvHD direction by determining whether or not they expressed HLA-C group 1 or 2, HLA-Bw4, or HLA-A3/-A11. Both HLA-C/-B KIR-ligand- and HLA-A-A3/-A11 KIR-ligand-incompatible CB HCT showed a trend to improved LFS ($p = 0.09$ and $p = 0.13$, respectively). Sixty-nine donor-patient pairs were HLA-A, -B, or -C KIR-ligand incompatible and 149 compatible. KIR-ligand-incompatible CB HCT showed improved LFS (hazards ratio = 2.05, $p = 0.0016$) and overall survival (OS) (hazards ratio = 2.0, $p = 0.004$) and decreased relapse incidence (RI) (hazards ratio = 0.53, $p = 0.05$). While the study noted the KIR effect is more pronounced in AML HCT, CB HCT for acute leukemia in CR from KIR-ligand-incompatible donors was associated with decreased RI and improved LFS and OS (Willemze et al. 2009).

In summary, there are major challenges in summarizing CB HCT outcomes for lymphoid malignancies in children since most studies report outcomes collectively for all hematological malignancies. However, from the data that are available, one can conclude that CB HCT is highly efficacious, providing patients with high-risk lymphoid malignancies the opportunity for cure.

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Part III

Cord Blood Transplants for Neoplastic Diseases in Adults: Data, Consensus and Challenges

Cord Blood Transplants for Myeloid Malignancies in Adults

10

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10.1 Introduction

Allogeneic hematopoietic cell transplantation (HCT) remains the mainstay of potentially curative therapy for the majority of myeloid malignancies including non-favorable risk groups of acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and higher-risk non-chronic myeloid leukemia myeloproliferative neoplasms (MPNs). While sibling donors are ideal, many patients lack a fully matched sibling and thus alternative donor options are sought. This chapter reviews the data illustrating the use of umbilical cord blood (UCB) donors for myeloid malignancies and highlights the outcomes and controversies surrounding its use in this patient population.

10.2 Cord Blood Transplant in Acute Myeloid Leukemia: Is This the Best Option When a Sibling Donor Is Not Available?

Potentially curative allogeneic HCT remains the standard of care for non-favorable risk AML. Historically, if no sibling donor was available, an adult HLA-matched unrelated donor (MUD) search was implemented to identify an alternative graft source. The advancing age of patients eligible for HCT, the limitation of

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_10

potential HLA-MUD options for patients of diverse ethnicity, and the time required to mobilize MUDs have prompted the development of additional alternative donor sources. Specifically, UCB transplantation has become a valuable donor source option. The proof of principle utilizing UCB as a graft source was first published by Gluckman et al. in 1989 after use in a child with Fanconi anemia (Gluckman et al. 1989) followed by Kurtzberg et al. in 1996 in the setting of AML (Kurtzberg et al. 1996), and in 2005 its use expanded to adults with the use of 1–2 cord units to provide adequate cell dose for the larger recipient (Barker et al. 2005). In 2010 Brunstein et al. presented the first risk-benefit analysis of alternative donor transplants comparing double UCB transplants with that of HLA-matched related donors (MRDs), HLA-matched unrelated donors, and HLA-mismatched unrelated donors (MMUDs) for patients with predominantly AML or ALL (acute lymphoblastic leukemia) (Brunstein et al. 2010). These data highlighted similar leukemia-free survival and grades II–IV acute graft-*versus*-host disease (aGvHD) with risks of prolonged neutrophil and platelet recovery and higher non-relapse mortality (NRM) with double UCB but diminished 2-year chronic GvHD (cGvHD) and possibly diminished relapse (Brunstein et al. 2010).

More recent publications have evaluated non-related donor sources in AML with attention to the impact of transplant disease status and conditioning intensity on outcome. A combined CIBMTR (Center for International Blood and Marrow Transplant Research) and Eurocord analysis compared the outcomes of patients with AML over age 50 transplanted in first complete remission (CR1) with a MUD, MMUD, or UCB donor and myeloablative (MA) or reduced intensity conditioning (RIC). Neutrophil recovery and platelet recovery were delayed with UCB graft source. Rates of aGvHD were similar across donor sources ranging from 35 to 44%, but rates of cGvHD were notably lower with UCB at 28% compared to >50% with HLA-MUD/MMUD ($p < 0.0001$) (Weisdorf et al. 2014). Non-relapse mortality (NRM) was higher in the UCB cohort within the first 3 months; however, beyond 3 months, NRM was higher only in the HLA-MMUD. Conditioning intensity and cytogenetic risk stratification influenced risk of relapse but donor source did not. After adjusting for both cytogenetic and conditioning, the 3-year probability of relapse was similar between UCB and HLA-MUDs around 35%. Donor source did influence survival; however, cytogenetic risk, age, and performance status were also significant determinants. Once adjusted for cytogenetics and age, 3-year survival for HLA-MUD was 43% compared to 30% for UCB ($p = 0.002$) (Weisdorf et al. 2014). The CIBMTR-Eurocord analysis demonstrated modestly inferior OS and higher early NRM with UCB in a homogeneous patient population of AML in CR1 but highlighted the similar rates of aGvHD and relapse and superior cGvHD rates with UCB.

Registry data has the benefit of larger patient numbers and resultant additional power to detect differences in outcome; however, heterogeneity in conditioning and supportive care approaches as well as treatment center expertise potentially creates variable outcomes. Reports from larger institutional studies with consistent conditioning regimens, supportive care, and clinical expertise contribute important outcome data. In a joint analysis between the University of Minnesota, University Hospital of Nantes, and Hospital Saint Louis Paris, outcomes of older adults (≥50 years) with AML undergoing RIC transplant with HLA-MSD, MUD, or UCB donors were compared. The majority of UCB transplants were conducted at the University of Minnesota, while the majority of HLA-MUD transplants were conducted at the University Hospital of Nantes and Hospital Saint Louis Paris. Sibling donor transplants were evenly mixed between the institutions. Acute GvHD rates were similar across donor types, and rates of cGvHD at 2 years revealed a nonsignificant trend toward diminished rates in UCB donors. Most notable was similar survival, relapse, and NRM between all donor sources (Peffault de Latour et al. 2013a). Expanding upon these data, the University of Minnesota and Hospital Saint Louis Paris extended the analysis to all adults with AML transplanted with MA or RIC between using HLA-MSD, UCB, HLA-MUD, or HLA-MMUD. Patient characteristics were similar across donor types with respect to gender, Karnofsky performance status, and age. UCB and MUD/MMUD had more cases with poor-risk cytogenetic/molecular profiles and those with sibling donors were more often transplanted in CR1. Conditioning intensity varied across donor sources with UCB more often receiving RIC. With extended follow-up, 6-year survival was similar across donor types. Donor type did not influence relapse or NRM; however, conditioning and age did impact survival, relapse, and NRM with superior outcomes in younger patients undergoing MA conditioning. These data highlight outcomes of a sizeable population of patients treated uniformly at two experienced centers and provide additional support for the use of alternative donors as a graft source for patients with non-favorable groups of patients with AML when a HLA-MSD is not available. These data suggest the importance of MA conditioning when possible to reduce risk of relapse (Warlick et al. 2015).

In summary, numerous alternative graft sources exist for adults with AML in need of a HCT and lacking a HLA-MSD. The composite data suggests modestly improved OS, NRM with HLA-MUD compared to UCB, but lower incidence of cGvHD with UCB (Table 10.1). Numerous patient variables, disease characteristics, and transplant variables will impact the decision-making for the optimal timing and choice of donor source for AML patients lacking a HLA-MSD with both HLA-MUD and UCB valuable alternatives (Fig. 10.1).

Table 10.1 UCB transplant outcomes for myeloid malignancies

Study and patients	Conditioning	OS % (95% CI)	Relapse % (95% CI)	NRM % (95% CI)	aGvHD % (95% CI)	cGvHD % (95% CI)
Acute myeloid leukemia (AML)						
Peffault de Latour et al. (2013a) *BBMT* Sib ($n = 82$) MUD ($n = 35$) UCB ($n = 80$)	RIC: 100%	Sib: 3 YR 51% (38–63) MUD: 3 YR 53% (28–78) UCB: 3 YR 45% (31–58) $p = 0.73$	Sib: 3 YR 33% (23–44) MUD: 3 YR 29% (13–46) UCB: 3 YR 43% (32–54) $p = 0.041$ Poor-risk cytogenetics associated with increased relapse	Sib: 3 YR 18% (10–28) MUD: 3 YR 14% (5–28) UCB: 3 YR 24% (15–34) $p = 0.22$	Day 100 Grades II–IV Sib: 29% (19–39) MUD:38% (22–54) UCB: 39% (28–50) $p = 0.18$	Sib: 3 YR 43% (31–54) MUD: 3 YR 41% (24–57) UCB: 3 YR 23% (15–34) $p = 0.14$
Weisdorf et al. (2014) *BBMT* MUD ($n = 441$) MMUD ($n = 84$) UCB ($n = 205$)	MA: 20% RIC: 80%	MUD: 3 YR 43% (38–48) MMUD: 3 YR 37% (27–46) UCB: 3 YR 30% (23–37) $p = 0.002$ MUD *vs.* UCB Adjusted for cytogenetic risk/ Age	MUD: 3 YR 35% (30–40) MMUD:3 YR 26% (18–35) UCB: 3 YR 35% (28–41) $p = 0.95$ MUD *vs.* UCB Adjusted for cytogenetic risk and conditioning intensity	MUD: 3 YR 27% (23–31) MMUD:3 YR 41% (31–51) UCB: 3 YR 35% (28–42) $p = 0.05$	Day 100 Grades II–IV MUD: 36% (32–41) MMUD: 44% (34–54) UCB: 35% (28–41) $p = 0.69$ (MUD *vs.* UCB)	MUD: 3 YR 53% (48–58) MMUD: 3 YR 59% (49–69) UCB:3 YR 28% (22–34) $p < 0.0001$ MUD *vs.* UCB

Warlick et. al (2015) *BBMT* Sib ($n = 187$) MUD ($n = 55$) MMUD ($n = 21$) UCB ($n = 151$)	MA: 54% RIC 46%	Sib: 6 YR 47% (39–53) MUD: 6 YR 54% (40–66) MMUD: 6 YR 51% (28–70) UCB: 6 YR 36% (28–44) $p < 0.11$	Sib: 2 YR 26% (19–32) MUD: 2 YR 20% (9–31) MMUD: 2 YR 33% (13–53) UCB: 2 YR 36% (28–45) $p < 0.05$	Sib: 1 YR 20% (14–25) MUD: 1 YR 25% (14–37) MMUD: 1 YR 14% (4–29) UCB: 1 YR 20% (14–26) $p < 0.53$	Day 100 Grades III–IV Sib: 9% (5–13) MUD: 15% (5–24) MMUD: 24% (6–42) UCB: 24% (17–31) $p < 0.01$	Overall 2 YR incidence 24% MVA: Risk higher in males and HCT during CR2/CR3 No impact of donor source
Myelodysplastic syndrome (*MDS*)						
Majhail et al. (2012) *BMT* Sib ($n = 38$) MDS $n = 12$ AML $n = 26$ UCB ($n = 60$) MDS $n = 15$ AML $n = 44$	RIC: 100%	Sib: 3 YR 37% (19–55) UCB: 3 YR 31% (19–44) $p = 0.21$	Sib: 2 YR 34% (17–50) UCB: 2 YR 47% (33–62) $p = 0.19$	Sib: 2 YR 25% (11–40) UCB: 2 YR 25% (13–37) $p = 0.82$	Day 100 Grades III–IV Sib: 26% (11–41) UCB: 21% (9–33) $p = 0.95$	Sib: 2 YR 61% (40–82) UCB: 2 YR 33% (20–46) $p = 0.04$
Robin et al. (2015) *BBMT* PB MUD: $n = 502$ (64% → to AML pre-HCT: Only 54% CR at HCT) UCB: $n = 129$ (71% → AML pre-HCT: Only 48% in CR at HCT)	RIC: 100%	MUD: 2 YR 50% (47–53) MMUD: 2 YR 43% (38–48) UCB: 2 YR 30% (26–34) $p < 0.0001$	MUD: 2 YR 23% (21–25) MMUD: 2 YR 28% (23–33) UCB: 2 YR 30% (24–34) $p = 0.47$	MUD: 2 YR 32% (30–34) MMUD: 2 YR 36% (31–41) UCB: 2 YR 42% (38–46) $p = 0.04$	Day 100 Grades II–IV MUD/MMUD: 29% UCB: 31% P value not given	MUD: 2 YR 44% (41–47) MMUD: 2 YR 37% (31–43) UCB: 2 YR 23% (19–27) $p = 0.004$

(continued)

Table 10.1 (continued)

Myelofibrosis						
Murata et al. (2014) *BMT* Related BM $n = 19$ Related PB $n = 25$ MUD BM $n = 28$ UCB $n = 11$	MA: 24% RIC: 76%	Related BM: 5 YR 63% (38–80) Related PB: 5 YR 43% (23–61) MUD BM: 5 YR 41% (21–59) UCB: 5 YR 0% 2 YR: 36% (11–63) $p = 0.15$	Related BM: 2 YR 5% (0–21) Related PB: 2 YR 8% (1–22) MUD BM: 2 YR 4% (0–18) UCB: 2 YR 0% p value not given	Related BM: 2 YR 33% (13–54) Related PB: 2 YR 45% (24–63) MUD BM: 2 YR 61% (38–77) UCB: 2 YR 64% (30–85) $p = 0.021$ UCB *vs.* Related BM	Day 100 Grades III–IV Related BM: 6% (0–22) Related PB: 16% (5–33) MUD BM: 14% (4–30) UCB: 0% $p < 0.001$	Related BM: 2 YR 35% (14–57) Related PB: 2 YR 52% (31–69) MUD BM: 2 YR 25% (11–42) UCB: 2 YR 18% (3–44) p value not given
Robin et al. (2014) *BBMT* PMF $n = 20$ SMF $n = 15$ 7 → AML pre-HCT and 4 achieved CR	MA: 31% RIC: 69%	UCB: 2 YR 44%	UCB: 15 of 35 relapsed (43%) at median of 7 months	UCB: 2YR 35%	Day 100 Grades II–IV Grade II: 7 (20%) Grade III: 2 (6%) Grade IV: 1 (3%)	Among 18 at risk for cGvHD: Limited: 6 (33%) Extensive: 1 (6%)

Sib sibling, *MUD* matched unrelated donor, *MMUD* mismatched unrelated donor, *UCB* umbilical cord blood, *BM* bone marrow, *PB* peripheral blood, *YR* year, *MA* myeloablative, *RIC* reduced intensity conditioning, *MDS* myelodysplastic syndrome, *AML* acute myeloid leukemia, *aGvHD* acute graft-*versus*-host disease, *cGvHD* chronic graft-*versus*-host disease, *PMF* primary myelofibrosis, *SMF* secondary myelofibrosis, *HCT* hematopoietic cell transplant, *CR* complete remission, *p* p value

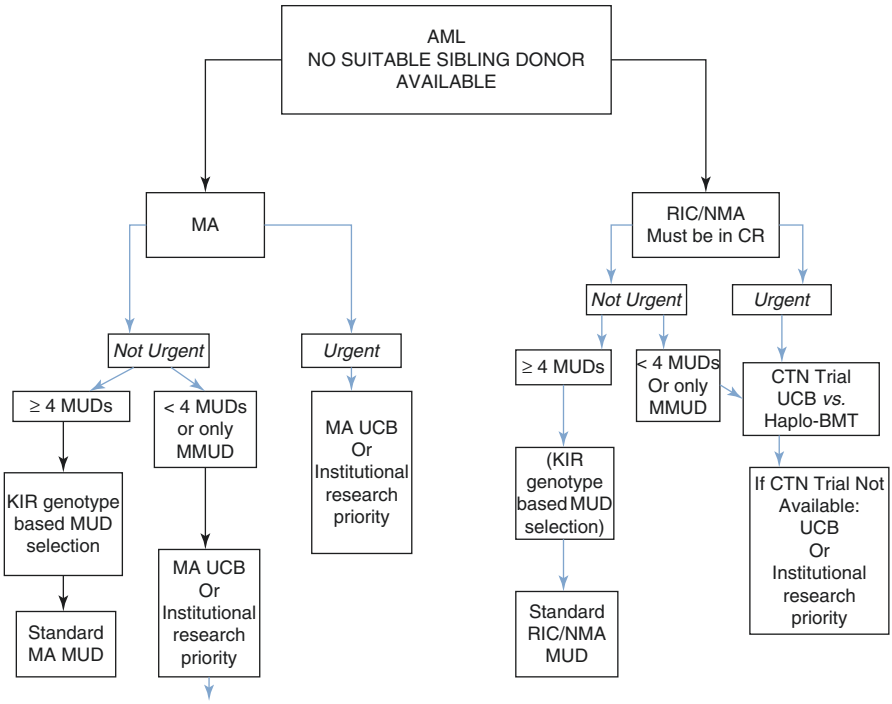

Fig. 10.1 Donor selection algorithm for AML

10.3 Cord Transplantation in Myelodysplastic Syndrome: Is This a Reasonable Choice When a HLA-Matched Sibling Donor Is Not Available?

Myelodysplastic syndromes (MDS) are a complex and heterogeneous group of clonal hematopoietic stem cell disorders for which the only known cure is allogeneic HCT (Sierra et al. 2002; Warlick et al. 2009). Numerous publications have identified the importance of prognostic stratification (Greenberg et al. 1997, 2012) for HCT timing decision-making in both the MA and RIC setting (Cutler et al. 2004; Koreth et al. 2013) and have identified the importance of cytogenetic risk stratification in transplant outcomes (Ustun et al. 2015; Della Porta et al. 2014; Trottier et al. 2015). Donor source also plays a role in MDS transplant outcomes. A recent CIBMTR analysis by Saber et al. compared the outcomes of adult MDS patients undergoing a HLA-MSD, HLA-MUD, or HLA-MMUD receiving MA or RIC. The data highlighted diminished adjusted 3-year survival, increased adjusted 1-year NRM, and increased incidence of severe aGvHD with MUDs/MMUDs *versus* HLA-MSD (Saber et al. 2013). In contrast, an EBMT analysis of 1333 MDS patients over the age of 50 receiving either a HLA-MSD or HLA-MUD donor transplant revealed similar 4-year survival at approximately 31% with advanced disease at HCT, the primary variable impacting survival, but increased NRM with unrelated donor source (Lim et al. 2010).

As noted above, comparisons of sibling and unrelated donor transplants in MDS are readily available; however, the literature comparing unrelated donors and UCB is less developed. Registry data provides the largest cohorts of patients for such outcome analysis comparing alternative donor HCT in MDS. Robin et al. presented the first larger report evaluating outcomes of UCB HCTs in MDS on behalf of Eurocord and EBMT comparing patients with either MDS ($n = 39$) or sAML ($n = 69$) undergoing MA or RIC UCB (Robin et al. 2011). They reported 2-year OS of 34%, 2-year DFS of 30%, and 2-year incidence of relapse of 21%. Severe aGvHD grades III–IV occurred in approximately 11%, 2-year cGvHD was 42%, and NRM was high at 49%. NRM was adversely influenced by MA conditioning, a longer time from diagnosis to transplantation, and GvHD prophylaxis regimen other than CsA/steroid or CsA/MMF (Robin et al. 2011). While this study was the first to report a larger cohort of patients undergoing UCB transplant for MDS, the number of patients with MDS in this cohort was small and the patient population/transplant approaches heterogeneous due to the retrospective registry nature of the study. Data from a larger Eurocord-EBMT comparison of MDS adults undergoing either UCB ($n = 129$) or peripheral blood (PB) HLA-MUD ($n = 502$) RIC HCT were reported (Robin et al. 2015). Of importance, 65% of the MDS patients within the entire cohort had progressed to AML prior to transplantation, and of those patients, only 52% had achieved remission prior to transplant. Two-year survival ranged from 30% for UCB to 50% for HLA-MUD ($p < 0.0001$) with similar relapse incidence across donors (23% MUD to 30% UCB) but increased NRM at 42% for UCB compared to 31% for PB HLA-MUD ($p = 0.03$). Incidence of cGvHD was lower with UCB donor source. Based on these data, the authors concluded that PB HLA-MUD donors were the preferred stem cell source compared to UCB in the absence of a HLA-MSD (Robin et al. 2015). This study is limited by the heterogeneity of patients/conditioning regimens/supportive care inherent to a registry study and the small number of true MDS patients not progressing to AML and receiving an UCB HCT ($n = 37$). Despite these limitations, the analysis importantly highlights surprisingly good outcomes of survival and relapse for such a high-risk group of patients with the majority of the MDS patients progressing to AML, only half in remission, and receiving only RIC. Thus, these data suggest that both HLA-MUD and UCB RIC transplants can be effective in curing even high-risk MDS and AML patients (Robin et al. 2015).

Single-institution studies provide additional important data when investigating comparative donor outcomes in the setting of more homogenous conditioning and supportive care platforms. In 2012, Majhail et al. published the outcomes of a cohort of patients at the University of Minnesota with MDS ($n = 28$) or AML ($n = 70$) undergoing RIC comparing the impact of donor source on post-HCT outcomes (Pourhassan et al. 2016). They reported similar 3-year OS, similar 2-year relapse, similar NRM, similar grades III–IV aGvHD, and lower rates of cGvHD with UCB as compared with siblings (Pourhassan et al. 2016). The University of Minnesota BMT program has recently updated outcomes of a larger cohort of MDS patients ($n = 89$, none of which had progressed to AML) undergoing MA or RIC HLA-MSD or UCB transplants. Outcomes based on donor source were similar: 5-year OS (33–41%), 1-year NRM (22–27%), and 2-year relapse incidence (27–30%) (Pourhassan et al. 2016). This analysis includes a much larger cohort of true MDS patients treated with consistent conditioning regimen platforms and consistent supportive care.

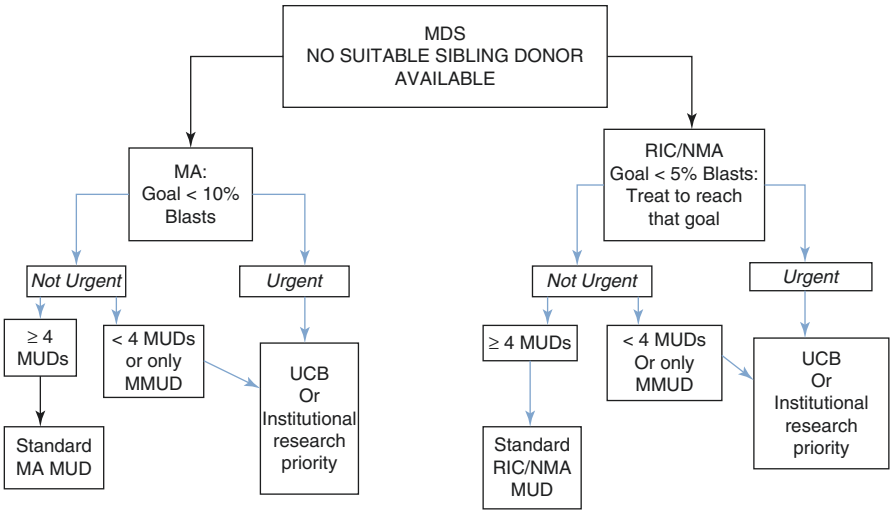

Fig. 10.2 Donor selection algorithm for MDS

In summary, the data on the use of UCB donor source in MDS is limited (Table 10.1). Larger registry studies suggest inferior outcomes with respect to NRM and OS; however, included patients who have progressed to AML, not in remission, and combine heterogeneous conditioning regimens and supportive care. Smaller single-institution studies with homogenous conditioning, with supportive care platforms, and with more homogenous MDS patient cohort show similar outcomes but lack power to detect a significant difference. Consequently, large-scale studies limiting the conditioning regimens and including only true MDS patients without progression to AML are needed to more definitively compare alternative donor source outcomes in MDS. Until that data is available, the data endorses HLA-MUD donors as the primary alternative donor source for MDS patients unless at an experienced center or in the context of a clinical trial (Fig. 10.2).

10.4 Cord Blood Transplant for Myelofibrosis: Is There Any Data Out There?

UCB HCT outcomes across hematologic malignancies reveal consistent delay in both neutrophil and platelet recovery with average delays of 1 and 4 weeks, respectively, and an incidence of graft failure of approximately 10% (Brunstein et al. 2010). Given the concern for slow engraftment and graft failure in MPNs such as myelofibrosis, the use of UCB donor sources in this context has been minimal. Recent limited published data now provides some insights on the expected outcomes of UCB HCT in myelofibrosis. Takagi et al. reported the first successful UCB engraftment in 14 patients with myelofibrosis (Takagi et al. 2010). The majority of the patients had grade III myelofibrosis (93%) and splenomegaly (79%). Neutrophil engraftment occurred in 93% and platelet engraftment in 43% of patients (Takagi et al. 2010). More recently

Eurocord-EBMT presented UCB HCT outcomes of adults with primary or secondary myelofibrosis. Forty-three percent underwent splenectomy prior to HCT and a small number had progressed to AML prior to HCT. The majority of patients underwent RIC, the most common regimen, a combination of total body radiation, cyclophosphamide, and fludarabine. Neutrophil recovery at day +60 was 80% and platelet recovery at day +100 was 54%. Graft failure rates were high at 40%. Interestingly, survival was similar between those with or without graft failure as some patients experienced autologous recovery. However, for those with aplastic graft failure and lack of neutrophil recovery, survival was dismal at 14%. Interestingly, all patients with TCF conditioning achieved platelet and neutrophil recovery. The majority of deaths were due to complications of transplant with disease recurrence the remaining. Despite the above, survival outcomes were better than expected with 2-year OS of 44%. These data reveal the possibility of engraftment with an UCB donor source in myelofibrosis and highlight a higher proportion of engraftment in those receiving a RIC with TCF (Robin et al. 2014). Murata et al. recently reported a retrospective comparison of adults with primary myelofibrosis undergoing related BM or PB *versus* HLA-MUD BM or UCB from the Japan Society for Hematopoietic Cell Transplantation (JSHCT) (Robin et al. 2014). Eight percent of patients died within 60 days of transplant without engrafting. Neutrophil and platelet engraftment were diminished in both UCB and MUD HCTs. Severe aGvHD and cGvHD were both lower in UCB (0% severe aGvHD and 18% cGvHD). Relapse was similar across donor sources but 2-year NRM was quite high in UCB (64%) and HLA-MUD BM (61%). Overall survival at 5 years was also inferior in UCB with 0% surviving (Murata et al. 2014). Based on these data, UCB should be avoided as a donor source for patients with primary myelofibrosis unless in the context of a clinical trial where no other donor options are available and extensive counseling regarding potential risks and benefits (Table 10.1).

10.5 UCB Transplants for Older Adults with Myeloid Neoplasms: Safe or Unwise?

As the age of eligibility for RIC HCT increases, so does the age of the potential HLA-MSD resulting in the possible situation of inability to use them due to medical comorbidities, age, or concerns for suboptimal graft. Consequently, alternative donors are often the donor source available for older patients undergoing HCT for hematologic malignancies. A number of registry and single-institution studies have recently evaluated the feasibility of unrelated adult and UCB donors in older patients undergoing HCT. Tanaka et al. reported a retrospective JSHCT analysis of AML, ALL, or MDS patients aged 50 and older undergoing MA or RIC HCT with HLA-MUD, HLA-MMUD, or UCB donors (Tanaka et al. 2015). Across the donor groups, UCB had higher percentage of patients aged 60+, higher percentage of AML, and higher percentage of RIC/NMA. Two-year adjusted OS was inferior with UCB (39% *versus* 49% with HLA-MUD donor); but interestingly, this survival difference by donor source was seen primarily in early-phase disease (AML or ALL in CR1 or RARS/RCMD MDS) and similar survival across donor sources in advanced disease (HLA-MUD 31%, HLA-MMUD 24%, UCB 25%). Two-year NRM was increased

in HLA-MMUD (40%) and UCB (38%). The incidence of relapse at 2 years was increased in UCB at 26% compared to 18% with MUD donors and 21% with MMUD; however, when adjusted for disease phase, the rates of relapse were similar across donor types. Additional analysis highlighted similar OS in those AML or ALL UCB recipients receiving a CD34 cell dose of $\geq 0.84 \times 10^5$ cells/kg as compared to a HLA-MUD (Tanaka et al. 2015). This retrospective analysis highlights outcomes with HLA-MUD, HLA-MMUD, and UCB in an older population of AML/ALL/MDS experiencing survival similar to expected outcomes for younger patients. These data confirm superior survival and NRM with HLA-MUD when compared to UCB but highlight similar survival outcomes when a threshold UCB $CD34^+$ cell dose is available. These data also importantly show that all donor sources can provide successful curative transplant outcomes in an older patient population with hematologic malignancies. The analysis included only Japanese transplant centers so those data may not be generalizable to other populations; however, a recent combined CIBMTR-Eurocord analysis in AML CR1 adults over age 50 found similar outcomes (Weisdorf et al. 2014). Future studies investigating the threshold $CD34^+$ UCB cell dose in other settings should be validated.

Another recent JSHCT retrospective analysis compared outcomes of patients aged 50 and older with AML, ALL, or MDS undergoing HCT with either HLA-MSD (BM or PB source) or UCB (Konuma et al. 2016). Those receiving an UCB graft were older and had higher DRI, higher RIC percentage, and more ABO mismatch. The authors reported lower risk of cGvHD in the UCB group, similar severe grades III–IV aGvHD across donor sources, and similar relapse but higher NRM in the UCB. Interestingly, GvHD-free/relapse-free survival (GRFS) was similar across donor sources supporting UCB as a viable donor source for older patients with AML/MDS (Konuma et al. 2016). Majhail et al. presented a retrospective single-center experience comparing HCT outcomes of patients with hematologic malignancies aged 55 and older transplanted with either a sibling or UCB donor and revealed similar OS, similar NRM, similar grades II–IV aGvHD, and lower cGvHD again highlighting the feasibility of UCB in an older patient population (Majhail et al. 2008). More recent analysis by Sandhu et al. presented HCT outcomes in an even older patient population (age 60–70+) with MDS and AML undergoing UCB and revealed 2-year NRM of 20% (95% CI, 1–44%) and relapse of 30% (95% CI, 0–52%) with 2-year OS of 60% (95% CI, 25–83%) (Sandhu et al. 2016).

In summary, these data show that UCB is feasible and reasonable in an older patient population when a HLA-MSD is not available and provide additional transplant options for a group of patients where donor options may be more limited.

10.6 Relapse After UCB Transplant in Myeloid Malignancies: What Does One Do?

Relapse of myeloid malignancies post-allogeneic HCT occurs in approximately 20–50% of patients depending upon disease status, conditioning intensity cytogenetic, and molecular risk profiles and portends a poor prognosis (Weisdorf et al. 2014; Ustun et al. 2015; Della Porta et al. 2014; Trottier et al. 2015; Saber et al.

2013; Peffault de Latour et al. 2013b). Historically, donor lymphocyte infusion (DLI) was considered for those relapsed patients with a HLA-MSD and MUD available with CR rates ranging from 25 to 50% (Warlick et al. 2012; Deol and Lum 2010). Unfortunately remission duration is often suboptimal with the majority of patients relapsing following post-DLI remission (Warlick et al. 2012).

In the setting of UCB HCT, there is no option for DLI upon relapse. Thus, alternatives for dealing with this substantial post-HCT complication are needed. An intriguing study by Bejanyan et al. compared outcomes of patients with relapsed AML post-HCT who had either HLA-MSD or UCB donors (Bejanyan et al. 2014). The median time to relapse in this cohort of patients was 152 days, and the majority of patients (60%) were still receiving immunosuppressive therapy at the time of relapse. Patients were tapered off immunosuppression. Supportive care alone was offered to one-third of patients. For those in good clinical condition who receive more intensive therapy, systemic chemotherapy, DLI +/− chemotherapy, or a second allogeneic HCT was offered. Systemic chemotherapy or second transplant was more often used in UCB HCT patients, whereas DLI was the most frequent approach in sibling donor patients. Complete remission rates after intervention for the post-HCT relapse were 35% (95% CI, 19–53%) in HLA-MSD compared to 21% (95% CI, 12–32%) in UCB donor transplants ($p = 0.16$) and by treatment type were 28% (95% CI, 13–47%) in those receiving chemotherapy alone, 58% (95% CI, 28–85%) for those receiving DLI, and 73% (95% CI, 39–94%) for those receiving a second transplant, whereas those receiving supportive care only (immunosuppressive withdrawal alone) was only 8% (95% CI, 2–22%) ($p < 0.01$) (Bejanyan et al. 2014). The 1-year survival after intervention for post-HCT relapse was similar across donor sources but was significantly different based on the specific relapse intervention. Survival was 35% (95% CI, 18–52%) for those getting chemotherapy alone, 50% (95% CI, 21–74%) for those receiving DLI, and 27% (95% CI, 7–54%) for those receiving a second Hematopoietic cell transplant (HCT) ($p < 0.01$) (Deol and Lum 2010). Importantly, this study showed that donor source did not compromise post-relapse survival, but the clinical condition of the patient allowing for more aggressive intervention did. Thus, intensive treatment for those patients fits enough to undergo the therapy that should be utilized whenever possible, and the lack of a posttransplant DLI option should not be a factor which dissuades the use of UCB donor source.

10.7 What Is the Best Alternative Graft Source in the Absence of HLA-MUD: Haploidentical Graft or Cord Blood Graft?

In the absence of a HLA-MSD and need for urgent transplant where time does not afford a HLA-MUD, both UCB and haploidentical (haplo) bone marrow donors are potential options. Reviewed above are numerous studies in myeloid malignancies evaluating UCB transplant outcomes individually and in comparison to HLA-MSD, HLA-MUD, and HLA-MMUD. What is the data regarding haplo donor outcomes? In a large retrospective analysis published by the Johns Hopkins group, patients aged 50 and older with hematologic malignancies underwent a NMA T-cell-replete

haplo bone marrow transplant with high-dose posttransplant cyclophosphamide (Kasamon et al. 2015). The majority of patients had intermediate- or high-risk disease as defined by the disease risk index (DRI) (Armand et al. 2008). One-year NRM was low across all age cohorts ranging from 10 to 14%. Overall survival at 3 years was 46% (95% CI, 40–53%) and was not impacted by age. Rates of severe aGvHD grades III–IV at 6 months were quite low at 3% (95% CI, 1–5%), and incidence of cGvHD was 10% (95% CI, 6–13%) at 1 year. Specific to AML, the 3-year incidence of relapse was high at 52% (95% CI, 39–65%), 3-year DFS 40% (95% CI, 29–54%), and 3-year OS 49% (95% CI, 37–64%). These data highlight very low NRM and severe GvHD with successful engraftment and reasonable survival at 3 years; however, relapse rates remain high and posttransplant relapse prevention studies are needed (Kasamon et al. 2015).

A national randomized Clinical Trials Network (CTN) study is underway comparing the Hopkins haplo-BMT with the University of Minnesota UCB approach. Until results from that study are published, the best comparison of the two alternative donor approaches is a retrospective Eurocord analysis by Ruggeri et al. evaluating the outcomes of adult AML and ALL undergoing haplo-BMT or UCB transplants (Ruggeri et al. 2015). Within the AML cohort, the follow-up was slightly longer in the UCB cohort, and there was a larger percentage of female recipients and lower percentage with advanced disease, whereas the haplo-BMT cohort more often received MA conditioning. Neutrophil engraftment in the AML cohort was 91% for the haplo-BMT compared with 84% with UCB with median day to ANC recovery of 17 and 23 days, respectively. Approximately 9% of AML haplo-BMT and 16% AML UCB did not engraft. The rates of severe aGvHD grades III–IV were similar at 11% (haplo) and 12% (UCB) ($p = 0.41$), and rates of cGvHD were 29% (haplo) and 24% (UCB) with the difference significant in multivariate analysis ($p = 0.008$). Relapse was similar between the two groups but was higher in those undergoing RIC or not in CR1 and lower in those with a shorter time from diagnosis to transplant. NRM was also similar between the two groups and again was higher in those with advanced disease status at transplant. Leukemia-free survival at 2 years for those with AML similar between donor sources with advanced disease status at transplant is the most significant influencing factor (Ruggeri et al. 2015). These findings highlight similar long-term outcomes of relapse, NRM, and LFS between the two donor sources and suggest that both are viable options when performed at experienced centers for patients who do not have a sibling donor and whose timeline does not allow HLA-MUD mobilization.

10.8 Impact of HLA Matching on AML and MDS UCB Transplant Outcomes

Outcomes of HCT in myeloid malignancies are dependent on numerous patient- and disease-related factors; however, transplant-related factors also impact results. As detailed above, HCT outcomes are different when comparing HLA-MSD, HLA-MUD, UCB, and haplo-BM donor sources and different depending on the conditioning intensity. Specifically with UCB donors, cell dose and HLA typing and

numerous immunogenetic factors impacting outcome exist: HLA disparity and anti-HLA-A antibodies (Ruggeri et al. 2016).

Details on UCB unit selection based on HLA matching and cell dose and other immunogenetic factors have been reviewed elsewhere in this book; however, specific outcome differences in the realm of AML and MDS UCB transplants with respect to HLA disparity are discussed here. In 2004, Gluckman et al. described the impact of HLA disparity on UCB transplant outcomes in a large retrospective analysis of 550 patients (including a subset of AML and MDS) within the Eurocord Registry. A number of HLA disparities had no impact on aGvHD grades II–IV or NRM, but 1-year relapse was lower in those using an UCB donor with 1, 2, or 3 disparities when compared with an HLA-matched UCB (Gluckman et al. 2004). More recently, Sanz et al. reviewed outcomes from AML patients undergoing MA UCB treated at a single institution with the following HLA UCB matching guidelines: "≥4–6 HLA matched with the recipient with HLA class I antigens (A and B) considering the antigen level and class II antigens (DRB1) considering the allele level" (Sanz et al. 2014). The Sanz group found results similar to Gluckman's Eurocord analysis: HLA disparity had no impact on aGvHD or cGvHD, had no impact on NRM, but did impact relapse. Relapse differences were only observed within mismatches in the GvH direction; 5-year incidence of relapse was 44% in those with ≥6/8 extended high-resolution HLA UCB match *versus* 22% with <6/8 UCB match ($p = 0.04$). This relapse difference extended to improve leukemia-free survival. In the setting of standard HLA typing and mismatches in the GvH direction, the leukemia-free survival (LFS) at 5 years was 45% (4/6 matches), 21% (5/6 matches), and 14% (6/6 matches). Multivariate analysis confirmed the impact of HLA disparity in the GvHD direction and CR1 at HCT on LFS (Gluckman et al. 2004). More recently Brunstein et al. retrospectively evaluated the impact of allele level HLA mismatch at the A, B, C, DRB1, and DQB1 level for both MA and RIC double UCB HCTs for patients transplanted for hematologic malignancies. Subset analysis in acute leukemia confirmed the impact of HLA disparity on outcomes of relapse with higher disparity associated with diminished relapse (Brunstein et al. 2016). In summary, greater HLA disparity, with adequate cell dose, may be a consideration in future UCB selection criteria in the context of a clinical trial for those undergoing UCB HCT for high-risk groups of patients with AML.

10.9 Expert Point of View

In summary, the current literature would suggest the following regarding UCB transplantation in myeloid malignancies:

1. For AML patients requiring a HCT and lacking a HLA-MSD, HLA-MUD donor source provides modestly improved OS and NRM compared to UCB, but cGvHD rates are superior with UCB. These factors, speed at which a transplant is needed, the risk profile of the AML, and experience of the transplant center, need to be considered when making a final decision on donor selection.

2. For MDS patients requiring a HCT and lacking a HLA-MSD, the data is less developed regarding alternative donor selection. The limited current data would suggest HLA-MUD as the donor source; however, single experienced institution studies suggest similar outcomes with UCB.
3. For patients with myelofibrosis, the use of UCB donor source should be avoided unless in the context of a clinical trial where no other transplant options exist.
4. UCB is feasible in an older patient population and remains a reasonable alternative when sibling donors are not available.

10.10 Future Directions

1. Large-scale comparisons of outcomes for MDS patients undergoing HCT with alternative donors (HLA-MUD, HLA-MMUD, UCB) are needed. Such analyses will require collaboration between the CIBMTR-EBMT-Eurocord.
2. Further refinement of UCB selection utilizing immunogenetic factors will be important to study in a large-scale manner. Such work may improve speed of engraftment leading to improve early NRM with UCB thus yielding equivalent outcomes with UCB and MUDs.
3. Further investigation into novel graft manipulation to improve UCB outcomes is needed.

Acknowledgment Special thanks to Dr. Claudio Brunstein for his critical review of the chapter.

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Cord Blood Transplants for Lymphoid Malignancies in Adults

11

Veronika Bachanova

11.1 Introduction

Allogeneic hematopoietic cell transplants (allo-HCT) have been shown to be a valuable and potentially curative strategy to treat patients with high-risk lymphoid malignancies such as acute lymphoblastic leukemia (ALL) and mature lymphoma. Donor availability is a potential barrier for patients who are candidates for allo-HCT but lack an adequately human leukocyte antigen (HLA)-matched and clinically suitable sibling donor. While Caucasian patients have a 60–70% probability of identifying an 8/8 allele-level HLA-matched unrelated donor (URD), for ethnic minority groups, fewer than 30% find a well-matched donor. In the past 10 years, a growing number of reports supported an expanding utilization of HLA-mismatched URD, umbilical cord blood (UCB), and partially HLA-matched family donors (haploidentical) as valuable alternatives to fill the gap in donor availability.

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_11

11.2 Acute Lymphoblastic Leukemia

11.2.1 Choosing the Optimal Donor for the ALL Patient Lacking HLA-Matched Sibling Donor

In choosing an alternative graft for high-risk ALL, physicians need to carefully consider HLA phenotype, the aggressiveness and pace of ALL, the patient's age and weight, and hence the UCB cell dose, as well as the experience of their transplant center. Currently, the best URD is allele HLA matched with the recipient at HLA-A, B, C, and DR (Flomenberg et al. 2004). URD HCT with ≥2 mismatched donors significantly compromises engraftment, GvHD risks, and survival. With nearly 14,000,000 donors available in registries, an 8/8 allele match is found for up to 75% of Caucasians; however, within ethnic and racial minorities, only 24–48% find a well-matched donor (www.marrow.org). Yet, identification, screening, and logistics of URD collection most often require 8–12 weeks even with an expedited search.

UCB grafts mismatched at 0–2 HLA loci with adequate cell dose (at least 2.5×10^7 nucleated cells/kg) can be considered as an alternative to mismatched or even well-matched URD for children and adults with high-risk ALL (Gluckman et al. 2004; Kumar et al. 2008). UCB units are generally immediately available after HLA match confirmation, often within 10–14 days. UCB grafts may therefore be particularly appealing for patients with detectable minimal residual disease (MRD) or evidence of subclinical molecular relapse. In the small window of opportunity (perhaps best including additional leukemia cytoreduction for those with MRD) to undergo transplantation, UCB grafts may permit urgent transplantation prior to another relapse and thereby reduce the patient's morbidity and overall risk.

11.2.2 Adult ALL

In contrast to pediatric leukemia where UCBT is now a standard option for management of ALL (Gluckman et al. 2008), experience in adults with ALL has been more cautious and only accelerated recently by feasibility of double UCBT in heavier adults and increasing need for readily available source of hematopoietic cells in patients without HLA-matched siblings. In contrast to URD adult bone marrow or peripheral blood hematopoietic cell (PBHC) grafts, UCB grafts permit the use of partially HLA-matched units for less common HLA haplotypes including mixed race and ethnicities. Growing experience using two partially matched HLA UCB units to provide an adequate $CD34^+$ cell dose in adults has successfully overcome the cell dose limitation and demonstrated reliable engraftment and effective graft-*versus*-leukemia (GvL) effect for various malignancies including ALL (Brunstein et al. 2010).

At the University of Minnesota, we described 623 consecutive patients with ALL treated over 25 years with a median follow-up of 8.3 years (Tomblyn et al. 2009). All patients received myeloablative, TBI containing conditioning (1320–1375 cGy) and either autologous (34%), sibling (45%), unrelated donor (16%), or umbilical cord blood (11%) grafts. Most patients were in CR1 (24%) or CR2 (50%). The

5-year OS after UCBT was 46%, which compares favorably to sibling grafts (35%) and URD (42%), while mismatched URD grafts yielded inferior survival of 21%. Acute GvHD was more frequent with URD ($p = 0.01$) but similar for sibling donor and UCB transplants. More recently, we published a single-center experience using RIC UCBT in 18 patients with ALL (Bachanova et al. 2009). All patients reached neutrophil engraftment and 100% donor chimerism at median of 10 and 23 days. OS, TRM, and relapse were 50%, 27%, and 35% at 3 years. We have not observed relapses beyond 2 years. The cumulative incidences of acute and chronic GvHD after RIC UCBT were 55% and 45%, respectively. HCT in CR1 ($n = 14$) led to significantly less TRM (8%, $p < .04$) and improved overall survival (81%, $p < .01$) (Bachanova et al. 2009). This initial result using RIC and UCB showed modest TRM and promising LFS for adult ALL and laid the basis for using RIC for older patients with ALL and those with comorbidities in whom mortality of conventional conditioning is prohibitively high (Goldstone et al. 2008).

Adult ALL has been examined in a large multi-institutional observational analysis comparing UCB with adult URD HCT facilitated by the CIBMTR. Marks et al. analyzed 802 adults with adult ALL in CR1 or CR2 (Marks et al. 2014). Two thirds of 546 peripheral blood and 140 bone marrow transplants were allele-level matched considering HLA-A, HLA-B, HLA-C, and HLA-DRB1. In contrast, 116 cord blood transplants were mismatched either in two (57%) or one (29%) locus. The 3-year probabilities of survival were similar between recipients of UCB (44%), matched URD (44%), and mismatched adult donor (43%) transplants. UCB transplants engrafted slower and were associated with less grades II–IV acute but similar chronic GvHD, relapse, and TRM.

The European Blood and Marrow Transplant Group (EBMT) performed a retrospective analysis on 421 adult patients who underwent UCBT for ALL (Tucunduva et al. 2014). Double units were used in 41% and MAC in most (75%). TRM at 2 years was 42%. Incidence of relapse was 28% and was significantly higher after RIC HCT. The best OS (32%) was observed in patients in CR1. Factors associated with improved LFS were age below 35 years, myeloablative conditioning, and CR1. A parallel study from the same group confirmed that the presence of minimal residual disease (MRD), particularly when tested by molecular-based methods in Ph-positive ALL, significantly increased the risk of post-UCBT relapse (Tucunduva et al. 2014).

An earlier EBMTR study compared UCB with adult URD transplants. Despite greater HLA disparity in UCB, acute GvHD was significantly less frequent after UCB compared to URD transplantation (26% *vs.* 39%) (Rocha et al. 2004). The estimated LFS was similar with either donor source (UCB RR 0.59; 95% CI 0.18–1.96). These results highlight that partially matched UCB grafts (usually four of six HLA locus matched) can be as successful as fully matched URD HCT without increased risks of either GvHD or mortality. Other reported series to date in large part reflect the multi-institutional learning curve in UCB graft selection and recipient supportive care (Table 11.1).

These encouraging results suggest that use of UCBT in therapy of adult ALL can be a promising alternative for patients with imminent need of allograft or those requiring an alternative donor source.

Table 11.1 Umbilical cord blood HCT which included patients with ALL

	Patients (*n*)	ALL (%)	CR1 (%)	TRM 3 years (%)	DFS 3 years (%)	Relapse (%)
IBMTR + NYBC (Laughlin et al. 2004)	250	22	40	46	35	18
Eurocord EBMT (Rocha et al. 2004)	584	46	34	42	42	23
UMinn UCB (Tomblyn et al. 2009)	66	100	24	18	46	24
Tokyo Series single cord UCB (Ooi et al. 2009)	27	100	–	–	57.2	27.4
CIBMTR (Marks et al. 2014)	116 UCB 546 8/8[a] 140 7/8[a]	100	49 59 59	42 31 39	44 (OS) 44 43	22 31 39

[a]7/8 or 8/8 matched URD

11.3 UCB in Hodgkin Lymphoma

Hodgkin lymphoma is often cured with standard or high-dose chemotherapy. However, allo-HCT remains a valuable alternative for relapsed/refractory Hodgkin lymphoma (HL). Data on the use of alternative donor in HL has been accumulating since we published the first cohort of 21 adult patients with advanced HL; nine received double-unit UCBT (Majhail et al. 2006). All had sustained donor engraftment by day 60. Two-year progression-free survival (PFS) and OS rates were 25% and 20% and were comparable between UCB and sibling HCTs. More recently, other groups reported their results using UCB HCT in HL as summarized in Table 11.2.

A group from MD Anderson reported 27 consecutive heavily pretreated patients with very high-risk, relapsed/refractory HL, most of whom having relapsed less than 6 months after a prior auto-HCT (Thompson et al. 2016). Patients received double UCBT between 2003 and 2014 using MAC ($n = 15$) or RIC. Antithymocyte globulin was used in most patients. All patients engrafted. Five-year TRM and relapse were 30% and 38%, respectively, leading to PFS of 32% with a median survival of 27 months.

The GITMO group examined 30 patients with HL who underwent single-unit UCBT and showed 90% myeloid engraftment (Pinana et al. 2016). The conditioning regimens consisted of thiotepa, busulfan, cyclophosphamide or fludarabine, and ATG. TRM at day 100 and 4 years was 30% and 47%, and the incidence of relapse at 4 years was 25%. EBV-related posttransplant lymphoproliferative disease (PTLD) accounted for more than one third of the transplant-related deaths. Four-year EFS and OS were rather low at 28% and 30%, respectively. Similar association of ATG and PTLD was demonstrated by others but remains controversial (Ponce et al. 2015; Brunstein et al. 2006).

Table 11.2 Umbilical cord blood HCT in patients with Hodgkin lymphoma

	Patients (*n*)	Prior auto (%)	Chemosensitive (%)	TRM 3 years (%)	Relapse (%)	PFS (%)	OS (%)
UMinn (Majhail et al. 2006)	21 (9 UCB)	78	77	N/A	N/A	25 (sib) *vs.* 20 (UCB) (2 years)	42 (sib) *vs.* 41 (UCB)
MD Anderson (Thompson et al. 2016)	27	100	x	38 (5 years)	30	31	n/a
GETH GITMO (Pinana et al. 2016)	30	90	x	30 (4 years)	25	28	30
UMinn (Hegerova et al. 2017)	72	82	90	16 *vs.* 6[a]	50 *vs.* 40	49 *vs.* 23	84 *vs.* 50

[a]2000–2008 *vs.* 2009–2013

We recently presented data on trends in presentation and transplant outcomes in 72 HL patients who received RIC allo-HCT and compared two transplant periods: before and after 2009 (52 *vs.* 20 patients) (Hegerova et al. 2017). The latter period coincides with the availability of novel CD30-targetting immunotoxin brentuximab vedotin. Grafts included UCB (56%) and HLA-matched sibling (32%). During the recent period, patients more often received brentuximab vedotin (60% *vs.* 2%) but also had less comorbidities (index 0: 60% *vs.* 12%) and more often attained complete remission pretransplant (50% *vs.* 23%). At median follow-up of 4 years, 3-year PFS significantly improved for patients treated after 2009 ($p = 0.01$) compared to the earlier time period, reflecting lower TRM and relapse rates in the recent era (Table 11.2). As previously shown, chemoresistance had the strongest adverse effect on OS; in addition, better OS occurred in the brentuximab treatment era with hazard ratio 0.24 (compared prior to 2009). Outcomes after sibling or UCB donor grafts were found to be similar. Importantly, this report demonstrated lower relapse rate and higher survival in patients with chronic GvHD, suggesting a graft-*versus*-HL effect.

In summary, recent studies suggest that in patients with relapsed/refractory HL, UCBT is feasible and results are comparable to HLA-matched donor allo-HCT, thus extending the alternative donor choice for patients who can benefit from allograft.

11.4 UCB Transplants in Non-Hodgkin Lymphoma

Allo-HCT remains the only curative modality for many patients with advanced lymphoma at this time. Indication for allo-HCT mainly includes relapsed follicular lymphoma, relapsed mantle cell lymphoma, advanced diffuse large B-cell lymphoma, and T-cell lymphoma. Suitable patients are expected to be in good health and prior to development of chemoresistance. Given the sustained and potent

graft-*versus*-lymphoma effect in non-Hodgkin lymphoma (NHL), the trends in transplant have shifted toward the use of RIC regimen. In 2009, Brunstein et al. reported outcomes of 65 patients with mature lymphoid malignancies who received UCBT after uniform RIC conditioning consisting of cyclophosphamide, fludarabine, and low-dose TBI followed by mycophenolate mofetil/cyclosporine GvHD prophylaxis (Brunstein et al. 2009). Median time to neutrophil recovery was 7.5 days, and cumulative incidence of grade II–IV acute GvHD was 57%. Chronic GvHD, however, was much less frequent (19%), and TRM was relatively low at 15%. Patients experienced promising 3-year PFS and OS of 49% and 55%. Data on relative efficacy of alternative donor HCT for adults with high-risk lymphoma were expanded by large retrospective registry studies. On behalf of CIBMTR, Bachanova et al. examined the use of UCB, 7/8 and 8/8 antigen-matched URD in almost 1600 NHL transplant recipients (Bachanova et al. 2015). While 3-year TRM was higher with 7/8 MUD (44%), it was similar between UCB and MUD (37% *vs.* 37%). It must be noted that UCB recipients had a 42% lower risk of acute and chronic GvHD as compared with URD groups. All three groups had comparable OS (43% matched URD, 37% 7/8 URD, and 41% UCB). While there was no difference in TRM, relapse, or PFS after transplantation between UCB and URD, UCB was associated with lower risk of chronic GvHD.

Similar data were obtained by EBMTR (Rodrigues et al. 2009, 2014). Rodrigues et al. used the Eurocord-Netcord database to analyze risk factors of outcomes after UCBT in adults with lymphoid malignancies (Rodrigues et al. 2009). They analyzed 104 adults with median age of 41 years who underwent UCBT using single- ($n = 78$) or double-unit grafts; two thirds were two-antigen mismatched. Diagnoses were NHL, HL, and chronic lymphocytic leukemia (only 14 patients). Incidences of TRM and relapse were 28% and 31% at 1 year. PFS at 1 year was 40% with improved survival in those with chemosensitive disease (49% *vs.* 34%), low-TBI containing conditioning (60% *vs.* 23%), and higher nucleated cell dose (49% *vs.* 21%). The EBMTR updated these data more recently and compared 645 adults with mature lymphoid malignancies after UCBT ($n = 104$) *versus* adult URD peripheral blood stem cells ($n = 541$) after a RIC regimen (Rodrigues et al. 2014). UCB recipients had more refractory disease. Consistent with findings from prior studies, chronic GvHD (26% *vs.* 52%; $p = 0.0005$) was less frequent after UCB than after HLA-matched URD, whereas no difference was observed in grades II–IV acute GvHD, TRM, relapse, PFS (43% *vs.* 58%), or OS (36% *vs.* 51%) at 3 years. Multivariate analysis (MVA) confirmed a high risk of poor neutrophil engraftment and twofold lower risk of chronic GvHD comparing UCB and sibling grafts; these results are remarkably consistent with the findings from CIBMTR.

These data demonstrate that successful allogeneic donor HCT can be available for all adult lymphoma patients, and lack of an HLA-matched donor should not be a barrier to an allograft. Our study supports prospective testing of UCBT in lymphoma and mandates wider access to alternative donor options.

11.5 UCB Transplants in Chronic Lymphocytic Leukemia

Experience using UCB in adults with chronic lymphocytic leukemia (CLL) is limited. At the University of Minnesota, we reported 26 consecutive allo-HCT recipients with CLL treated between 2000 and 2009 (McClune et al. 2012). Donors were either HLA-identical MRD ($n = 12$) or double UCB ($n = 14$) units matched at 4/6–6/6 HLA loci to recipients and to each other. All patients received cyclophosphamide (50 mg/kg intravenously day −6), fludarabine (40 mg/m^2 intravenously daily days −6 to −2), and total body irradiation (200 cGy day −1). Graft failure occurred in three (21%) UCB HCT cases and two sustained autologous recovery. OS at 3 years was 51% while PFS at 3 years was 38%; relapse rate at 3 years was 25%. Notably, UCB demonstrated a trend toward less chronic GvHD. These data indicate that allo-HCT can result in long-term remissions for heavily treated CLL patients using either MRD or UCB HCT; however, the risk of non-engraftment is higher in CLL compared to other malignant diseases and requires further study focused on understanding of the risk factors.

Conclusions

Identifying the patient with a high-risk phenotype to plan timely allo-HCT is critical in improving survival in adults with lymphoid malignancies. Particularly in high-risk ALL, initiation of a donor search while initial remission induction is underway can facilitate transplantation during CR1, when the long-term results are best. Finding a suitable matched sibling donor, or alternatively the choice between a well-matched URD adult and a UCB graft, can permit the curative potential of allogeneic transplantation. The data presented here suggest that UCB should be considered a valid alternative source of stem cells in the absence of a matched URD for adults with high-risk ALL and relapsed mature lymphoid malignancies. UCB can be particularly beneficial for urgent transplants such as high-risk ALL with MRD or high-risk NHL in partial remission. Disease control prior to allograft is critical, and novel therapies should be used to achieve this goal. Importantly, lower risk of GvHD and greater HLA mismatch in UCBT does not compromise the alloreactivity against lymphomas. As GvHD contributes to morbidity and mortality and can compromise the quality of life of long-term survivors, a lower risk of GvHD after UCB HCT may be an additional favorable feature influencing donor choice. UCB transplants were used more frequently for ethnic minorities since suitable UCB units mismatched in one or two HLA loci can provide a graft for 90–95% of patients with minority backgrounds, who are less likely to have a HLA-MUD (Ustun et al. 2014).

Decisions to use UCB HCT among other alternative donor choices should be tailored to individual patient risk, disease biology, age, expertise of the transplant center, and availability of clinical trials.

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Immune Reconstitution After Cord Blood Transplantation

12

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Definitions

ALC	Absolute lymphocyte count
CMV	Cytomegalovirus
CTL	Cytolytic T-cell
CTLA-4	Cytotoxic T-lymphocyte-associated (protein) 4
EBV	Epstein-Barr virus
FLT3	Fms-related tyrosine kinase 3
HHV-6	Human herpesvirus 6
HLA	Human lymphocyte antigen (system)
IFN-γ	Interferon-γ
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-7	Interleukin-7
IL-10	Interleukin-10
IL-15	Interleukin-15
IL-22	Interleukin-22
KGF	Keratinocyte growth factor

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_12

LHRH-A	Luteinizing hormone-releasing hormone analog
LMP	Lymphoid-myeloid progenitors
LPS	Lipopolysaccharide
NFAT	Nuclear factor of activated T-cells
NK	Natural killer
NSG	NOD SCID gamma (mouse strain)
RTE	Recent thymic emigrants
SCF	Stem cell factor
sIgM	Surface immunoglobulin M
Tc1/2	T cytotoxic 1/T cytotoxic 2
TEC	Thymic epithelial cells
TGF-β	Transforming growth factor-β
Th1/2	T helper 1/ T helper 2
TNF-α	Tumor necrosis factor-α
TREC	T-cell receptor excision circles
Treg	T regulatory
VZV	Varicella zoster virus

12.1 Unique Characteristics of Immune Cells in the UCB Graft

The unique composition and properties of cells contained in UCB units (Table 12.1) may account, at least in part, for the distinct patterns of hematopoietic and immune reconstitution after UCBT. UCB contains higher numbers of $CD4^+$ and $CD8^+$ T-cells

Table 12.1 Properties of hematopoietic cells in UCB grafts *versus* HSPC grafts from peripherally mobilized adult donor HSPC (pmHSPC)

	UCB	pmHSPC
Hematopoietic progenitors		
Absolute numbers of $CD34^+$ stem cells	Limited	Higher (by 1–2 logs)
Proportion of hematopoietic progenitors	Higher	Reduced
Proliferation of $CD34^+$ cells in response to cytokines	Higher	Reduced
Production of hematopoietic factors[a]	Reduced	Higher
Innate immune cells		
Dendritic cells		
Expression of MHC class II	Reduced	Higher
Expression of CD80/CD86	Reduced	Higher
Cytokine response to stimulation	Reduced	Higher
Plasmacytoid to myeloid ratio	Higher	Reduced
Induction of Treg differentiation	Higher	Reduced
B lymphocytes		
Proportion of naïve B-cells ($CD5^+CD23^-$)	Higher	Reduced
Ig class switching	Reduced	Higher

Table 12.1 (continued)

	UCB	pmHSPC
Susceptibility to apoptosis	Higher	Reduced
NK cells		
Proportion of $CD56^+$ NK-cell subsets	Comparable	
Proportion of $CD56^-$ NK-cell precursors	Higher	Reduced
Expression of granzyme B	Reduced	Higher
Expression of adhesion molecules	Reduced	Higher
NK-cell cytolytic activity	Reduced	Higher
Adoptive immune cells		
T lymphocytes		
Proportion of naïve $CD4^+CD45RA^+$ T-cells	Majority of T-cells	Mixed $CD45RA^+/CD45RO^+$ T-cells
T-cell responses to mitogens/alloantigens	Reduced/moderate	High/vigorous
Production of IFN-γ, TNF-α, IL-2 from activated T-cells	Reduced	Higher
Cytotoxic activity of alloactivated T-cells	Reduced	Higher
Expression of NFAT1	Reduced	Higher
Expression of CD40L	Reduced	Higher
Expression of perforin	Reduced	Higher
Expression of Fas L	Reduced	Higher
Bias toward Th2/Tc2 differentiation	Reduced	Higher
Treg cells		
Naïve subset	Higher	Reduced
Foxp3 expression	Comparable	
Suppression function	Reduced	Higher
CTLA4 expression after activation	Higher	Reduced
Expansion capacity after stimulation	Higher	Reduced

[a]G-CSF, GM-CSF, IL-3, M-CSF, TGF-β1, MIF-1α

compared to adult peripheral blood (PB) (Szabolcs et al. 2003), although the absolute counts of immune cells are lower compared to peripherally mobilized or bone marrow (BM) adult stem cell grafts (Storek 2008). In addition, the majority of UCB $CD4^+$ and $CD8^+$ T-cells are phenotypically naïve ($CD45RA^+CD62L^+$), in contrast to adult PB, which contains predominantly memory T-cells (Szabolcs et al. 2003; Chalmers et al. 1998). UCB T-cells produce less IL-2, IL-4, IFN-γ, and TNF-α in response to mitogens (Chalmers et al. 1998). The deficient production of cytokines by stimulated UCB T-cells may be due to the reduced expression of NFAT1 or NFAT-associated genes (Kadereit et al. 1999; Kaminski et al. 2003). While UCB T-cells display vigorous proliferation after allogeneic stimulation, they have decreased antigen-specific cytotoxicity (Risdon et al. 1994). This might be explained by aberrations in the Fas ligand pathway and the decreased perforin expression (Sato et al. 1999; Berthou et al. 1995). Despite an intrinsic Tc2-Th2 bias (Szabolcs and Niedzwiecki 2007), UCB T-cells can rapidly acquire Tc1/Th1 function and mediate enhanced antitumor effects compared to adult PB T-cells in a murine model of B-cell lymphoma (Hiwarkar et al. 2015).

UCB is enriched in $CD4^+CD25^+$ T regulatory cells (Tregs), which express Foxp3 and exhibit potent suppression function, independent of IL-10 or TGF-β (Godfrey et al. 2005). UCB Tregs are characterized by higher expression of the naïve markers CD45RA and CD38 and lower expression of activation markers CD45RO and HLA-DR in comparison to adult PB Tregs. Freshly isolated UCB Tregs are incapable of suppressing alloantigen-induced proliferation (Wing et al. 2003; Chang et al. 2005), a property that has been attributed to their naïve state, but can effectively suppress T-cell responses after stimulation (Wing et al. 2003; Takahata et al. 2004). Interestingly, after allogeneic stimulation, CTLA4 expression on Tregs derived from UCB $CD4^+CD25^-$ T-cells is induced at a higher rate compared to $CD4^+CD25^-$ T-cells derived from adult HSPC grafts (Chang et al. 2005). UCB Tregs also have a higher expansion capacity to anti-CD3, anti-CD28 and IL-2, which correlates with overexpression of genes involved in proliferation and chromatin remodeling (Torelli et al. 2012).

Similarly to T-cells, UCB B-cells are predominantly naïve and are characterized by the expression of CD5 and sIgM (B1 cells), whereas CD23-expressing B-cells are less frequent compared to adult PB (Szabolcs et al. 2003). These UCB B-cells can produce poly-reactive IgM antibodies but display decreased capacity for Ig class switching and production of IgA or IgG upon stimulation (Hayward and Lawton 1977; Durandy et al. 1990). Furthermore, UCB B-cells have increased susceptibility to spontaneous apoptosis (Kessel et al. 2006). $CD56^{bright}$ and $CD56^{dim}$ NK subsets are present at similar proportions in UCB and PB, although UCB also contains a significant population of NK progenitors, including $CD16^+CD56^-$ cells (Verneris and Miller 2009; Gaddy and Broxmeyer 1997; Gaddy et al. 1995). UCB NK cells have high-level expression of the inhibitory receptor NKG2A/CD94 and low-level expression of granzyme B, resulting in decreased NK-cell activity compared to adult NK cells (Wang et al. 2007). Decreased expression of adhesion molecules may also account for the decreased NK-cell activity (Tanaka et al. 2003). UCB dendritic cells (DCs) also display unique properties and are characterized by lower surface expression of major histocompatibility complex (MHC) and cell adhesion molecules (Hunt et al. 1994), decreased upregulation of costimulatory molecules or activation markers (CD80, CD86, CD86), and markedly decreased production of IL-12 in response to LPS stimulation (Langrish et al. 2002; Wong et al. 2005). Thus, UCB DCs are functionally immature with impaired ability to stimulate T-cell responses. Instead, UCB DCs efficiently induce $CD4^+$ T-cells with a Treg phenotype (Encabo et al. 2007). UCB is also characterized by a higher ratio of plasmacytoid to myeloid DCs compared to adult PB (Crespo et al. 2004). These observations support the notion that UCB DCs are biased against Th1 responses and toward induction of immune tolerance (Langrish et al. 2002; Wong et al. 2005; Crespo et al. 2004).

12.2 Mechanisms and Pathways of T-Cell Reconstitution After UCBT

While the innate immune system recovers within weeks after transplantation, the restoration of the adaptive T-cell immunity is delayed for several months. Recovery of T-cells after HSCT follows two different pathways, one *peripheral* or

thymus-independent and one *central* or *thymus-dependent pathway*. These two pathways of T-cell reconstitution act in parallel but follow different kinetics (Williams et al. 2007) (Fig. 12.1).

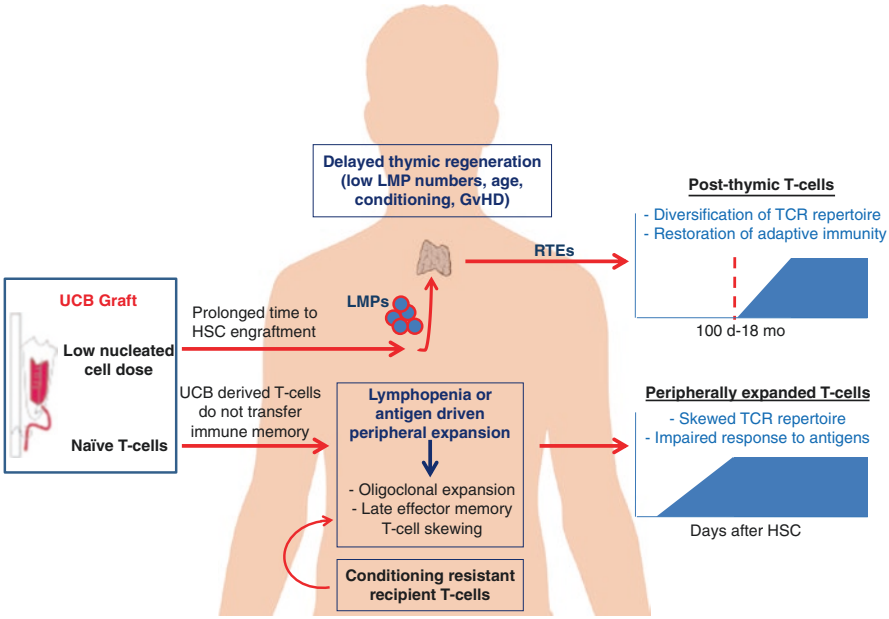

Fig. 12.1 Reconstitution of the T-cell compartment after UCBT. Conditioning chemoradiation prior to UCBT results in profound lymphopenia and immunodeficiency of the host. T-cell reconstitution after UCBT is achieved by two independent mechanisms: The thymus-independent pathway of T-cell reconstitution predominates in the early post transplant period and is mediated by adoptively transferred UCB T-cells, which are uniformly naïve and do not transfer protective immune memory, or recipient T-cells that survive conditioning. These T-cell populations undergo peripheral expansion in response to lymphopenia and high cytokine levels (IL-7, IL-15 etc), or oligoclonal proliferation upon interaction with cognate antigen. Overtime, this early peripheral T-cell expansion results in late effector memory T-cell skewing and contraction of the T-cell repertoire diversity, and is associated with impaired immunologic responses to antigens. Reconstitution of a functionally competent T-cell compartment with broad antigenic specificity eventually requires the de novo production of naïve T-cells by the thymus of the USCT recipient. This thymus-dependent pathway of T-cell reconstitution is a prolonged multistep process. LMPs contained in the UCB graft or arising from the engrafted donor-derived HSCs migrate via circulation and repopulate the thymus with thymocyte precursors that can reconstitute thymopoiesis. The thymus provides the essential microenvironment (stroma) that supports T-cell proliferation, selection and differentiation into Recent Thymic Emigrants. Several factors can delay the recovery of thymopoiesis after UCBT, including low number of LMPs (as a result of low nucleated cell dose of UCB and delayed engraftment), advanced recipient age with resultant thymic involution and thymic damage from the conditioning chemoradiation or GvHD. Although slow, the thymus-dependent mechanism is imperative for the renewal of peripheral T-cell pool and constant export of new naïve T-cells with broad TCR repertoire diversity, capable of responding to a great spectrum of antigens. (This research was originally published in Blood. Politikos I and Boussiotis VA. The role of thymus in T-cell immune reconstitution after umbilical cord blood transplantation. Blood. 2014; Vol 124:3201–3211. © the American Society of Hematology)

12.2.1 Peripheral or Thymus-Independent Pathway of T-Cell Reconstitution

In the early posttransplant period, the *thymus-independent pathway* predominates and is mediated by adoptively transferred donor T-cells contained in the graft or recipient T-cells surviving conditioning chemotherapy. These T-cells proliferate following antigen encounter (Mackall et al. 1996) or undergo lymphopenia-driven homeostatic peripheral expansion (HPE) through low-affinity interactions with self-peptide MHC complexes under the influence of homeostatic cytokines, which are highly elevated during this posttransplant period (Goldrath and Bevan 1999). Among these cytokines, IL-7 is a critical regulator of the thymus-independent pathway (Fry et al. 2001). Studies in HSCT recipients have shown that IL-7 levels rise in the posttransplant period and inversely correlate with T-cell counts, most likely due to decreased consumption (Bolotin et al. 1999; Dean et al. 2008). In double unit UCBT (dUCBT) recipients, IL-7 levels remain elevated for a prolonged time compared to recipients of adult donor HSPC, and inversely correlate with T-cell counts, especially $CD4^+$ T-cells (Politikos et al. 2015).

In contrast to BM or peripherally mobilized HSPC grafts that contain a large number of memory T-cells, UCB grafts contain T-cells that are almost exclusively naïve. As a consequence, UCB T-cells display a complete T-cell receptor (TCR) repertoire at birth (Garderet et al. 1998). However, they are subject to the stringent requirements of naïve T-cells during mounting of primary immune responses which cannot provide efficient protection, in contrast to memory T-cells which develop rapid antigen-specific recall responses. As a result, protection against pathogens during the early posttransplant period is impaired and depends on memory T-cells of host origin that survive conditioning chemotherapy (Chalandon et al. 2006). During HPE, UCB T-cells lose their naïve phenotype and acquire an effector or memory-like phenotype in an antigen-independent manner (Mackall et al. 1993; Cho et al. 2000). At the same time, antigen-driven proliferation of UCB T-cells circulating in the UCBT recipient results in oligoclonal expansion and contraction of TCR repertoire diversity. Moreover, apoptotic cell death of the activated T-cells during homeostatic activation and/or antigen exposure may lead to contraction of the T-cell compartment over time (Hakim et al. 1997). As a result of these concurrent mechanisms, the *thymus-independent pathway* cannot sustain a diverse TCR repertoire with broad antigenic specificity that ensures immune competence (Roux et al. 1996).

12.2.2 Central or Thymus-Dependent Pathway of T-Cell Reconstitution

The *central* or *thymus-dependent pathway* of T-cell recovery involves the de novo production of T-cells by the thymus, which is critical for successful long-term immune reconstitution (Politikos and Boussiotis 2014). This pathway generates naive T-cells with broad antigenic specificity, and it is essential for the restoration of TCR repertoire diversity (Roux et al. 2000; Dumont-Girard et al. 1998). Restoration

of thymopoiesis is a multistep process that begins with the seeding of the thymus with lymphoid-myeloid progenitors (LMPs) derived from the engrafted donor HSPCs. Upon entry in the thymic cortex, LMPs undergo T-cell lineage commitment under the influence of Notch signaling and interactions with cortical thymic epithelial cells (TECs) (De Smedt et al. 2002; Taghon et al. 2009). During intrathymic T-cell development, thymocytes undergo sequential rearrangement of the TCR beta (TCRB) and alpha (TCRA) loci and develop into double-positive (DP) thymocytes, which are subject to positive selection based on their ability to bind self-peptide/self-MHC complexes with appropriate affinity. Positively selected DP thymocytes continue their development into CD4 or CD8 single-positive (SP) thymocytes, migrate to the medulla via the upregulation of the chemokine receptor CCR7, and undergo negative selection by medullary TECs, which have the unique capability to promiscuously express tissue-restricted antigens (TSAs) under the influence of the transcription factor Aire (Anderson et al. 2002, 2005). The negative selection process ensures the elimination of self-reactive lymphocytes. The small number of T-cells that successfully proceed through these intrathymic steps is exported to the periphery and is termed recent thymic emigrants (RTEs).

12.3 Factors Influencing T-Cell Immune Reconstitution After UCBT

12.3.1 Graft-Related Factors

Several factors can affect the timing and efficiency of immune reconstitution after UCBT. *Graft-related factors* such as limited number of stem cells and lymphocytes, T-cell naivety, immaturity, and lack of memory T-cells have the greatest impact on the *thymus-independent pathway* of T-cell recovery. Specifically, total nucleated cell (TNC) and $CD3^+$ T-cell content in UCB units positively correlate with lymphocyte recovery in the early posttransplant period after UCBT (Niehues et al. 2001; Castillo et al. 2016). It is conceivable that the low stem cell dose of UCB grafts and the delayed engraftment result in delayed emergence of LMPs that repopulate the thymus and restore thymopoiesis. Thus, the properties of the UCB graft may also affect the *thymus-dependent pathway* of immune reconstitution. Interestingly, although the number of HSPC clones contributing to thymic seeding and intrathymic T-cell development is restricted, this very limited number of HSPC clones can give rise to a fully diverse TCR repertoire. Thus, immunodeficiency after UCBT may not be a consequence of low HSPC numbers but may rather be related to intrathymic events (Brugman et al. 2015). An additional important mechanism regulating immune reconstitution is related to the frequent HLA discordance between the UCB graft and the host, due to the less stringent requirements for HLA matching during UCBT. The intrathymic positive and negative selection is dictated by MHC expression of thymic epithelial cells, which remain of host origin after transplantation, in contrast to the donor-derived LMPs that repopulate the thymus (Krenger and Hollander 2008). As a result, MHC mismatches between graft and the host might

adversely impact immune reconstitution, by compromising thymic selection and leading to skewed TCR repertoire diversity (Roux et al. 2000; Krenger and Hollander 2008; Eyrich et al. 2003).

12.3.2 Host-Related Factors

Host-related factors also influence immune reconstitution. Recipient's age has a critical impact on T-cell reconstitution. Compared to adults, pediatric patients receive higher TNC and $CD3^+$ cell doses adjusted per body weight resulting in faster neutrophil engraftment and lymphocyte recovery. In addition, the decreased thymic function that occurs with aging as a result of thymic involution (Mackall et al. 1995; Douek et al. 1998) might pose a rate-limiting step in the immune reconstitution after HSCT. In support of this hypothesis, the recipient's age is inversely associated with the onset and extent of thymopoietic recovery after HSCT or UCBT (Klein et al. 2001; Clave et al. 2009; Uhlin et al. 2012). Furthermore, the pretransplant thymic function of the host plays a role in the rate of thymic recovery (Mackall et al. 1995; Chen et al. 2005; Clave et al. 2005).

12.3.3 Treatment-Related Factors

Factors related to the *transplantation procedure* or *posttransplant complications* may affect the rate and success of immune reconstitution. The use of *in vivo* T-cell depletion by means of ATG has a detrimental effect on the early *thymus-independent* T-cell recovery (Admiraal et al. 2015; Lindemans et al. 2014). A negative effect of ATG on the *thymus-dependent pathway* has also been implied (Sairafi et al. 2012). Reduced intensity conditioning (RIC) regimens may provoke less damage to the peripheral niches in which T-cell proliferation takes place, thus allowing for a faster immune recovery (Uhlin et al. 2012; Sairafi et al. 2012; Chao et al. 2002). Furthermore, radiation-based conditioning regimens may lead to delayed *thymus-dependent* T-cell reconstitution, likely by inducing greater thymic destruction (Glowala-Kosinska et al. 2016; Chung et al. 2001). It should also be noted that the thymus is a target organ of graft-*versus*-host disease (GvHD), which affects both its lymphoid and stromal compartments (Krenger and Hollander 2008). Importantly, the development of acute and chronic GvHD has been associated with delayed T-cell reconstitution (Clave et al. 2009; Uhlin et al. 2012; Weinberg et al. 2001) not only because the thymus is a target organ of GvHD, but also because corticosteroid therapy for the treatment of GvHD can lead to lymphodepletion.

12.4 Assessment of Immune Reconstitution After UCBT

Several tools are available for the study of immune recovery after transplantation, both in the clinical or research settings. Hemograms provide an assessment of myeloid engraftment and quantitative recovery of lymphocytes by measurement of

absolute lymphocyte count (ALC). Flow cytometry provides a means for the enumeration of lymphocyte subsets and monitoring of $CD4^+$, $CD8^+$, B, and NK cells is commonly used in the clinical practice to guide decisions regarding the management of transplant recipients, such as discontinuation of prophylactic antiviral therapy and timing of vaccination. Flow cytometry is also a valuable tool for the study of thymopoieisis because it allows quantification of RTEs based on the expression of the CD45 isoform, CD45RA (Mackall et al. 1993). Additional surface markers, such as CCR7, CD31, CD62L, and CD27, have improved the differentiation between naïve and memory T-cells (McFarland et al. 2000). Quantification of T-cell receptor excision circles (TRECs), which are DNA by-products of the intrathymic TCR sequential rearrangement events, also provides an excellent assessment of the thymic function (Politikos and Boussiotis 2014; Douek et al. 1998). Additionally, because diversification of the T-cell repertoire after HSCT depends on the *de novo* production of naïve T-cells, assessment of the TCR diversity by CDR3 spectratyping or by sequencing of the TCR β chain can also provide information about the ability of thymopoiesis to reconstitute a competent T-cell immune system (Douek et al. 2000; Robins et al. 2009). Because these assays provide only quantitative assessment, approaches to evaluate pathogen-specific T-cell responses such as cytokine flow cytometry (CFC) or enzyme-linked immunosorbent spot (ELISpot) are valuable tools for the assessment of immune competence.

12.5 Quantitative Immune Reconstitution After UCBT

12.5.1 Immune Reconstitution in Adults After Single-Unit UCBT

Adult patients undergoing single-unit CBT (sUCBT) after ATG-containing myeloablative conditioning (MAC) have delayed neutrophil engraftment and slower ALC recovery during the first 2 months after transplantation compared to matched unrelated donor (MUD) transplant recipients. However, ALC recovery in the sUCBT group surpassed that of MUD recipients beyond day 180 and reached normal range by day +200. While bacterial infections were more common in the sUCBT group early posttransplant, there was no significant difference in the infection rates between the two groups beyond day 50 (Hamza et al. 2004). However, adult patients who receive sUCBT after ATG-containing conditioning may have protracted lymphopenia for up to 2 years posttransplant (Klein et al. 2001). This is most pronounced for the $CD4^+$ T-cells, which remain below normal range until 2 years, whereas $CD8^+$ T-cells reach low normal levels by 1 year. The kinetics of T-cell recovery in adult patients is markedly delayed compared to pediatric UCBT recipients. Moreover, the majority of T-cells during the first year have a memory phenotype.

An important observation is that TREC levels in adult sUCBT recipients remain below the age-adjusted normal range for up to 3 years after transplantation, suggesting a profound thymic deficiency compared to the pediatric control group (Klein et al. 2001). This finding explains, at least in part, the protracted delay in T-cell recovery. Similarly, sUCBT after ATG-containing RIC is associated with extremely

suppressed $CD4^+$ and $CD8^+$ numbers at least through 6 months post-UCBT (Komanduri et al. 2007). Under these conditions, $CD8^+$ T-cells approach near-normal levels at 1 year after transplantation, but $CD4^+$ T-cells remain low. Thymopoiesis, as assessed by sjTRECs, is nearly absent in the first year and is associated with a paucity of naïve and central memory T-cells and expansion of terminally differentiated T effector cells. In contrast, NK and B-cells develop a marked expansion to supranormal levels, as early as 30 days post-UCBT.

12.5.2 Immune Reconstitution in Adults After Double-Unit UCBT

The use of dUCBT has been employed to circumvent the cell dose limitation in adult patients and to enhance hematopoietic and immune reconstitution. Analysis of immune reconstitution in adult patients undergoing dUCBT (Cutler et al. 2011) and comparison between dUCB and peripherally mobilized HSPC MUD transplantation after RIC (Jacobson et al. 2012) revealed that $CD3^+$, naïve and memory $CD4^+$, $CD8^+$, and $CD4^+CD25^+$ Treg numbers were significantly lower in dUCBT recipients during the first 6 months after transplantation. The inclusion of ATG only in the dUCBT group may have contributed to this observation. By 12 months, no significant difference was observed between the two groups, and, by 24 months, the median number of naïve $CD4^+$ T-cells in the dUCBT cohort surpassed that in the MUD cohort. In addition, dUCBT recipients seemed to have higher $CD123^+$ plasmacytoid DCs in the first year compared to MUD, but the difference disappeared at later time points. Infections were more frequent in the dUCBT group, but the nonrelapse mortality (NRM) was not different (Jacobson et al. 2012). In addition, ATG may also cause a delay in the emergence of IFN-γ-secreting T-cells in response to immunogenic viral antigens, suggesting that the early post-UCBT period after ATG-containing conditioning is characterized not only by quantitative but also by qualitative/functional immunologic defect in adaptive immunity (Saliba et al. 2015).

Comparison of immune reconstitution patterns between adult patients undergoing dUCBT and patients undergoing HSCT from matched sibling donors (MSD) or HSCT from MUD after ATG-free MAC regimens has provided evidence that ALC is lower in dUCBT recipients at 1.5 months but reaches similar levels to those in MSD/MUD recipients by 3 months (Kanda et al. 2012). $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+CD25^+CD62L^+$ (Treg), and $CD4^+CD45RA^+CD62L^+$, which represent RTE counts, are significantly lower after dUCBT during the first 3–6 months, but at 1 year after transplantation, there is no difference in T-cell subsets between dUCBT and MSD/MUD recipients. As a result of the delayed T-cell recovery, dUCBT recipients have a significantly higher incidence of CMV reactivation and CMV disease, compared to MSD/MUD recipients. Notably, at 6 months after transplantation, an increase in TRECs was comparable in dUCBT and MSD/MRD recipients. Moreover, at 12 months, TCRβ repertoire was comparable between the two cohorts. A vigorous recovery of B and NK cells was detected in the dUCBT cohort in this study (Kanda et al. 2012), consistent with previous observations (Jacobson et al. 2012; Brown et al. 2010). UCBT is mainly reserved for patients who lack an MSD or

MUD but may have mismatched unrelated donors (MMUD). Comparison between recipients of UCBT and 9/10 MMUD HSCT, after various conditioning regimens, showed largely similar kinetics of lymphocyte recovery and infectious complications (Servais et al. 2014).

Memorial Sloan Kettering Cancer Center (MSKCC) has reported their experience in adult patients after ATG-free dUCBT and RIC to high-intensity MAC (Sauter et al. 2011). Median ALC was within normal range by day 60. Median $CD4^+$ T-cell count >200 cells/uL was reached by day 120, and both $CD4^+$ and $CD8^+$ median counts were at the lower limit of normal range by day 180. The infectious risk was heightened during the first 4 months, especially in the context of GvHD, but no infection-related deaths were seen beyond that time point. The use of deep sequencing technology to assess TCR diversity showed that dUCBT recipients have the highest $CD4^+$ and $CD8^+$ TCR diversity compared to recipients of conventional or T-cell-depleted (TCD) HSCT at 6 and 12 months after transplantation (van Heijst et al. 2013). This increased TCR diversity correlated with a substantially greater fraction of naive $CD4^+$ T-cells. An important observation of the study is that diversity of $CD4^+$ T-cells was 50 times higher than that of $CD8^+$ T-cells in all patients. Notably, acute GvHD and steroid therapy did not restrict repertoire recovery, whereas CMV or EBV infections had a negative impact.

An important benefit of double UCB unit infusion is the recovery of thymopoiesis, as assessed by increasing TREC levels, by 6 months post-UCBT. This is in marked contrast to the thymopoietic failure seen in adult patients who receive single UCB unit infusion, especially after *in vivo* T-cell depletion by ATG (Komanduri et al. 2007). Early thymopoietic recovery is observed in adult dUCBT recipients both after ATG-containing RIC or MAC regimens (Politikos et al. 2015; Brown et al. 2010; Ballen et al. 2012). In both groups, more than half of the patients had detectable, yet below normal range, TRECs at 6 months. By 1 year, median TREC levels reached low normal values. Interestingly, circulating levels of SCF inversely correlate with TRECs at 6 and 12 months post-dUCBT, as well as $CD4^+CD45RA^+$ naïve T-cells at 1 year (Politikos et al. 2015). This outcome is likely due to consumption of SCF in the early intrathymic stages of T-cell development, consistent with the critical role of this cytokine in the *thymus-dependent* pathway of immune reconstitution.

12.6 Prognostic Significance of Immune Reconstitution for Clinical Outcomes After UCBT

As a result of the delayed neutrophil engraftment and the lack of adoptive immunoprotection due to the naivety of UCB lymphocytes, UCBT is associated with increased risk of infectious complications, which contribute greatly to the early transplant related mortality (TRM) after UCBT (Rubinstein et al. 1998; Laughlin et al. 2004; Rocha et al. 2004). Reactivation of latent viruses that require intact cellular immunity for their control is a major concern after UCBT. Among them, CMV is one of the most commonly reactivated viruses and is associated with

significant morbidity and mortality (Szabolcs and Niedzwiecki 2007; Sauter et al. 2011; Dahi et al. 2015; Beck et al. 2010). The rate of CMV reactivation after UCBT ranges widely from 21 to 100% and the rate of CMV disease between 6 and 27% (Beck et al. 2010; Milano et al. 2011; Matsumura et al. 2007; Takami et al. 2005) depending on the characteristics of the study population, such as the number of seropositive patients at risk for reactivation (Albano et al. 2006; Mikulska et al. 2012); method of monitoring CMV reactivation, e.g., pp65 antigenemia *vs.* PCR viral load monitoring (Dahi et al. 2015; Milano et al. 2011); type of prophylaxis; and use of preemptive treatment (Milano et al. 2011). With the use of sensitive PCR assays essentially all seropositive UCBT recipients develop CMV viremia, although this can be mitigated with intensified antiviral prophylaxis (Dahi et al. 2015; Milano et al. 2011). Notably, the omission of *in vivo* T-cell depletion by ATG appears to provide protection against CMV reactivation or CMV disease after UCBT (Sauter et al. 2011; Dahi et al. 2015).

Similarly to CMV reactivation, the use of ATG is associated with increased risk of EBV reactivation and an incidence of post-transplant lymphoproliferative disorder (PTLD) as high as 21% (Cutler et al. 2011; Brunstein et al. 2006; Dumas et al. 2013). The risk can be greatly reduced by the avoidance of ATG, close monitoring of EBV viral load, and preemptive therapy with rituximab (Dumas et al. 2013; Blaes et al. 2010; Barker et al. 2001). An increased frequency of HHV-6 reactivation and related complications has also been observed after UCBT (Scheurer et al. 2013; Hill et al. 2012, 2015; Le Bourgeois et al. 2014), although the incidence of encephalitis appears to be low when ATG is omitted (Olson et al. 2014). Similarly, UCBT is associated with increased rate and severity of VZV disease (Vandenbosch et al. 2008), BK viremia, and increased risk of BKV disease (Rorije et al. 2014; Satyanarayana et al. 2015; Silva Lde et al. 2010), as well as higher risk for disseminated adenovirus infection (Robin et al. 2007).

The increased risk for infectious complications in UCBT recipients is most pronounced in the early posttransplant period, as studies have shown comparable infection-related mortality in recipients of UCBT and HSCT from adult donors beyond the first 3–4 months (Sauter et al. 2011; Laughlin et al. 2004; Parody et al. 2006; Barker et al. 2005a). The restoration of adaptive immunity via the *thymus-dependent* pathway, as well as the lower rates of GvHD, may account for this observation. Furthermore, dUCBT has been associated with enhanced GvL effect and low relapse risk (Ponce et al. 2011; Verneris et al. 2009; Brunstein et al. 2010; Rodrigues et al. 2009). As a result, long-term outcomes of UCBT are comparable to HSCT from conventional graft sources (Ponce et al. 2011).

The prognostic effect and the pathophysiologic link between different immune reconstitution metrics on the clinical outcomes of UCBT have been extensively investigated both in pediatric and adult patients undergoing UCBT. It has been determined that ALC > 200 × 10^6/L at day 30 after MAC and at day 42 after RIC is independently associated with superior 2-year overall survival (OS) and progression-free survival (PFS) (Burke et al. 2011). For the MAC cohort, ALC > 200 × 10^6/L at day 30 was also associated with lower number of infections prior to day 30 and lower TRM. However, no significant relationship between ALC and relapse has

been observed. An ALC > 150 × 10^6/L on day 30 after dUCBT is also highly predictive of TRM and OS (Saliba et al. 2015). However, no difference between patients who did or did not develop viral reactivations or infections was identified. Because ALC offers only a crude assessment of immune reconstitution, other studies have focused on lymphocyte subsets to identify the most clinically relevant cell types for successful transplantation outcomes. Among such subsets, $CD4^+$ T-cells are of great importance, and their impact on TRM and survival outcomes after allo-HCT from conventional donors (Berger et al. 2008) or sUCBT in children (Admiraal et al. 2015; Bartelink et al. 2013) has been established. Furthermore, in adult recipients of sUCBT, it has been observed that preservation of $CCR7^+$ T-cells, which identify naïve and central memory T-cell subsets within the $CD4^+$ T-cell compartment, has been associated with significantly longer survival (Komanduri et al. 2007).

The most objective measure of successful immune reconstitution is the ability of the new immune system to provide protection against infections and leukemia relapse. The presence of a proliferative response against herpes viruses has been associated with decreased risk of infection-related death, but is also associated with marked reduction in leukemia relapse, and improved PFS (Parkman et al. 2006). Detection of CMV-specific $CD4^+$ and $CD8^+$ T-cells by CFC in the early post-UCBT period (Komanduri et al. 2007; McGoldrick et al. 2013) suggests that UCB-derived T-cells are primed early after transplantation and can initiate a primary immune response against CMV. However, the development of antigen-specific T-cell responses does not correlate with the recovery of ALC and lymphocyte subsets (Saliba et al. 2015; Cohen et al. 2006), suggesting that quantitative T-cell recovery is not an optimal surrogate of functional immunity after transplantation. Moreover, CMV-specific T-cells detected early after UCBT fail to control viral reactivation *in vivo* (McGoldrick et al. 2013).

Our group has examined the immunological mechanisms involved in the restoration of successful anti-CMV immunity and control of CMV viremia in a cohort of dUCBT recipients (Brown et al. 2010). CMV-specific effectors were detected by IFN-γ ELISpot assay as early as 8 weeks posttransplant. In spite of the presence of CMV-specific effectors, clearance of CMV viremia was not achieved at this early time point after transplantation but was increasingly observed after 6 months. Notably, clearance of CMV was associated with the recovery of naïve $CD4^+CD45RA^+$ T-cells, a finding consistent with the need of $CD4^+$ T-helper cells for the development of a functional $CD8^+$ T-cell response (Shedlock and Shen 2003). Clearance of CMV viremia was associated with the emergence of thymopoiesis as assessed by TREC levels, and patients who attained normal TREC levels (>2000 copies/ug) were more likely to display absence of CMV viremia (Brown et al. 2010). These findings suggest a critical contribution of the thymopoiesis on $CD4^+$ T-cell recovery, anti-CMV immunity, and *in vivo* control of the virus. Further analysis was performed to examine whether the development of CMV-specific responses and thymic reconstitution might be linked to improved long-term outcomes after dUCBT, which might correlate with restoration of immune responses against other pathogens or tumor antigens. CMV-specific immune responses were associated with improved PFS and OS. Furthermore, patients with TREC levels >2000 copies/ug DNA had

superior OS compared with patients whose TRECs remained below 2000 copies/ug (Brown et al. 2010).

These observations were extended in a subsequent study, which assessed the role of thymic reconstitution on long-term outcomes in adult dUCBT recipients. In time-dependent regression analysis, higher TREC levels were associated with lower TRM and superior PFS and OS (Politikos et al. 2015). In contrast, high plasma levels of SCF, a cytokine critically involved in the early intrathymic T-cell development, inversely correlated with TREC levels and predicted for higher TRM and worse OS. No association of TREC levels with relapse was detected. This is in contrast to a study in pediatric recipients of UCBT or haploidentical HSCT, in which low levels of βTREC and sjTRECs before transplantation and at 3–6 months posttransplant were associated with increased incidence of relapse (Clave et al. 2013). These discrepancies between pediatric and adult recipients of UCBT might be related to an age-dependent effect on the *thymus-dependent* pathway of immune reconstitution and suggest that impaired thymic reconstitution might have a stronger negative prognostic value for the prediction of long-term clinical outcomes in pediatric than in adult UCBT recipients.

12.7 Expert Point of View

UCB is a valuable source of HSPC and has extended allograft availability to patients who lack suitable adult donors. UCB grafts contain a limited number of stem cells and lymphocytes with unique proliferative and functional properties compared to their adult counterparts. As a result of the UCB composition and transplantation practices, immune reconstitution following UCBT is characterized by the absence of transferred immune memory and prolonged T-cell lymphopenia, with compensatory marked expansion of NK and B-cells in the early posttransplant period. The omission of pharmacologic *in vivo* T-cell depletion from the conditioning regimen has led to faster quantitative recovery during the *thymus-independent* phase of immune reconstitution. However, increased infectious risk and TRM remain a concern early post-UCBT. Recovery of thymopoiesis is critical for the restoration of peripheral T-cell compartment and diversification of T-cell repertoire. The kinetics and efficiency of the *thymus-dependent* immune reconstitution after sUCBT in children and dUCBT in adults appear to be at least as efficient as that observed after allo-HCT from adult donors and provide excellent reconstitution of long-term T-cell immunity. Whether this is due to inherent properties of UCB-derived lymphoid progenitors or due to the lower rates and severity of GvHD, thereby diminishing the immunosuppression burden, remains unknown. Several measures of quantitative, thymus-specific, and pathogen-specific recovery correlate with infectious complications and long-term clinical outcomes of UCBT and have allowed the identification of patients at greatest risk for poor outcome after UCBT. Newer technologies, such as deep sequencing, may allow a global and more comprehensive assessment of the immune system. Future efforts should focus on the development of strategies to enhance the immune reconstitution process in order to improve outcomes of UCB transplantation.

12.8 Future Directions

Because immune reconstitution has a significant impact on morbidity and mortality at the early posttransplant stage but also on long-term clinical outcomes of UCBT, several approaches have been developed to shorten the period of immune deficiency and to improve reconstitution of adaptive immunity. The use of two UCB units in adults resulted in improved recovery of innate immunity, by enhancing neutrophil engraftment and reducing the risk of graft failure (Barker et al. 2005b; Ballen et al. 2007). Moreover, dUCBT may lead to faster thymic reconstitution compared to sUCBT (Komanduri et al. 2007; Kanda et al. 2012; Brown et al. 2010). This outcome might be related to a cell-dose effect or yet unidentified mechanisms, considering that the majority of patients display single-unit chimerism within 1–3 months post-dUCBT (Barker et al. 2005b) and long-term hematopoiesis and lymphopoiesis by a single UCB unit.

Several groups have investigated methods to reduce the duration of neutropenia after UCBT, either by *ex vivo* culture of the UCB graft to increase the stem cell dose or by *ex vivo* priming of UCB grafts to improve homing (Cutler et al. 2013; Delaney et al. 2010; de Lima et al. 2012; Wagner et al. 2016; Popat et al. 2015). Most of these strategies have been tested in the setting of dUCBT platform, in which patients receive one manipulated and one unmanipulated UCB unit. While these approaches have resulted in faster recovery of neutrophils derived from the ex vivo-manipulated UCB unit, long-term hematopoietic engraftment is provided by the unmanipulated unit. Moreover, a beneficial effect on reconstitution of adaptive immunity has not been observed to date.

Cytokine-based approaches to improve posttransplant immune reconstitution are being investigated in preclinical models or early clinical trials, albeit not yet in the setting of UCBT. KGF is a mitogen of TECs and has been shown to enhance thymopoiesis and peripheral T-cell reconstitution by protecting TECs from damage induced by radiation, chemotherapy, or GvHD (Min et al. 2002). A beneficial effect of KGF administration has been observed in murine experimental UCBT models (Wang et al. 2011). However, in human trials, administration of KGF has not shown a discernible benefit on immune recovery (Rizwan et al. 2011). IL-7 is a lymphopoietic cytokine and in mouse studies of allogeneic BMT it has been shown to promote both the *thymus-dependent* and *thymus-independent* pathways of T-cell regeneration, as well as B-cell recovery (Mackall et al. 2001). Administration of rhIL-7 has been tested in a phase I trial of patients undergoing T-cell-depleted HSCT and resulted in expansion of effector memory T-cells, increase of virus-specific T-cell responses, and broader TCR diversity (Perales et al. 2012). Sex steroid ablation with LHRH-A in murine BMT models is associated with increased numbers of LMPs in the bone marrow and enhanced peripheral T-cell recovery, perhaps by reversing thymic atrophy (Goldberg et al. 2009). In humans undergoing autologous or allogeneic transplantation, LHRH-A administration before transplant resulted in enhanced naïve and total $CD4^+$ T-cell recovery, diversification of T-cell repertoire, and improved T-cell function (Sutherland et al. 2008). Other candidate cytokines include FLT3L (Fry et al. 2004), IL-15 (Alpdogan et al. 2005), IL-22 (Dudakov et al. 2012),

SCF (Wils et al. 2011), and several combinations, which often display synergistic effects (Wils et al. 2012; Kelly et al. 2008). These approaches may form the basis of new clinical trials that might improve reconstitution of adoptive immunity and provide clinical benefit in UCBT recipients.

A different approach to overcome immunodeficiency due to delayed immune reconstitution after UCBT involves the adoptive transfer of cell products that can confer protection against pathogens. In that regard, multi-specific cytotoxic T lymphocytes (CTLs) recognizing multiple viruses including CMV, EBV, HHV-6, BK, and adenovirus can be generated from UCB T-cell populations (Papadopoulou et al. 2014). These multi-specific CTLs may be highly beneficial for the prevention or treatment of viral disease in UCBT recipients. The use of third party, partially HLA matched, *in vitro*-expanded EBV-specific CTLs have already been used with success in the treatment of post-UCBT EBV-associated PTLD (Barker et al. 2010). This "off-the-shelf" approach, which is currently being extended to the generation of CTL with specificity for other pathogens, might be particularly beneficial to UCBT recipients, who cannot rely on donor-derived CTLs for transfer of protective immunity. Adoptive transfer of UCB NK cells to prevent relapse is also being evaluated in early studies (Verneris and Miller 2009). As mentioned above, a major complication of UCBT is the prolonged impairment of thymic function. Recently, it was discovered that human progenitor T-cells, differentiated *in vitro* from UCB stem cells with the use of OP9-DL1 culture system, could be a source of thymus-seeding progenitors when infused in NSG mice. Importantly, a subset of *in vitro*-derived progenitor human T-cell populations could also restore thymic architecture and, when co-infused with HSPCs, were able to promote HSPC-derived T-cell lymphopoiesis (Awong et al. 2013). Together these ongoing research approaches are expected to form the basis for the development of new clinical trials. Such novel strategies to improve either the thymus-dependent or thymus-independent pathways of immune reconstitution may further enhance the outcomes of UCB transplantation.

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Cord Blood Therapies for Genetic and Acquired Brain Injuries

13

Jessica M. Sun and Joanne Kurtzberg

Over the past two decades, human umbilical cord blood (CB) has emerged as an alternative source of hematopoietic stem cells for use in hematopoietic stem cell transplantation (HSCT). It is readily available, can be collected without risk to the mother or infant donor, and is significantly less likely to transmit infectious diseases transmissible through the blood. In addition, it can be transplanted across partially mismatched HLA barriers and is less likely to cause acute and chronic graft-*versus*-host disease (GvHD) than allogeneic bone marrow transplantation. CB has also recently been identified as a promising source of cells for use in novel cellular therapies. This chapter will focus on the use of CB therapies for neurologic conditions, including inherited metabolic disorders (IMDs) of childhood and emerging applications in the treatment of acquired brain injuries.

13.1 Hematopoietic Stem Cell Transplant as a Treatment for Inherited Metabolic Disorders

The inherited metabolic disorders (IMDs) are a heterogeneous group of genetic diseases. In most of these diseases, a single gene mutation causes an enzyme defect, which leads to the accumulation of substrates that are toxic and/or interfere with normal cellular function. Many affected patients appear normal at birth. During infancy, however, they begin to exhibit disease manifestations, often including progressive neurological deterioration associated with absent or abnormal brain myelination. The ultimate result is death in later infancy or childhood.

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_13

In the 1960s, Elizabeth Neufeld demonstrated that co-culture of fibroblasts from patients with two different IMDs (Hunter syndrome and Hurler syndrome) cross-corrected each other (Fratantoni et al. 1968), establishing the basis for enzyme replacement therapy (ERT) and for utilizing cellular therapy for that purpose. While ERT is available for selected IMDs and can be effective in ameliorating certain systemic disease manifestations, it does not effectively cross the blood-brain barrier and therefore does not alter the progression of neurologic symptoms (Shull et al. 1994; Tokic et al. 2007). Intrathecal ERT (Dickson et al. 2015) and gene therapy (Biffi et al. 2013) are currently being investigated to attempt to address this shortcoming. Currently, however, the only effective therapy to halt neurologic progression of disease is allogeneic HCT, which serves as a source of permanent cellular ERT (Krivit et al. 1999).

Following successful HCT, engrafted donor-derived hematopoietic stem cells serve as a constant endogenous source of the missing enzyme throughout the body, including the peripheral tissues and the central nervous system (Di Ferrante et al. 1971; Knudson et al. 1971). Donor microglia cells of the brain, which are of myeloid origin, are thought to be the source of such ERT after HCT (Krivit et al. 1995). These donor-derived cells not only act as normal scavengers in the central nervous system but they also secrete a portion of their lysosomal enzymes that can then be taken up by neighboring cells, thereby cross-correcting the metabolic defect in affected host cells (Krivit et al. 1995; Unger et al. 1993; Neufeld 2001). It is also possible that donor cells exert anti-inflammatory and pro-neurogenic effects through paracrine signaling. The timing of migration to, and engraftment of, donor-derived microglia cells in the brain after HCT is not known. Based on clinical observations, however, it is likely several months following hematologic engraftment.

13.2 Mucopolysaccharidoses

The mucopolysaccharidoses (MPS) consist of seven distinct clinical syndromes and numerous subtypes with a wide spectrum of clinical manifestations, representing approximately 35% of all lysosomal storage diseases. In these conditions, progressive accumulation of incompletely degraded glycosaminoglycans (previously called mucopolysaccharides) in lysosomes leads to disease manifestations including psychomotor retardation, musculoskeletal manifestations, vision and hearing impairment, and life-threatening cardiopulmonary failure.

The first HCT for a lysosomal storage disease was performed in 1980 in a 1-year-old child with Hurler syndrome (MPS1) using bone marrow from his parents (Hobbs et al. 1981). Since then, more than 500 HCTs have been performed worldwide in patients with Hurler syndrome, making it the most transplanted IMD (see Fig. 13.1). Numerous reports have demonstrated the efficacy of both bone marrow and CB transplantation in Hurler syndrome including improvements in neurocognitive function, joint integrity, motor development, growth, hydrocephalus, corneal

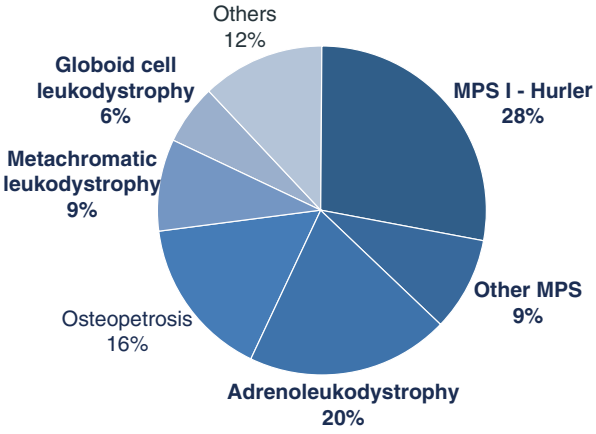

Fig. 13.1 *HCT in IMDs.* Frequencies reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) from 2000 to 2013 (CIBMTR 2014)

clouding, cardiac function, hepatosplenomegaly, hearing, visual and auditory processing, and overall survival (Staba et al. 2004; Peters et al. 1998; Souillet et al. 2003; Bjoraker et al. 2006; Boelens et al. 2007; Aldenhoven et al. 2008, 2015a). Despite improvements in both symptomatology and life expectancy, survivors still experience a variable degree of residual disease burden (Aldenhoven et al. 2015b). Factors associated with superior clinical outcomes include transplantation early in the course of the disease and the ability to attain full-donor chimerism and normal enzyme levels posttransplant (Aldenhoven et al. 2015b; Boelens et al. 2013).

In addition to CB's ready availability and more liberal HLA matching criteria, recent studies demonstrate that CB has additional advantages in the transplantation of MPS. Since most MPS are inherited in an autosomal recessive fashion, many related donors are heterozygous carriers and therefore have lower than normal levels of the affected enzyme. When CB is used as the donor source, potential units are screened and only used if they are noncarriers. A recent retrospective European Group for Blood and Marrow Transplantation (EBMT) study analyzed risk factors for graft failure in 146 HS patients (Boelens et al. 2007). While this study showed no significant difference in survival and engraftment between the use of CB, bone marrow, and peripheral blood stem cells, significantly more patients receiving CB achieved full-donor chimerism (93% *versus* 67%) and normal enzyme levels (100% *versus* 72%) compared to patients receiving bone marrow or peripheral blood stem cells. Other studies using CB as a cell source also showed high incidences of full-donor chimerism and normal enzyme levels in Hurler syndrome patients, (Staba et al. 2004; Church et al. 2007; Martin et al. 2006; Prasad et al. 2008) as well as in other lysosomal storage diseases (Martin et al. 2006; Prasad et al. 2008; Escolar et al. 2005). Among patients receiving CBT for Hurler syndrome, a shorter interval between diagnosis and CBT (<4.6 months 82% *versus* >4.6 months 57%) and a

conditioning regimen containing busulfan and cyclophosphamide (busulfan/cyclophosphamide 75% *versus* other 44%) were associated with a significantly higher event-free survival (Boelens et al. 2009).

Based on these observations, the EBMT developed transplantation guidelines for HCT in MPS patients in 2005. In these guidelines, CB was prioritized as a donor source in the absence of a noncarrier-matched sibling or fully matched unrelated donor, and myeloablative conditioning with busulfan/cyclophosphamide (later replaced with busulfan/fludarabine) with exposure-targeted intravenous busulfan was recommended. Since the guidelines were instituted, transplant outcomes in patients with Hurler syndrome have improved significantly. Engrafted survival rates are now 95%, with low transplant-related toxicity (Aldenhoven et al. 2015a). As a result, fully matched CB grafts are now considered one of the most appealing cell sources, if not the most appealing cell source, for HCT in patients with MPS.

A recent international, multicenter study of the long-term outcome of 217 patients with Hurler syndrome with a median of 9 years of post-HCT follow-up again demonstrated increased life expectancy for decades after transplantation with overall improvement in neurodevelopmental, cardiac, and other organ systems, though most patients continued to develop orthopedic complications (Aldenhoven et al. 2015b). Cognitive function was preserved/normalized in patients transplanted in the first year of life who had normal to mildly impaired cognitive function at the time of transplant, confirming that early transplantation is crucial. Normal post-transplant enzyme level was a predictor of superior long-term outcome in most organ systems. Thus, the use of noncarrier donors and achievement of full-donor chimerism were recommended. As CB is readily available and is known to result in high rates of full-donor chimerism and normal enzyme levels, it was identified as an attractive source for HCT in Hurler syndrome in this long-term study.

HCT in other MPS types has also been performed in small numbers and suggests benefit in Hunter and Sanfilippo syndromes (Prasad et al. 2008; Tanaka et al. 2012). Outcomes in other series have been variable, raising the possibility that some MPS diseases may be more responsive to HCT than others (Martin et al. 2006; Boelens 2006; Annibali et al. 2013; Guffon et al. 2009; Jester et al. 2013; Peters and Steward 2003; Vellodi et al. 1992, 1999). Lessons learned from the HCT experience in Hurler syndrome and the ability to achieve high rates of engraftment and survival may help inform investigations in other MPS (sub)types.

13.3 Leukodystrophies

The leukodystrophies are a group of disorders caused by genetic defects in the production or maintenance of myelin. Many affected children may appear normal at birth or early in infancy but then develop progressive deterioration in muscle tone, movements, gait, speech, ability to eat, vision, hearing, and behavior. The majority of HCTs for leukodystrophies have been performed in patients with adrenoleukodystrophy (ALD), metachromatic leukodystrophy (MLD), and Krabbe disease (globoid leukodystrophy), conditions in which HCT has been shown to halt or

slow disease progression in selected patients. Prognosis is impacted by the age of the patient, onset of their disease presentation, and stage of disease at the time of transplant, with children undergoing HCT in presymptomatic or early disease stages faring better than those in symptomatic or advanced stages (Musolino et al. 2014). Therefore, all of these factors weigh heavily in deciding whether to proceed with HCT.

13.3.1 Adrenoleukodystrophy

X-linked ALD is a peroxisomal disorder in which a defect in the *ABCD1* gene leads to accumulation of very long chain fatty acids in tissues and plasma. Symptoms include cerebral demyelination, adrenal insufficiency, and progressive neurological deterioration. The largest series of HCT (including 53 CBT) for boys with ALD was reported from the University of Minnesota in 2011 (Miller et al. 2011), and Duke University described their experience in CBT for 12 boys with ALD, ages 2–11 years, in 2007 (Beam et al. 2007). In both series, boys with less disease manifestations (clinical and radiographic) at the time of transplantation had superior outcomes in terms of overall survival and neurologic outcomes, whereas symptomatic children demonstrated lower survival and rapid deterioration of neurologic function despite HCT. The Loes score, a severity scale used to determine the extent and damage to myelin in the brain via MRI, was also strongly predictive of posttransplantation developmental outcome and therefore can be used to counsel families who are considering transplantation regarding prognosis. Transplantation of these patients should be performed in centers that have experience transplanting patients with adrenal insufficiency, which is the other common manifestation of this disease.

13.3.2 Metachromatic Leukodystrophy

In MLD, a deficiency of the lysosomal enzyme arylsulfatase A (ASA) leads to sphingolipid accumulation, resulting in a rapidly progressive loss of myelinating cells. The time of onset and severity of symptoms including spasticity, neuropathy, dementia, and seizures correlate with residual ASA activity, making it feasible to transplant babies with the early infantile form of the disease only if the diagnosis is known at birth. In 2015, the University of Minnesota published a series of 40 patients who underwent HCT for MLD between 1984 and 2013 (Boucher et al. 2015). Their cohort included patients with late-infantile ($n = 4$), juvenile ($n = 27$), or adult-onset ($n = 9$) MLD transplanted at 0–44 years of age. Donor sources consisted of related bone marrow ($n = 11$), unrelated bone marrow ($n = 14$), and CB ($n = 15$). Transplant-related mortality was high (23%) and may reflect the inclusion of patients from an earlier transplant era. Overall survival was 59% at 5 years and was not related to MLD subtype or the presence of symptoms at the time of HCT, though there were relatively few patients with late-infantile or asymptomatic disease included. Patients who received transplants utilizing matched-related bone

marrow or CB showed a trend for improved survival compared to those from unrelated bone marrow.

The largest series of CBT in children with MLD included 27 children, ages 4 months to 16 years old, with the late-infantile ($n = 10$) or juvenile ($n = 17$) forms of the disease who underwent CBT at Duke University after myeloablative conditioning from 1997 to 2011 (Martin et al. 2013). With a median follow-up of 5 years, 20/27 patients were survivors, for a 5-year survival probability of 74%. The late-infantile group had a 5-year survival probability of 60%, *versus* 82% in the juvenile group. Significant disease progression was noted in ten patients (six late-infantile onset, four juvenile onset). In the late-infantile-onset group, only asymptomatic patients with minimal disease burden benefited from transplantation. Despite transplantation, many patients with MLD continue to experience progression of peripheral nervous system disease, although at a slower rate than expected by natural history.

13.3.3 Krabbe Disease

Krabbe disease, caused by mutations in the lysosomal enzyme GALC, leads to accumulation of psychocine, which in turn causes apoptosis of myelin-forming oligodendrocytes and Schwann cells in both the central and peripheral nervous systems. In the most common early infantile form, babies develop symptoms including irritability, spasticity, developmental regression, and seizures within the first 6 months of life and die within 2 years. As the early infantile form progresses particularly rapidly, CB has been used most commonly as a graft source for HCT due to its ready availability. In 2005, the outcomes of 11 asymptomatic babies with Krabbe disease transplanted in the first month of life were reported along with 14 infants transplanted after the onset of symptoms (Escolar et al. 2005). With a median follow-up of 3 years, survival was dramatically increased in babies who underwent CBT prior to the development of symptoms (100% *vs.* 42.8%). Symptomatic infants stabilized but did not demonstrate neurologic improvement. Newborns who underwent transplant with minimal to no symptoms of disease exhibited substantial neurodevelopmental gains in all areas of development compared to symptomatic infants and untreated patients. Nonetheless, some degree of gross motor function deficit became apparent in all the children. Recent analysis of late outcomes shows that babies transplanted younger than 30 days of age have superior outcomes than those transplanted at greater than 30 days (Allewelt et al. 2016).

In the United States, the success of CBT in infantile Krabbe disease and the need to intervene as early as possible led to the development of newborn screening programs for the disease, initiated in New York State in 2006. Approximately 2 million babies have been screened in New York, with five cases (two siblings) of early infantile Krabbe disease detected (Orsini et al. 2016). Four patients underwent HCT; two died of transplant-related complications, and two are alive with neurologic impairments 5 and 8 years later. In addition, approximately 100 novel mutations in the GALC gene have been detected in babies who appear to be healthy at

the present time. Whether or not these individuals will eventually develop later onset disease remains to be seen. While newborn screening may lead to diagnosis in the presymptomatic state, thereby enabling transplantation earlier in life, it has also highlighted the challenges of implementing a diagnostically challenging screening program and counseling families and providers regarding indeterminate results.

As described above, results of CBT for IMDs suggest that greater benefit is likely when the transplant is performed early in the disease course prior to the development of clinical neurologic and other manifestations (Boelens et al. 2007; Martin et al. 2013; Escolar et al. 2005). However, damage to the central nervous system occurs prenatally in some of these disorders. In addition, neurologic progression often occurs during and in the early months following HCST before sufficient numbers of donor cells engraft in the brain and produce adequate levels of the deficient enzyme. As a result, patients often experience a progressive loss of neurologic function for the first few months after transplantation before the disease stabilizes, and most patients are left with some residual and irreversible neurologic impairment. Even when complete donor hematopoietic chimerism and normal blood enzyme levels are obtained and survival is extended for decades, emerging long-term data suggest that eventual neurologic decline, particularly in motor function, commonly occurs later in life. Additional approaches are necessary to fully address the multifaceted tissue pathology in these diseases and normalize functional outcomes for patients. Augmented cellular therapies, such as CB-derived microglial-like cells (DUOC-01) (Tracy et al. 2008, 2011; Kurtzberg et al. 2015) and others, (Koc et al. 2002) gene therapies, (Biffi et al. 2013) supplemental enzyme therapy, (Li and Sands 2014) and chaperone therapy, alone or in combination with HCT, are all being investigated in the laboratory and/or clinic for that purpose.

13.4 Investigations in the Treatment of Acquired Brain Injuries with Umbilical Cord Blood

Aside from acting as cellular enzyme replacement, donor CB cells in patients with IMDs may also play a role in replacing damaged cells, secreting supportive factors, and immunoregulation. These additional possible mechanisms led to the hypothesis that CB might also be beneficial in patients with acquired brain injuries. Over the past several years, CB cells have been investigated in preclinical models of stroke, neonatal hypoxic ischemic encephalopathy (HIE), cerebral palsy, traumatic brain injury, and spinal cord injury. Numerous animal models have demonstrated neuroprotection, (Vendrame et al. 2004) neovascularization, (Taguchi et al. 2004) and neuronal regeneration (Taguchi et al. 2004) after xenogeneic CB administration leading to both neurological and survival benefits (Vendrame et al. 2004; Chen et al. 2001; Meier et al. 2006; Nan et al. 2005; Lu et al. 2002; Zhao et al. 2004; Nishio et al. 2006). Based on these observations, early phase clinical trials of CB have begun in human patients with acquired brain injuries, utilizing either autologous or allogeneic CB cells.

13.4.1 Hypoxic Ischemic Encephalopathy

Neonatal HIE results from a recent lack of oxygen to the infant brain, typically attributed to events during labor and delivery. Babies with moderate and severe HIE are standardly treated with therapeutic hypothermia. Despite this intervention, moderate-to-severe HIE is fatal in approximately 25% of cases and causes neurologic sequelae in at least another 25%. In a phase I trial of newborns with HIE at birth conducted at Duke University, fresh, non-cryopreserved, volume- and RBC-reduced autologous CB was infused in 1, 2, or 4 doses of 1–5 × 10^7 (Di Ferrante et al. 1971) nucleated cells/kg within the first 48–72 h of life in babies with moderate-to-severe encephalopathy qualifying for systemic hypothermia (Cotten et al. 2014). These babies ($n = 23$) were compared to a concomitant group of babies who were also cooled at Duke University but did not receive CB cells ($n = 83$). Infusions were found to be safe in these critically ill babies, and babies receiving cells had increased survival rates to discharge (100% *vs.* 87%, $p = 0.12$) and improved function at 1 year of age (72% *vs.* 41% with development in the normal range, $p = 0.05$). Based on these findings, a phase II, randomized, placebo-controlled multicenter study is underway to further investigate the utility of CB infusion in conjunction with therapeutic hypothermia for babies with HIE.

13.4.2 Cerebral Palsy

In the majority of cases, cerebral palsy results from an *in utero* or perinatal brain injury such as stroke, hypoxic insult, or hemorrhage. Affected children have varying degrees of functional impairments ranging from mild limitations in advanced gross motor skills to severely limited self-mobility despite the use of assistive technology. Clinical studies evaluating the use of CB in children with cerebral palsy are ongoing in several countries. In Russia, 80 pediatric patients received multiple intravenous doses of allogeneic, ABO-matched, and HLA-unmatched CB, with improvements noted in children who received at least four doses (Romanov et al. 2015). However, many children with cerebral palsy are expected to make some gains over time, and there was no control group for comparison. A double-blind study was conducted in Korea in 93 children, who were randomized to receive erythropoietin, erythropoietin + cyclosporine + allogeneic CB (≥4/6 HLA-matched, ≥3 × 10^7 (Di Ferrante et al. 1971) TNC/kg), or placebo (Min et al. 2013). They reported greater improvements in cognitive and select motor functions in children who received CB and erythropoietin *versus* controls, with higher cell doses associated with greater improvement. A CB-only group was not included.

In the United States, investigations of CB in children with cerebral palsy have focused on intravenous infusions of autologous CB that had been banked at the time of the child's birth. An initial safety study of 184 infants and children with cerebral palsy (76%), congenital hydrocephalus (12%), and other brain injuries (12%) identified a temporary hypersensitivity reaction (i.e., hives and/or wheezing) in approximately 1.5% of patients as the only side effect (Sun et al. 2010). A randomized,

double-blind, placebo-controlled study was subsequently conducted in 63 children, ages 1–6 years old, to evaluate the efficacy of this approach. In that study, there was no difference in motor improvement between placebo and treated groups as a whole. However, patients receiving $\geq 2 \times 10^7$ (Di Ferrante et al. 1971) TNC/kg demonstrated greater improvement of motor function than subjects receiving smaller cell doses, which is consistent with the minimum dose used for allogeneic HCT (Sun et al. 2016).

13.4.3 Stroke

Stroke represents a significant public health concern and a leading cause of morbidity and mortality among adults. Studies of cell therapy in older patients who have suffered a stroke have focused on autologous bone marrow-derived cells. The only published randomized trial, conducted in 120 patients in India, administered autologous bone marrow MNCs (mean 280×10^6 (Krivit et al. 1999) cells) as a single intravenous dose 7–30 days after acute stroke (Prasad et al. 2014). There was no difference in functional outcomes or infarct volume between groups at 6 months. Additional studies are underway or planned using autologous and allogeneic cells (Hess et al. 2014). There are concerns regarding the functionality and feasibility of autologous bone marrow-derived cells, as they must be collected from a typically ill, elderly patient, and early administration may not be possible if the cells must be cultured or processed for any length of time. Therefore, a CB-derived off-the-shelf therapy is an attractive alternative to autologous bone marrow, as it would be readily available and avoid the need for a potentially risky bone marrow harvest. A phase I safety study administering ABO-matched, HLA-unmatched allogeneic CB intravenously to adults within 10 days of acute ischemic stroke recently completed accrual and did not demonstrate any acute safety issues related to the CB infusion (Kurtzberg et al. 2016). A phase II study to evaluate efficacy is planned.

Intravenous infusion of CB is also currently being investigated in young children with stroke, traumatic brain injury, and autism. The field of cell therapy as a possible treatment for acquired neurologic conditions is still evolving, and there is much work to be done in both the preclinical and clinical arenas before any cellular products may be established for proven use. The relative availability, favorable safety profile, and pluripotential nature of CB, however, make it a prime source of stem cells for such emerging therapies.

Conclusion

Over the past 25 years, CB has been established as a viable and, at times, preferred source of donor cells for hematopoietic reconstitution in allogeneic transplantation for all of the clinical indications treated with adult hematopoietic stem cells. The ready availability of CB, its ability to deliver high cell doses to pediatric patients, and the high incidence of full-donor chimerism and normal post-transplant enzyme levels make it an especially attractive option for children with IMDs that affect the brain. CB also has potential for use in the treatment of

acquired brain injuries, though efficacy has yet to be clearly proven in clinical trials. Looking ahead, CB and CB-derived products hold promise for use in the emerging fields of cellular therapies and regenerative medicine.

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Cord Blood Transplants *Versus* Other Sources of Allografts: Comparison of Data in Adult Setting

14

Rohtesh S. Mehta and Claudio G. Brunstein

14.1 Introduction

About two-thirds of patients undergoing hematopoietic cell transplantation (HCT) lack a human leukocyte antigen (HLA)-matched related donor. The probability of finding an HLA-matched unrelated donor (URD) varies from 75% for white Europeans to 16–19% for black Americans (Gragert et al. 2014). As the degree of HLA mismatch is relatively well tolerated with umbilical cord blood (UCB) transplantation (Laughlin et al. 2004; Wagner et al. 2002; Rocha et al. 2001; Barker et al. 2001; Rubinstein et al. 1998; Gluckman et al. 1997; Kurtzberg et al. 1996), it provides an excellent alternative graft source for patients who do not have a suitable HLA-matched donor. The likelihood of finding 4/6 or higher HLA-matched UCB units is more than 80% for African-Americans and almost 100% for white Europeans (Gragert et al. 2014). As compared with other donor sources, UCB transplantation can be performed in a short time period (Barker et al. 2002), carries minimal risk of infection, and is associated with lower risk of acute or chronic graft-*versus*-host disease (GvHD) (Laughlin et al. 2004; Rocha et al. 2004; Takahashi et al. 2007; Atsuta et al. 2009; Brunstein et al. 2010, 2012; Marks et al. 2014; Chen et al. 2012; Jacobson et al. 2012; Robin et al. 2015; Rodrigues et al. 2014; Weisdorf et al. 2014; Warlick et al. 2015). However, because of its naive immune system (Garderet et al. 1998) and significantly lower total nucleated cell (TNC) content (Laughlin et al. 2004; Rocha et al. 2004; Takahashi et al. 2007; Atsuta et al. 2009; Brunstein et al. 2010, 2012; Marks et al. 2014; Chen et al. 2012; Jacobson et al. 2012; Robin

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_14

et al. 2015; Rodrigues et al. 2014; Weisdorf et al. 2014; Warlick et al. 2015; Eapen et al. 2010; Majhail et al. 2008) as compared with other graft sources, it has traditionally been associated with slower myeloid and platelet recoveries and graft failure, which translate into higher non-relapse mortality (NRM) (Brunstein et al. 2010; Jacobson et al. 2012; Eapen et al. 2010; Majhail et al. 2008).

14.2 Hematopoietic Recovery Is Prolonged After UCB Transplantation

While most patients achieve hematopoietic recovery after UCB transplantation, it may take 7–10 days longer than other graft sources (Laughlin et al. 2004; Rocha et al. 2004; Atsuta et al. 2009; Eapen et al. 2010). After myeloablative conditioning, neutrophil engraftment is achieved in about 25 days with UCB as compared with 14–18 days after peripheral blood progenitor cells (PBPC) and bone marrow (BM) grafts, respectively. Similarly, platelet engraftment (>20 × 10^9/L), which occurs in about 20 days after PBPC and 25 days after BM grafts, takes about 60 days after UCB transplantation (Takahashi et al. 2007; Brunstein et al. 2010; Eapen et al. 2010; Barker et al. 2005, 2015; Liu et al. 2014; Ruggeri et al. 2014; Sanz et al. 2012; Verneris et al. 2009). Despite the remarkable proliferative capacity of UCB, slower engraftment is explained to some extent by about a log less TNC and $CD34^+$ cells in UCB grafts than in other grafts (Laughlin et al. 2004; Rocha et al. 2004; Atsuta et al. 2009; Eapen et al. 2010). The use of two partially matched UCB grafts overcomes the low cell dose for larger patients who would not have a single UCB unit with sufficient cell dose to proceed to transplantation (Barker et al. 2005). Although double-unit UCB grafts increase the total cell content of the graft, time to neutrophil engraftment still remains delayed as compared to adult donors and is similar to an adequately dosed single-unit UCB graft (Ruggeri et al. 2014; Verneris et al. 2009; Wagner et al. 2014; Kindwall-Keller et al. 2012; Scaradavou et al. 2013). Recent prospective and retrospective data do not support the use of double-unit UCB grafts when a single-unit UCB with a cryopreserved TNC dose of 2.5–3.0 × 10^7/kg is available (Wagner et al. 2014; Scaradavou et al. 2013). Moreover, UCB has a risk of graft failure, which is higher than that of PBPC or BM from HLA-matched related or unrelated donors, but similar to mismatched adult donors. The reported risk of UCB graft failure varies from 5 to 15% depending upon the experience of the transplant center and whether the study was performed at a single institution *versus* multi-institutional or a registry-based analysis.

In the reduced-intensity conditioning (RIC) setting, time to neutrophil engraftment is faster than what is observed after myeloablative regimens and approaches that is of transplantation with other grafts (Brunstein et al. 2012; Chen et al. 2012; Majhail et al. 2008; Barker et al. 2003; Ballen et al. 2007; Uchida et al. 2008; Cutler et al. 2011; Sandhu et al. 2016; Oran et al. 2011). A part of this is due to transient autologous reconstitution. Neutrophil recovery with the TCF regimen is faster than with other RIC regimens (Brunstein et al. 2012; Barker et al. 2003) and is reported to take a median of 9–17 days, which is similar to that of HLA-matched PBPCs

(median 13–16 days). The time is longer after more intensive RIC regimens (Chen et al. 2012; Ponce et al. 2013; Gyurkocza et al. 2014; Mehta et al. 2015). The risk of graft failure is particularly greater in patients receiving RIC who have an intact immune system, such as those who have not received prior chemotherapy or autologous transplantation (Brunstein et al. 2012; Weisdorf et al. 2014; Malard et al. 2015; Peffault de Latour et al. 2013; Le Bourgeois et al. 2013).

In summary, there is delayed hematopoietic recovery and an increased risk of graft failure after UCB transplantation. While delayed engraftment is more notable in the myeloablative setting, graft failure is more significant in the RIC setting. Hematopoietic recovery and engraftment are directly dependent on the quality of the UCB graft. Thus, the selection of UCB units considering factors beyond cell dose and HLA matching will directly affect engraftment and overall transplant outcomes. Additionally, studies of ex vivo UCB expansion demonstrated faster engraftment and lower risk of graft failure and, if made widely available, may favorably impact how UCB transplantation compares to other donor types. For detailed discussion on these topics, please refer to Chaps. 3 and 4 on UCB selection and Chap. 5 on ex vivo cord blood manipulation.

14.3 HLA Mismatch with UCB Transplantation Is Well Tolerated

In URD transplantation, the risk of GvHD directly correlates with the degree of HLA mismatch (Lee et al. 2007). In contrast, the risks of acute and chronic GvHD after UCB transplantation are relatively low despite a higher degree of HLA mismatch (Laughlin et al. 2004; Rocha et al. 2004; Takahashi et al. 2007; Atsuta et al. 2009; Brunstein et al. 2010, 2012; Marks et al. 2014; Chen et al. 2012; Jacobson et al. 2012; Robin et al. 2015; Rodrigues et al. 2014; Weisdorf et al. 2014; Warlick et al. 2015; Liu et al. 2014; Malard et al. 2015; Le Bourgeois et al. 2013; Konuma et al. 2014). Current UCB graft selection criteria permit up to two HLA mismatches to the patient, with antigen-level matching performed at HLA-A and HLA-B and allele-level matching at HLA-DRB1. If we were to revise this and incorporate allele-level matching for HLA-A, HLA-B, HLA-C, and HLA-DRB1 as used for URD selection, some UCB units would be as low as 2–4/8 HLA-matched to the recipient (Brunstein et al. 2016; Oran et al. 2015; Eapen et al. 2014). However, studies consistently suggest that allele-level matching for UCB transplantation does not impact the risk of GvHD (Brunstein et al. 2016; Oran et al. 2015; Eapen et al. 2014). Thus, for the purpose of comparison with other donor types, all UCB recipients are typically regarded as a single group regardless of the HLA match.

Several other factors influence the risk of GvHD after UCB transplantation and must be considered when interpreting data in comparison with other donor sources. For example, the risk of GvHD after UCB transplantation may vary depending on whether the patient received a single- or double-unit UCB graft. Initial studies demonstrated a higher risk of GvHD after double-unit graft than after single-unit UCB graft (MacMillan et al. 2009). This was later confirmed in a prospective randomized

comparison between single- and double-unit UCB transplantation in children, which showed a higher risk of acute, but not chronic, GvHD in the double-unit UCB group (Wagner et al. 2014). In contrast, in a retrospective registry study, the risk of acute and chronic GvHD was similar in adults receiving single- or double-unit UCB grafts (Scaradavou et al. 2013). This discrepancy can be partly explained by more frequent use of ATG as a part of the conditioning regimen in single- but not in double-unit UCB transplantation.

In studies that compared the outcomes of *single-unit UCB* transplantation with HLA-matched donor, the risks of grade II–IV acute GvHD and chronic GvHD were similar or lower after UCB transplantation (Laughlin et al. 2004; Rocha et al. 2004; Atsuta et al. 2009; Eapen et al. 2010) (Table 14.1). Some studies (Laughlin et al. 2004; Eapen et al. 2010) showed similar risk of grade II–IV acute GvHD but significantly lower risk of chronic GvHD, while others (Rocha et al. 2004; Atsuta et al. 2009) showed lower risk of acute GvHD but similar risk of chronic GvHD after single-unit UCB transplantation. As compared with single-unit UCB transplantation, the incidence of acute, but not chronic, GvHD is noted to be somewhat higher in studies of *double-unit UCB* transplantation in the myeloablative or nonmyeloablative setting. Yet, the risk of acute GvHD is comparable after double-unit UCB or matched URD transplantation in the myeloablative (Takahashi et al. 2007; Brunstein et al. 2010; Konuma et al. 2014) (Table 14.2) or RIC setting (Chen et al. 2012; Weisdorf et al. 2014; Majhail et al. 2008, 2012; Malard et al. 2015; Peffault de Latour et al. 2013; Le Bourgeois et al. 2013) (Table 14.3). In contrast, a registry study from the Center for International Blood and Marrow Transplant Research (CIBMTR) showed a higher risk of grade II–IV acute GvHD after double-unit UCB transplantation as compared with 8/8 HLA-matched PBPCs, but the excess events were grade II skin GvHD (Brunstein et al. 2012). The risk of chronic GvHD has been consistently found to be lower after UCB transplantation than with other donor types (Takahashi et al. 2007; Brunstein et al. 2010, 2012; Chen et al. 2012; Weisdorf et al. 2014; Majhail et al. 2008, 2012; Le Bourgeois et al. 2013).

In summary, the risk of acute and chronic GvHD after single- and double-unit UCB transplantation is similar or lower than after 7–8/8 HLA-matched adult donor transplantation. UCB transplantation has this improved risk profile despite HLA mismatching at up to two antigens as compared to other graft sources. This observation is thought to result from the immunologically naïve T-cells present in UCB grafts (Garderet et al. 1998).

14.4 Non-Relapse Mortality (NRM) After UCB Transplantation Is Influenced by Study Population and Conditioning Regimen

In the *myeloablative setting*, the risk of NRM after UCB transplantation is similar or higher than that of other donor types. This is influenced, among other factors, by the comparison population as illustrated by three studies with nonoverlapping populations (Takahashi et al. 2007; Brunstein et al. 2010; Eapen et al. 2010). A large

Table 14.1 Comparison of single-unit umbilical cord blood transplantation with other graft sources

Reference	Graft (sample size)	Age median (range), in years	Acute GvHD, grade II–IV	Acute GvHD, grade III–IV	Chronic GvHD	Extensive chronic GvHD	NRM/TRM	Relapse	Disease-free survival	Overall survival/ HR of mortality
Laughlin et al. (2004)	*s*UCBT ($n = 150$)	Unknown (16–60)	HR 0.81 (95% CI 0.59–1.1), $p = 0.17$ *vs.* matched BM HR 0.66 (95% CI 0.44–0.99; $p = 0.04$ *vs.* mismatched BM		HR 1.62 (95% CI 1.08–2.42), $p = 0.02$ *vs.* matched BM HR 1.12 (95% CI, 0.63–2.02); $p = 0.69$ *vs.* mismatched BM	*33% (95% CI NR)* ($p = 0.03$)	HR 1.89 (95% CI 1.45–2.48) $p < 0.001$ *vs.* matched BM HR 0.99 (95% CI 0.70–1.40) $p = 0.96$ *vs.* mismatched BM	HR 0.73 (95% CI 0.46–1.14) $p = 0.16$ *vs.* matched BM HR 0.85 (95% CI 0.43–1.70), $p = 0.65$ c/w mismatched BM	*Treatment failure* HR 1.48 (95% CI 1.18–1.86), $p = 0.001$ *vs.* matched BM HR 0.94 (95% CI 0.69–1.28), $p = 0.69$ *vs.* mismatched BM	26% (95% CI 19–32) at 3 years
	6/6-HLA BM URD ($n = 367$)	Unknown (16–60)				*52% (95% CI NR)*				35% (95% CI 30–39) at 3 years, $p < 0.001$
	5/6-HLA BM URD ($n = 83$)	Unknown (16–60)				*71% (95% CI NR)*				20% (95% CI 12–28) at 3 years, $p = 0.62$

(continued)

Table 14.1 (continued)

Reference	Graft (sample size)	Age median (range), in years	Acute GvHD, grade II–IV	Acute GvHD, grade III–IV	Chronic GvHD	Extensive chronic GvHD	NRM/TRM	Relapse	Disease-free survival	Overall survival/ HR of mortality
Rocha et al. (2004)	*s*UCBT ($n = 98$)	24.5 (15–55)	RR 0.57 (95% CI 0.37–0.87), $p = 0.01$		RR 0.64 (95% CI 0.37–1.1), $p = 0.11$		RR 1.13 (95% CI 0.78–1.64), $p = 0.50$	RR 1.02 (95% CI 0.63–1.65), $p = 0.93$	RR 0.95 (95% CI 0.72–1.25), $p = 0.70$	RR 0.95 (95% CI 0.71–1.27), $p = 0.75$
	6/6-HLA BM URD ($n = 584$)	32 (15–59)								
Takahashi et al. (2007)	*s*UCBT ($n = 100$)	38 (16–55)	HR 1.09 (95% CI 0.71–1.68), $p = 0.69$	HR 0.38 (95% CI 0.15–0.95), $p = 0.04$	HR 1.43 (95% CI 0.95–2.14), $p = 0.09$	HR 0.49 (95% CI 0.29–0.85), $p = 0.01$	HR 0.49 (95% CI 0.19–1.24), $p = 0.13$	HR 0.72 (95% CI 0.37–1.41), $p = 0.34$	HR 0.74 (95% CI 0.44–1.25), $p = 0.26$	
	BM or PBPC from URD (76% 6/6-HLA match)	40 (16–58)								

Atsuta et al. (2009)	*s*UCBT ($n = 173$) – AML	38 (16–69)	RR 0.80 (95% CI 0.56–1.15), $p = 0.23$		RR 0.94 (95% CI 0.63–1.42), $p = 0.79$	RR 0.36 (95% CI 0.18–0.72), $p = 0.004$	RR 1.5 (95% CI 1.0-2.3), $p = 0.085$	RR = 1.2 (95% CI 0.8–1.9), $p = 0.38$	Treatment failure HR = 1.5 (95% CI 1.1–2.0), $p = 0.012$	HR = 1.5 (95% CI 1.0–2.0), $p = 0.028$
	6/6-HLA BM from URD ($n = 311$) – AML	38 (16–60)								
	4–6/6-HLA *s*UCBT ($n = 114$) – ALL	34 (16–58)	RR 0.61 (95% CI 0.39–0.95), $p = 0.028$		RR 1.08 (95% CI 0.66–1.77), $p = 0.77$	RR 0.58 (95% CI 0.28–1.20), $p = 0.14$	RR 1.0 (95% CI 0.6–1.7), $p = 0.98$	RR 1.4 (95% CI 0.8–2.4), $p = 0.19$	HR = 1.2 (95% CI 0.9–1.8), $p = 0.28$	HR = 1.1 (95% CI 0.7–1.6), $p = 0.78$
	6/6-HLA BM from URD ($n = 222$) – ALL	32 (16–59)								

Abbreviations: *ALL* acute lymphoid leukemia, *AML* acute myeloid leukemia, *BM* bone marrow, *CI* confidence interval, *dUCBT* double-unit umbilical cord blood transplantation, *DFS* disease-free survival, *GvHD* graft-*versus*-host disease, *HLA* human leukocyte antigen, *HR* hazards ratio, *MA* myeloablative, *MMUD* mismatched unrelated donor, *MRD* matched related donor, *MUD* matched unrelated donor, *NMA* nonmyeloablative, *NR* not reported, *NRM* non-relapse mortality, *NS* nonsignificant, *OR* odd ratio, *PBPC* peripheral blood progenitor cell, *PFS* progression-free survival, *RD* related donor, *RIC* reduced-intensity conditioning, *RR* relative risk, *TCD* T-cell depleted, *TRM* transplant-related mortality, *UCBT* umbilical cord blood transplantation, *URD* unrelated donor

Table 14.2 Comparison of double-unit cord blood transplantation with adult donor HSC transplantation after myeloablative conditioning

Reference	Graft (*n* patients)	Age median (range), in years	II–IV acute GvHD	III–IV acute GvHD	Chronic GvHD	Extensive chronic GvHD	NRM/TRM	Relapse	Disease-free survival	Overall survival/HR of mortality
Gutman et al. (2009)	CBT (*n* = 31; dUCBT 27)	22 (0.6–42)	80.6% (95% CI 61.9–90.8)	29.0% (95% CI 14.5–45.3)			20.6% (95% CI 8.2–36.9) at 2 years	3.2% (95% CI 0.2–14.1) at 2 years	76.2% (95% CI 56–88) at 2 years	74.5% (95% CI 53–87.2) at 2 years
	MMUD (*n* = 31)	25 (1–48)	87.1% (95% CI 69.2–95)	35.5% (95% CI 19.4–51.9)			29.2% (95% CI 14.6–45.5) at 2 years	23% (95% CI 10.1–38.9) at 2 years	47.8% (95% CI 29.5–64) at 2 years	50% (95% CI 31–66.2) at 2 years
	MUD (*n* = 31)	25 (0.9–41)	67.7% (95% CI 48.4–81.2)	12.9% (95% CI 4.1–27.0)			17% (95% CI 6.2–32.5) at 2 years	25.8% (95% CI 12.1–41.8) at 2 years	57.1% (95% CI 37.7–72.5) at 2 years	59.7% (95% CI 39.8–74.9) at 2 years
Brunstein et al. (2010)	dUCBT (*n* = 128)	25 (10–46)	60% (95% CI 50–70)	22% (95% CI 15–29)	26% (95% CI 15–35) at 2 years		34% (95% CI 25–42) at 5 years	15% (95% CI 9–22) at 5 years	51% (95% CI 41–59) at 5 years	
	MRD (*n* = 204; 92% PBPC)	40 (12–67)	65% (95% CI 57–73)	13% (95% CI 9–18)	47% (95% CI 39–55) at 2 years		24% (95% CI 17–39) at 5 years	43% (95% CI 35–52) at 5 years	33% (95% CI 26–41) at 5 years	
	MUD (*n* = 152; 58% PBPC)	31 (10–57)	80% (95% CI 70–90)	14% (95% CI 9–20)	43% (95% CI 34–52) at 2 years		14% (95% CI 9–20) at 5 years	37% (95% CI 29–46) at 5 years	48% (95% CI 40–56) at 5 years	
	MMUD (*n* = 52; 65% PBPC)	31 (10–51)	85% (95% CI 68–100)	37% (95% CI 23–50)	48% (95% CI 32–64) at 2 years		27% (95% CI 15–39) at 5 years	35% 35 (95% CI 21–48) at 5 years	38% (95% CI 25–51) at 5 years	

Konuma et al. (2014)	UCBT ($n = 66$)	49 (45–55)	HR 0.90 (95% CI 0.49–1.64), $p = 0.76$)	9.2% (95% CI 3.7–17.8)	HR 0.94 (95% CI 0.55–1.62), $p = 0.84$		3% (95% CI 0.6–9.4) at day +100 16% (95% CI 7.6–26.6) at 5 years HR 0.29 (95% CI 0.08–0.99), $p = 0.04$	22% (95% CI 12.7–33) at 5 years HR 2.02 (95% CI 0.63–6.42), $p = 0.23$		67.4% (95% CI 55.7–81.6) at 5 years HR 0.72 (95% CI 0.30–1.73), $p = 0.47$
	RD ($n = 31$; BM = 26; PBPC = 5)	48 (45–58)		16.1% (95% CI 5.7–31.2), $p = 0.35$ HR 1.08 (.49–2.35), $p = 0.34$	HR 1.08 (95% CI 0.49–2.35), $p = 0.84$	HR 1.08 (95% CI 0.49–2.35), $p = 0.84$	6.5% (95% CI 1.1–18.9) at day +100 32.7% (95% CI 16.8–49.6) at 5 years	16.7% (95% CI 5.9–32.3) at 5 years		55.2% (95% CI 39.4–77.4) at 5 years
Liu et al. (2014)	UCBT ($n = 70$)	23 (16–46)	40% (*95% CI NR*) OR 3.80 (95% CI 1.87–7.70), $p < 0.00001$	4% (*95% CI NR*) OR 2.53 (95% CI 0.77–8.30), $p = 0.207$	20.7% (*95% CI NR*) OR 0.36 (95% CI 0.17–0.75), $p = 0.005$	3.4% (*95% CI NR*) OR 0.21 (95% CI 0.05–0.94), $p = 0.026$	34.2% (95% CI 34.15–34.42) at 6 months 35.7% (95% CI 35.06–36.36%) at 2 years HR 0.96 (95% CI 0.31–3.00), $p = 0.682$	11.9% (95% CI 11.59–12.22) at 3 years HR 0.46 (95% CI 0.19–1.11), $p = 0.083$	55% (95% CI 43–67) HR 0.67 (95% CI 0.28–1.60), $p = 0.668$	
	MRD ($n = 115$; 59% PBPC, 50% PBPC + BM, 6% BM)	32 (16–58)	15% (*95% CI NR*)	10% (*95% CI NR*)	42.2% (*95% CI NR*)	14.6% (*95% CI NR*)	8.7% (95% CI 8.45–8.83) at 6 months 25.3% (95% CI 25.06–25.62) at 2 years	16.2% (95% CI 15.95–16.44) at 3 years	60% (95% CI 51–69)	

(continued)

Table 14.2 (continued)

Reference	Graft (*n* patients)	Age median (range), in years	II–IV acute GvHD	III–IV acute GvHD	Chronic GvHD	Extensive chronic GvHD	NRM/TRM	Relapse	Disease-free survival	Overall survival/HR of mortality
Marks et al. (2014)	UCBT ($n = 116$)	25 (16–59)	27% (95% CI 19–35)	9% (95% CI 5–15),	39% (95% CI 30–49) at 3 years	29/44; 66%	42% (95% CI 33–51), at 3 years	22% (95% CI 15–31) at 3 years		44% (95% CI 34–54), at 3 years
	8/8 matched ($n = 546$; 33.3% BM, 66.6% PBPC)	32 (16–59)	47% (95% CI 39–55) HR 1.57 (95% CI 1.07–2.31), $p = 0.021$	16% (95% CI 13–20) HR 1.70 (95% CI 0.90–3.22), $p = 0.10$	42% (95% CI 34–50) at 3 years	197/247; 80%	31% (95% CI 27–35) at 3 years HR 0.74 (95% CI 0.53–1.03), $p = 0.07$	25% (95% CI 19–33) at 3 years HR 1.35 (95% CI 0.88–2.09), $p = 0.17$	Treatment failure HR 0.97 (95% CI 0.75–1.26), $p = 0.82$	44% (95% CI 40–48) at 3 years HR 0.89 (0.68–1.16), $p = 0.38$
	7/8 matched ($n = 140$; 40% BM, 60% PBPC)	33 (16–59)	41% (95% CI 37–45) HR 1.89 (95% CI 1.22–2.92), $p = 0.004$	24% (95% CI 18–32) HR 2.55 (95% CI 1.27–5.10), $p = 0.008$	45% (95% CI 41–49) at 3 years	54/60; 90%	39% (95% CI 31–47) at 3 years HR 0.83 (95% CI 0.56–1.23), $p = 0.35$	28% (95% CI 25–32) at 3 years HR 1.37 (95% CI 0.82–2.31), $p = 0.24$	HR 1.03 (95% CI 0.76–1.41), $p = 0.85$	43% (95% CI 35–51) at 3 years HR 0.96 (95% CI 0.70–1.32), $p = 0.81$

Abbreviations: *ANC* absolute neutrophil count, *BM* bone marrow, *CI* confidence interval, *dUCBT* double-unit umbilical cord blood transplantation, *DFS* disease-free survival, *GvHD* graft-*versus*-host disease, *HLA* human leukocyte antigen, *HR* hazards ratio, *MA* myeloablative, *MMUD* mismatched unrelated donor, *MRD* matched related donor, *MUD* matched unrelated donor, *NMA* nonmyeloablative, *NR* not reported, *NRM* non-relapse mortality, *NS* nonsignificant, *OR* odd ratio, *PBPC* peripheral blood progenitor cell, *PFS* progression-free survival, *RD* related donor, *RIC* reduced-intensity conditioning, *RR* relative risk, *TCD* T-cell depleted, *TRM* transplant-related mortality, *UCBT* umbilical cord blood transplantation, *URD* unrelated donor

Table 14.3 Comparison of double-unit cord blood transplantation with adult donor HSC transplantation after reduced-intensity conditioning

Reference	Graft (*n* patients)	Age median (range), in years	II–IV acute GvHD	III–IV acute GvHD	Chronic GvHD	Extensive chronic GvHD	TRM	Relapse	Disease-free survival	Overall survival/HR of mortality
Majhail et al. (2008)	UCBT ($n = 43$; 88% DCBT)	59 (55–69)	49% (95% CI 32–65)		17% (95% CI 5–29) at 1 year		28% (95% CI 14–41) at day +180		34% (95% CI 19–48) at 3 years HR 1.31 (95% CI 0.73–2.32) $p = 0.36$	34% (95% CI 17–50) at 3 years HR 1.78 (95% CI 0.93–3.42) $p = 0.08$
	MSD (total $n = 47$; 94% PBPC)	58 (55–70)	42% (95% CI 27–57)		40% (95% CI 23–56) at 1 year ($p = 0.02$)		23% (95% CI 11–36) at day +180		30% (95% CI 16–44) at 3 years	43% (95% CI 29–58) at 3 years
Majhail et al. (2012)	UCBT ($n = 60$)	61 (55–69)	45% (95% CI 31–58)	21% (95% CI 9–33)	33% (95% CI 20–46)		25% (95% CI 13–37) at 2 years RR 1.22 (95% CI 0.56–2.67) $p = 0.61$	47% (95% CI 33–62) at 2 years RR 0.66 (95% CI 0.33–1.30) $p = 0.23$	22% (95% CI 12–35) at 3 years RR 1.23 (95% CI 0.73–2.09) $p = 0.44$	31% (95% CI 19–44) at 3 years RR 1.31 (95% CI 0.75–2.29), $p = 0.34$
	MSD ($n = 38$)	63 (56–70)	38% (95% CI 22–55), $p = 0.19$	26% (95% CI 11–41), $p = 0.95$	61% (95% CI 40–82), $p = 0.04$		25% (11–40%) at 2 years, $p = .82$	34% (17–50%) at 2 years, $p = 0.19$	34% (17–52%) at 3 years, $p = 0.23$	37% (19–55%) at 3 years, $p = 0.21$

(continued)

Table 14.3 (continued)

Reference	Graft (*n* patients)	Age median (range), in years	II–IV acute GvHD	III–IV acute GvHD	Chronic GvHD	Extensive chronic GvHD	TRM	Relapse	Disease-free survival	Overall survival/HR of mortality
Brunstein et al. (2011)	dUCBT ($n = 50$)	58 (16–69)	40% (95% CI 26–54)	21% (95% CI 6–37)	25% (95% CI 12–39) at 1 year		24% (95% CI 11–36) at 1 year	31% (95% CI 17–44) at 1 year	46% (95% CI 31–60) at 1 year	54% (95% CI, 38–67) at 1 year
	Haplo-BM ($n = 50$)	48 (7–70)	32% (95% CI 19–45)	0%	13% (95% CI 3–23) at 1 year		7% (95% CI 0–15) at 1 year	45% (95% CI, 30–61) at 1 year	48% (95% CI 32–62)	62% (95% CI 44–76) at 1 year
Brunstein et al. (2012)	dUCBT-TCF regimen ($n = 121$)	55 (23–68)	50% (95% CI 41–58)	17% (95% CI 11–25)	34% (95% CI, 25–43) at 2 years		19% (95% CI 11–26) at 2 years	49% (95% CI 38–59) at 2 years	31% (95% CI 22–41) at 2 years	37% (95% CI 28–48) at 2 years
	dUCBT-other regimen ($n = 40$)	48 (21–67)	33% (95% CI 19–48)	18% (95% CI, 8–31)	36% (95% CI 22–51) at 2 years		52% (95% CI 33–71) at 2 years	35% (95% CI 19–50) at 2 years	15% (95% CI 2–28) at 2 years	19% (95% CI 4–34) at 2 years
	8/8-PBPC ($n = 313$)	59 (23–69)	33% (95% CI 28–39)	14% (95% CI 10–18)	56% (95% CI 50–62) at 2 years		21% (95% CI 16–25) at 2 years	44% (95% CI 38–51) at 2 years	35% (95% CI 30–41) at 2 years	44% (95% CI 38–50) at 2 years
	7/8-PBPC ($n = 111$)	58 (21–69)	40% (95% CI 31–49)	23% (95% CI 16–32)	54% (95% CI 44–64) at 2 years		28% (95% CI 20–37) at 2 years	44% (95% CI 33–54) at 2 years	29% (95% CI 21–38) at 2 years	37% (95% CI 27–46) at 2 years
Chen et al. (2012)	UCBT ($n = 64$)	53 (19–67)	14.1% *(95% CI NR)* at day +200	3.1% *(95% CI NR)*	21.9% *(95% CI NR)* at 2 years		26.9% *(95% CI NR)* at 2 years HR 3.55 (95% CI 1.82–6.92), $p = 0.0002$	42.7% *(95% CI NR)* at 3 years HR 0.68 (95% CI 0.44–1.04), $p = 0.08$	30% *(95% CI NR)*at 3 years HR 1.19 (95% CI 0.83–1.72), $p = 0.35$	46% *(95% CI NR)* at 3 years HR 1.34 (95% CI 0.89–2.03), $p = 0.16$

	URD ($n = 221$; 97% PBPC)	58 (19–73)	20.3% *(95% CI NR)* at day +200; $p = 0.32$	6.8% *(95% CI NR); $p = 0.29$*	53.9%*(95% CI NR)* at 2 years; $p < 0.0001$		10.4% *(95% CI NR)* at 2 years; $p = 0.0009$)	49.8% *(95% CI NR)* at 3 years; $p = 0.09$	40% *(95% CI NR)* at 3 years; $p = 0.47$	50% *(95% CI NR)* at 3 years; $p = 0.49$
Jacobson et al. (2012)	dUCBT ($n = 42$)	49 (20–67)	21% *(95% CI NR)*		24% *(95% CI NR)*		11% *(95% CI NR)* at 2 years	40% *(95% CI NR)*at 2 years	49% *(95% CI NR)* at 2 years	66% *(95% CI NR)* at 2 years
	PBPC – MUD ($n = 102$)	56 (20–73)	12% *(95% CI NR), $p = 0.19$*		54% *(95% CI NR), $p < 0.001$*		11% *(95% CI NR)* at 2 years, $p = 0.63$	32% *(95% CI NR)* at 2 years, $p = 0.87$	57% *(95% CI NR)* at 2 years, $p = 0.88$	68% *(95% CI NR)* at 2 years, $p = 0.93$
Robin et al. (2015)	UCBT ($n = 129$; 62% dUCBT)	57 (20–72)	31% *(95% CI NR)*		23% (95% CI 19–27) at 2 years		42% (95% CI 38–46) at 2 years	30% (95% CI 24–34)	28% (95% CI 24–32) at 2 years	30% (95% CI 26–34) at 2 years
	10/10-HLA PBPC MUD ($n = 379$)	60 (24–76)			44% (95% CI 41–47)at 2 years		32% (95% CI 30–34) at 2 years HR 0.57 (95% CI 0.39–0.83), $p = 0.003$	23% (95% CI 21–25) HR 0.57 (95% CI 0.37–0.90), $p = 0.02$	45% (95% CI 42–48) at 2 years HR 0.57 (95% CI 0.43–0.76), $p = 0.0002$	50% (95% CI 47–53) at 2 years HR 0.56 (95% CI 0.41–0.75), $p = 0.0001$
	9/10-HLA PBPC MUD ($n = 107$)	61 (20–74)			37% (95% CI 31–43) at 2 years, $p = 0.004$		36% (95% CI 31–41) at 2 years, $p = 0.04$ HR 0.71 (95% CI 0.44–1.15), $p = 0.16$	28% (95% CI 23–33), $p = 0.47$ HR 0.70 (95% CI 0.39–1.23), $p = 0.21$	36% (95% CI 30–42) at 2 years, $p < 0.0001$ HR 0.70 (95% CI 0.49–1.01), $p = 0.06$	43% (95% CI 38–48) at 2 years, $p < 0.0001$ HR 0.73 (95% CI 0.50–1.06), $p = 0.10$

(continued)

Table 14.3 (continued)

Reference	Graft (*n* patients)	Age median (range), in years	II–IV acute GvHD	III–IV acute GvHD	Chronic GvHD	Extensive chronic GvHD	TRM	Relapse	Disease-free survival	Overall survival/HR of mortality
Le Bourgeois et al. (2013)	dUCBT-TCF ($n = 39$)	55.5 (22–69)	26% *(95% CI NR)*	8% *(95% CI NR)*	26% *(95% CI NR)*		Day 100: 13% *(95% CI NR)* 2 years: 26% *(95% CI NR)*		50.5% *(95% CI NR)* at 2 years	61% *(95% CI NR)* at 2 years
	PBPC ($n = 52$; 58% sibling donors)	59 (22–70)	31%*(95% CI NR)*, p = N.S.	15%*(95% CI NR)*, p = N.S.	35% *(95% CI NR)*, p = N.S.		Day 100: 0% *(95% CI NR)* $p < 0.01$ at 2 years: 6% *(95% CI NR)*; $p = 0.02$ HR 0.24 (95% CI 0.1–0.7) $p = 0.01$		50% *(95% CI NR)* at 2 years HR 0.75 (95% CI 0.4–1.3) $p = 0.34$	62% *(95% CI NR)* at 2 years HR 0.81 (95% CI 0.4–1.6) $p = 0.5$
Rodrigues et al. (2014)	UCBT ($n = 104$)	48 (18–67)	29% *(95% CI NR)*		26% *(95% CI NR)*at 3 years		29% *(95% CI NR)* at 3 years	28% *(95% CI NR)* at 3 years; P = N.S.	41% *(95% CI NR)* at 3 years	56% *(95% CI NR)* at 3 years
	MUD ($n = 541$)	50 (18–70)	32% *(95% C.I. NR)*, p = N.S.		52% *(95% CI NR)* at 3 years, $p < 0.0001$ HR 2.22 (95% CI 1.45–3.03), $p = 0.0002$		28% *(95% CI NR)* at 3 years; p = N.S. HR 2.22 (95% CI 1.45–3.03), $p = 0.0002$	35% *(95% CI NR)* at 3 years; p = N.S. HR 1.23 (95% CI 0.82–1.89), p = N.S.	36% *(95% CI NR)* at 3 years; p = N.S. HR 1.06 (95% CI 0.80–1.41), p = N.S.	49% *(95% CI NR)* at 3 years; p = N.S. HR 1.14 (95% CI 0.82–1.57), p = N.S.

Weisdorf et al. (2014)	UCBT ($n = 205$; 60% dUCBT)	59 (50–71)	35% (28–41); p = N.S.		28% (95% CI 22–34) at 3 years, $p < 0.0001$		35% (95% CI 28–42) at 3 years Day 100 TRM: HR 2.83 (95% CI 1.73–4.62) $p < 0.0001$ [*vs.* 8/8-HLA URD] Delayed TRM (>100 days): HR 1.00 (95% CI 0.68–1.47) $p = 0.99$ [*vs.* 8/8-HLA URD]	35% (95% CI 28–41) at 3 years HR 1.15 (95% CI 0.85–1.54) $p = 0.36$ [*vs.* 8/8-HLA URD]	28% (95% CI 22–35) Treatment failure HR 1.35 (95% CI 1.09–1.65) $p = 0.005$ [*vs.* 8/8-HLA URD]	30% (95% CI, 23–37) at 3 years HR 1.43 (95% CI 1.16–1.76) $p = 0.0008$ [*vs.* 8/8-HLA URD]
	8/8-HLA URD ($n = 441$; 86% PBPC)	58 (50–75)	36% (32–41)		53% (95% CI 48–58) at 3 years		27% (95% CI, 23–31) at 3 years	35% (95% CI, 30–40) at 3 years	39% (95% CI 34–43)	43% (95% CI 38–48) at 3 years
	7/8-HLA URD ($n = 94$; 85% PBPC)	58 (50–72)	44% (34–54)		59% (95% CI 49–69) at 3 years		41% (95% CI 31–51), at 3 years Day 100 TRM: HR 1.19 (95% CI 0.52–2.72) $p = 0.68$ [*vs.* 8/8-HLA URD] Delayed TRM (>100 days): HR 1.73 (95% CI 1.18–2.54) $p = 0.005$ [*vs.* 8/8-HLA URD]	26% (95% CI 18–35) at 3 years HR 0.86 (95% CI 0.57–1.29) $p = 0.47$ [*vs.* 8/8-HLA URD]	34% (95% CI 24–43) Treatment failure HR 1.18 (95% CI 0.91–1.53) $p = 0.22$ [*vs.* 8/8-HLA URD]	37% (95% CI 27–46) at 3 years HR 1.24 (95% CI 0.95–1.62) $p = 0.12$ [*vs.* 8/8-HLA URD]

(continued)

Table 14.3 (continued)

Reference	Graft (*n* patients)	Age median (range), in years	II–IV acute GvHD	III–IV acute GvHD	Chronic GvHD	Extensive chronic GvHD	TRM	Relapse	Disease-free survival	Overall survival/HR of mortality
Warlick et al. (2015)	UCBT ($n = 151$)	18–74		24% (95% CI 17–31) HR 3.2 (95% CI 1.6–6.2), $p < 0.01$			20% (95% CI 14–26) at 1 year HR 1.4 (95% CI 0.8–2.3), $p = 0.40$	36% (95% CI 28–45) at 2 years HR 1.3 (95% C.I. 0.9–1.9), $p = 0.34$	34% (95% CI 27–42) at 6 years	36% (95% CI 28–44) at 6 years HR 1.3 (95% CI 1.0–1.8), $p = 0.28$
	MRD ($n = 187$; 76% PBPC)	18–74		9% (95% CI, 5% to 13%)			20% (95% CI 14–25) at 1 year	26% (95% CI 19–32) at 2 years	44% (95% CI 37–52) at 6 years	47% (95% CI 39–54) at 6 years
	MUD ($n = 55$; 64% PBPC)	18–74		15% (95% CI 5% to 24%),			25% (95% CI 14–37) at 1 year	20% (95% CI 9–31) at 2 years	50% (95% CI 36–63) at 6 years	54% (95% CI 40–66); at 6 years
	MMUD ($n = 21$; 57% PBPC)	18–74		24% (95% CI 6% to 42%)			14% (95% CI 4–29) at 1 year	33% (95% CI 13–53) at 2 years	39% (95% CI 18–60) at 6 years	51% (95% CI 28% to70%) at 6 years

Malard et al. (2015)	UCBT (n = 205; 52% dUCBT)	49.0 (19.3–69.1)		11.7% *(95% CI NR)*		5.8% *(95% CI NR)* at 2 years	18.6% *(95% CI NR)* at 2 years	39.6% *(95% CI NR)* at 2 years	42.0% (95% CI 35.2–48.7) at 2 years	47.9% (95% CI 40.9–54.6) at 2 years
	9/10-HLA MUD PBPC (n = 99)	55.1 (19.0–68.2)		20.2% *(95% C.I. NR)* 2.61 (95% CI 1.30–5.23) p = 0.007		10.1% *(95% CI NR)* at 2 years HR 1.84 (95% CI 0.68–4.95) p = 0.23	23.2% *(95% CI NR)* at 2 years HR 1.58 (95% CI 0.88–2.83) p = 0.13	27.3% *(95% CI NR)* at 2 years HR 0.62 (95% CI 0.37–1.03) p = 0.07	49.5% (95% CI, 39.3–58.9) at 2 years HR 1.17 (95% CI 0.86–1.65) p = 0.29	50.5% (95% CI 40.3–59.8) at 2 years HR 0.98 (95% CI 0.66–1.45) p = 0.92
	10/10-HLA MUD PBPC (n = 347)	57.1 (19.0–70.3)		13% *(95% CI NR)* HR 1.72 (95% CI 0.093–3.19) p = 0.08		11.5% *(95% CI NR)* at 2 years HR 2.15 (95% CI 0.93–4.97) p = 0.08	16.6% *(95% CI NR)* at 2 years HR 1.05 (95% CI 0.62–1.78) p = 0.85	29.6% *(95% CI NR)* at 2 years HR 0.60 (95% CI 0.39–0.92) p = 0.02	54.0% (95% CI, 48.6–59.1) at 2 years HR 1.10 (95% CI 0.84–1.42) p = 0.49	56.7% (95% CI 51.2–61.8) at 2 years HR 0.74 (95% CI 0.52–1.03) p = 0.08

Abbreviations: *ANC* absolute neutrophil count, *BM* bone marrow, *CI* confidence interval, *dUCBT* double-unit umbilical cord blood transplantation, *DFS* disease-free survival, *GvHD* graft-*versus*-host disease, *HLA* human leukocyte antigen, *HR* hazards ratio, *MA* myeloablative, *MMUD* mismatched unrelated donor, *MRD* matched related donor, *MUD* matched unrelated donor, *NMA* nonmyeloablative, *NR* not reported, *NRM* non-relapse mortality, *NS* nonsignificant, *OR* odd ratio, *PBPC* peripheral blood progenitor cell, *PFS* progression-free survival, *RD* related donor, *RIC* reduced-intensity conditioning, *RR* relative risk, *TCD* T-cell depleted, *TRM* transplant-related mortality, *UCBT* umbilical cord blood transplantation, *URD* unrelated donor

registry study analyzed data from the 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 (Eapen et al. 2010). In this study, the recipients of single-unit UCB transplantation had significantly higher NRM than recipients of BM or PBPC transplantation from 8/8 HLA-matched URDs; however, recipients of single-unit UCB transplantation had similar NRM as compared to recipients of BM or PBPC transplantation from 7/8 HLA-matched URDs (Table 14.1). Of note, 72% of patients in the UCB group received antithymocyte globulin (ATG) as compared with 18% and 28% in the PBPC and BM groups, respectively. In another study from the University of Minnesota and the Fred Hutchinson Cancer Research Center, recipients of myeloablative double-unit UCB transplantation experienced significantly higher early NRM than recipients of grafts from MRDs, matched URDs, or even mismatched URDs (Brunstein et al. 2010) (Table 14.2). Notably, in this report double-unit UCB transplantation recipients who engrafted after 26 days had NRM of 41% as compared to 16% in patients who achieved neutrophil engraftment before 26 days, which was similar to that of other donor types. In contrast, in a Japanese registry study (Takahashi et al. 2007), the risk of NRM after single-unit UCB (n = 171) transplantation was similar to that of matched URD (n = 100) or MRD (n = 71) groups (Table 14.1).

In the setting of *RIC* UCB transplantation, the type of conditioning regimen appears to be an important determinant of outcomes. Studies from the University of Minnesota showed that patients who received RIC with fludarabine 200 mg/m^2, cyclophosphamide 50 mg/kg, and 2 Gy total body irradiation (TCF regimen) had lower NRM at day 100 than patients who received RIC with busulfan with or without cladribine-containing regimens (28% *vs.* 48%) (Majhail et al. 2008, 2012; Barker et al. 2003). This result was also observed in a CIBMTR registry study (Brunstein et al. 2012), which compared double-unit UCB transplantation (n = 161) to PBPCs from 7/8 (n = 111) and 8/8 HLA-matched URDs (n = 313). While NRM was similar after double-unit UCB transplantation with TCF conditioning and 8/8 HLA-matched PBPC groups, patients undergoing double-unit UCB transplantation with other conditioning regimens had higher risk of NRM than those receiving the TCF regimen or the PBPC group. However, the group of patients receiving double-unit UCB transplantation with other conditioning regimens was small (n = 40) and heterogeneous. Similar NRM was observed among recipients of double-unit UCB transplantation and recipients of matched URD grafts in studies by the EBMT and the Eurocord (Rodrigues et al. 2014), where 74% of patients received the TCF regimen, and the Société Française de Greffe de Moelle et de Therapie Cellulaire (Malard et al. 2015), where diverse types of RIC regimens were used (Table 14.3). Recent variations of the TCF regimen including thiotepa (Ponce et al. 2013) or treosulfan (Gyurkocza et al. 2014) have also demonstrated encouraging results with similar NRM, but better disease control (see under Relapse section).

In contrast, other studies in the RIC setting showed significantly higher NRM with double-unit UCB transplantation than with matched URD transplantation

(Chen et al. 2012; Weisdorf et al. 2014; Le Bourgeois et al. 2013). One of these was an analysis of the CIBMTR and the Eurocord registry data comparing double-unit UCB transplantation ($n = 205$) to 8/8 ($n = 441$) and 7/8 ($n = 94$) HLA-matched URD transplantation in older patients with acute myeloid leukemia (AML) (Weisdorf et al. 2014). There were certain differences in the baseline characteristics of different groups, with a higher proportion of unfavorable cytogenetic risk among AML patients in the UCB transplantation group (37%) than in the 8/8 HLA-matched URD group (30%). About 20% of patients in the UCB transplantation group, and 50% in the 8/8 HLA-matched URD group received myeloablative conditioning. Yet, NRM at day 100 (but not beyond that period) was significantly higher in the UCB transplantation group than in the 8/8 HLA-matched URD group. Two other studies including patients with diverse hematological malignancies—one using the TCF regimen (Le Bourgeois et al. 2013) and the other one using fludarabine, melphalan, and ATG (Chen et al. 2012)—also showed higher NRM in recipients of double-unit UCB transplantation than in recipients of PBPCs from HLA-matched URDs.

In summary, the risk of NRM after UCB transplantation is higher than in other donor types, particularly in the myeloablative setting where hematopoietic recovery is prolonged. Older patients, who typically have associated comorbidities and are less likely to tolerate prolonged pancytopenia, are also at risk of higher NRM after UCB transplantation. These observations demonstrate the importance of newer strategies of UCB expansion that now consistently show time to neutrophil engraftment around 15 days (Wagner et al. 2016; Horwitz et al. 2014; de Lima et al. 2012; Delaney et al. 2010). As these techniques are not widely available, appropriate selection of graft and conditioning regimen will at least partly minimize the risk of prolonged pancytopenia and graft rejection, thus reducing the risk of NRM.

14.5 Risk of Relapse Is Not Increased After UCB Transplantation and May in Fact Be Lower After Myeloablative Double-Unit UCB Transplantation as Compared with Other Grafts

In the early days of UCB transplantation, the use of naive T-cells in the graft raised concerns about their ability to mount a graft-*versus*-tumor (GvT) effect. However, extensive data demonstrate that the GvT effect is intact, and relapse rates after UCB transplantation are similar to that of other donor types. This observation is supported by several retrospective studies comparing single- or double-unit UCB to adult donor types in the myeloablative setting (Laughlin et al. 2004; Rocha et al. 2004; Takahashi et al. 2007; Atsuta et al. 2009; Brunstein et al. 2010; Eapen et al. 2010; Liu et al. 2014; Konuma et al. 2014; Gutman et al. 2009) (Tables 14.1 and 14.2). In many studies, the adjusted risk of relapse was similar despite a higher proportion of patients with high-risk or more advanced disease in the UCB group. Some studies suggested a lower risk of relapse after double-unit as compared to single-unit UCB

grafts (Verneris et al. 2009; Rodrigues et al. 2009). Others have also shown a lower risk of relapse after double-unit UCB transplantation as compared to matched related or unrelated donors (Brunstein et al. 2010; Gutman et al. 2009), even after adjusting for the higher risk of early deaths in the UCB group (Brunstein et al. 2010; Gutman et al. 2009). Recent data suggests that the lower risk of relapse after UCB transplantation is explained, at least to some extent, by the higher degree of HLA mismatch typical of this donor type (Wagner et al. 2014; Brunstein et al. 2016).

In the *RIC setting*, most studies have shown that the risk of relapse with UCB transplantation is similar to that of other grafts (Brunstein et al. 2012; Chen et al. 2012; Weisdorf et al. 2014; Majhail et al. 2008, 2012; Malard et al. 2015; Peffault de Latour et al. 2013; Le Bourgeois et al. 2013) (Table 14.3). In contrast, in a retrospective study by the Société Française de Greffe de Moelle et de Therapie Cellulaire (Malard et al. 2015), patients transplanted with 10/10 HLA-matched PBPCs from unrelated donors had a significantly lower risk of relapse (30% at 2 years; $n = 347$) than patients transplanted with double-unit UCB grafts (40% at 2 years; $n = 205$). However, this observation may have been influenced by a higher proportion of patients in the double-unit UCB transplantation group receiving ATG as part of their conditioning regimen (96% *vs.* 24%, $p < 0.001$). Other UCB studies that used more intensive variations of the TCF regimen incorporating thiotepa (Ponce et al. 2013) or treosulfan (Gyurkocza et al. 2014) demonstrated better disease control (2-year relapse rates of 11–27%), which approximates the relapse rates seen after myeloablative regimens (Brunstein et al. 2010; Marks et al. 2014; Liu et al. 2014; Konuma et al. 2014).

14.6 Survival After Double-Unit UCB Transplantation Is Similar to That of Other Donor Types

Most studies found similar disease-free (DFS) and overall survival (OS) after transplantation with UCB or other donor types. In the *double-unit UCB transplantation* setting, survival has been consistently found to be similar to other donor types after myeloablative (Takahashi et al. 2007; Brunstein et al. 2010; Liu et al. 2014; Konuma et al. 2014; Gutman et al. 2009) (Table 14.2) or RIC regimens (Brunstein et al. 2012; Chen et al. 2012; Weisdorf et al. 2014; Majhail et al. 2008, 2012; Malard et al. 2015; Peffault de Latour et al. 2013; Le Bourgeois et al. 2013) (Table 14.3). The findings of studies comparing *single-unit UCB transplantation* to other donor types after myeloablative conditioning have been less consistent (Table 14.1). Some studies showed DFS and OS similar to matched URD (Rocha et al. 2004; Eapen et al. 2010), while other studies showed survival slightly inferior to HLA-matched, but similar to HLA-mismatched URD (Laughlin et al. 2004; Atsuta et al. 2009). The inclusion of more patients with high-risk disease in the UCB transplantation group may have accounted for some of these discrepancies. Also, as survival may differ between patients with myeloid and lymphoid malignancies, differences in the proportion of underlying diseases between UCB and other donor types may have also affected the results.

14.7 Expert Point of View

In the absence of an HLA-matched related donor, UCB is a valuable graft source, and available evidence supports that its outcomes are similar to those of matched or mismatched URD transplantation. The rapid availability of single- or double-unit UCB grafts for almost all patients may favor its utilization in cases where transplantation is urgent (Atsuta et al. 2009; Marks et al. 2014; Rodrigues et al. 2009). The lower risk of chronic GvHD after UCB transplantation should also be taken into consideration, as it reduces late posttransplantation morbidity as compared with matched and mismatched URD. The use of UCB is further supported by emerging data that suggests similar NRM, relapse, DFS, and OS but significantly lower risk of chronic GvHD after UCB transplantation than after 8/8 HLA-matched URD transplantation when matched for disease risk index (Bejanyan et al. 2015). Therefore, upfront UCB transplantation should be considered particularly for those with high-risk hematological malignancies who need transplantation urgently. In addition, data demonstrating similar long-term outcomes when transplantation is nonurgent suggests that the use of UCB or another alternative adult donor will depend on center expertise and open protocols.

14.8 Future Directions

The main barrier to successful UCB transplantation remains delayed hematopoietic recovery as compared to other donor types. Novel ex vivo expansion techniques, which yield similarly rapid engraftment as that of PBPC or BM grafts, have the potential to improve short and long-term outcomes and improve the efficacy of UCB transplantation.

Similar to UCB transplantation, partially matched related (haploidentical) HCT can also be performed on an urgent basis and extends donor availability almost universally. Although these two approaches are the most common mismatched HCT currently pursued, their comparative data are currently lacking. Limited data from two parallel studies by the Blood and Marrow Transplant Clinical Trials Network suggested similar outcomes after these two types of transplantations in the RIC setting (Brunstein et al. 2011). However, the small number and heterogeneity of patients enrolled in each study limit conclusions. These studies provided the equipoise for the ongoing phase III randomized study comparing partially matched related and double-unit UCB donors in the RIC setting, the results of which are eagerly awaited (NCT0159778).

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Limitations and Unique Complications of Umbilical Cord Blood Transplantation

15

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15.1 Limitations of Umbilical Cord Blood Transplantation

Over the past three decades, the efficacy of UCB transplant (UCBT) has significantly advanced. In both the pediatric and adult settings, and in spite of delayed neutrophil and platelet engraftment which do lead to increased early non-relapse mortality (NRM), reported outcomes after UCBT have been comparable (overall survival (OS) and disease-free survival (DFS)) or in some cases superior (chronic GvHD and relapse) to that of a HLA-matched related (MRD) or HLA-matched unrelated (MUD) donor transplant in retrospective analyses (Wagner et al. 1996, 2002; Kurtzberg et al. 1996; Gluckman et al. 2004; Locatelli et al. 1999; Bizzetto et al. 2011; Rocha et al. 2001; Eapen et al. 2007; Hwang et al. 2007). It offers equivalent or even superior outcomes to HLA-mismatched unrelated transplants and appears to have a lower relapse rate than does haploidentical transplant for advanced hematologic malignancies. Recently reported is improved immune reconstitution as measured by total lymphocyte count and achievement of full donor chimerism after UCBT, as compared to mismatched related or haploidentical HCT (Fernandes et al. 2012). The availability of more banked UCB units and the improved understanding of graft selection have increased the chances of finding an optimal UCB graft for more recipients, and time to transplant is decreased due to readily available units in established cord banks. In addition, reduced-intensity conditioning (RIC) has extended UCBT to older and less fit individuals, making it appealing for those 70%

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_15

of the transplant-eligible population lacking an HLA-matched related donor. Yet there are both unique challenges and complications associated with this form of transplantation that deserve attention from those who perform these procedures.

15.1.1 Importance of Cell Dose

Unlike living donors, the dose of UCB cells at collection is fixed. Typically, UCB grafts given to adults have a one log lower total nucleated cell dose and a five log fewer number of lymphocytes as compared to living adult donors. Remarkably, engraftment and hematopoietic reconstitution still occur and may be explained by the higher concentration of more proliferative stem cells in UCB and other biological aspects of UCB stem cells, including longer telomeres, more rapid exit from the growth phases of the cell cycle, and increased sensitivity to cytokines and paracrine activity (Mold and McCune 2012; Mayani et al. 1993; Burlingham 2009; Vormoor et al. 1994). Initially limited to children because of smaller recipient size with lower cell dose requirements, UCBT has now been successfully employed in adult transplantation as one of the most popular alternative donor sources.

The first UCBT experience in adults reported a high day 100 transplant-related mortality (TRM) of 40% (Laughlin et al. 2001). Analysis of this group of adults showed that more rapid neutrophil recovery was associated with higher total nucleated cell (TNC) dose and higher CD34 cell dose was associated with improved event-free survival ($p = 0.05$). Other groups have confirmed the importance of cell dose, showing a correlation between cell dose (specifically CD34/kg dose) and rapidity of neutrophil and platelet recovery, as well as cell dose and reliable engraftment and lower TRM (Locatelli et al. 1999; Laughlin et al. 2001; Sobol et al. 2015; Migliaccio et al. 2000; Sanz et al. 2001; Ooi et al. 2002). Median time to neutrophil recovery for unmanipulated single UCB transplants is now in the day +20–25 range, exceeding that typically reported in living donor allograft transplants. Median time to platelet engraftment is in the day +40–50 day range, again exceeding that typically occurring in adult allograft transplants (Brunstein and Laughlin 2010; Sanz et al. 2010; Satwani et al. 2013; Eapen et al. 2010; Le Bourgeois et al. 2013; Laughlin et al. 2004). This delayed engraftment and hematopoietic recovery is associated with increased non-relapse-related mortality (NRM), as well as increased length of hospital stay, transfusion needs, and costs. Thus, centers acknowledge the shortcomings of UCB to be largely associated with its limited cell doses. Low cell doses are associated not only with delayed engraftment and graft failure but also invasive fungal infection, death from GvHD, other infection, and relapse (Bortin et al. 1983; Ringden et al. 1985, 2003; Ringden and Nilsson 1985; Storb et al. 1977; Tollemar et al. 1989). Subsequent UCBT experiences achieved improved outcomes with more attention to cell dose, along with better supportive care, and optimized patient selection (Cohen et al. 2011; Ooi et al. 2008; Robin et al. 2011; Sanz et al. 2012).

However, cell dose alone does not account for the entire picture, with the degree of HLA match contributing to outcomes. In a large retrospective review of 1067 single UCB transplants, greater HLA mismatch required higher cell doses, when considering TRM as the endpoint (Barker et al. 2010). Specifically, a minimum cell dose of TNC 5.0 × 10(7)/kg with 4/6 HLA-matched UCB units was required to match the TRM of 5/6 HLA-matched UCB units with minimum cell dose of TNC >2.5 × 10 (7) /kg. It should be noted though that this study employed intermediate resolution typing at HLA-A and -B and high resolution typing only at HLA-DRB1, which is the current standard. More recently, it has been reported that less HLA-matched UCB units (<6/8 *versus* = >6/8) at the allele level were for the match in the graft-*versus*-host direction, associated with decreased relapse and improved leukemia-free survival for patients undergoing myeloablative conditioning (Sanz et al. 2014a). Continued efforts utilizing registry-sized studies are needed to further clarify the best cell dose/HLA-matching algorithm to select the optimal UCB unit.

However HLA match, cell, and CD34/kg infused still do not explain all of the 5–20% of cases of delayed, incomplete, or total failure of engraftment. Page et al. have found however that in contrast to NC and CD34/kg, a more robust *in vitro* growth of cells both before and after cryopreservation as measured by colony-forming units (CFU) was predictive of engraftment with post-thaw CFU having the highest correlation for both neutrophil and platelet engraftment (Page et al. 2011). They subsequently developed a composite score that predicted engraftment of neutrophils based on pre- and post-thawing graft characteristics, including NC, $CD34^+$, mononuclear cells, CFU cell doses, and volume of CB collected (Page et al. 2012). It is important to note that a lack of growth in CFU after thawing is usually an indication for graft failure.

Nonetheless, NC dose continues to define units for transplantation with UCB transplant programs having specified minimum cell dose requirements, which range from 1.0 to 2.5 × 10(7) TNC/kg depending on the program's approach, in order to maximize rate of engraftment and minimize time to count recovery and TRM. Many programs advocate a minimum UCB dose of 2.5 × 10(7) TNC/kg and a 4/6 NHL match. Using these criteria upward of 90% of adults will have at least a single donor found for them. Engraftment is slower for those at the lower end as described leading a number of initiatives as described earlier in this volume, including infusing two UCB units (Cutler et al. 2011; Ballen et al. 2007), co-infusing another cell graft (such as a peripheral blood haploidentical graft) (Liu et al. 2011; Fernandez et al. 2003; Kwon et al. 2014), ex vivo expanding a UCB graft (Delaney et al. 2010; de Lima et al. 2012), and techniques aimed at improving homing, such as intraosseous administration of UCB cells (Frassoni et al. 2008; Brunstein et al. 2009; Rocha et al. 2013), administration of agents to enhance homing, such as with fucosylation (Xia et al. 2004; Robinson et al. 2012), or by prostaglandin E2 modulation (Hoggatt et al. 2009; Cutler et al. 2013). There has been some success with these various approaches, and the best approach remains unclear. Efforts to advance and improve on UCBT outcomes continue, and numerous clinical trials are underway. Table 15.1 summarizes the clinical implications and counteractive strategies that apply to UCB cell dose limitations.

Table 15.1 UCB cell dose implications

Clinical consequences	Counteractive strategies
Delayed engraftment (Bortin et al. 1983; Ringden et al. 2003, 1985; Ringden and Nilsson 1985; Storb et al. 1977; Tollemar et al. 1989) ↑ NRM ↑ Rates of infection ↑ Transfusion requirements Longer hospital stay	Minimum cell dose requirement: 1.5–2.5 × 10(7) TNC/kg dUCB transplant (Cutler et al. 2011; Ballen et al. 2007) Co-infusion of another graft source (Liu et al. 2011; Fernandez et al. 2003; Kwon et al. 2014) Ex vivo expansion of UCB unit (Delaney et al. 2010; de Lima et al. 2012) Techniques to improve homing (Frassoni et al. 2008; Brunstein et al. 2009; Rocha et al. 2013)

Abbreviations: *NRM* non-relapse mortality, *TNC* total nucleated cells, *dUCB* double umbilical cord blood

15.1.2 Graft Failure

Graft failure (GF) is one of the most feared complications after allogeneic Hematopoietic cell transplant (HCT). Variably defined in the literature by failure to achieve neutrophil engraftment and/or full or majority donor chimerism by a milestone day (typically for living donors as day +28), it invariably represents failure of sustained hematopoietic function by the intended graft. Particularly in UCB transplant, time to engraftment can be prolonged, making it difficult to establish a universal definition of GF.

Factors associated with increased risk of graft failure after allogeneic HCT include the degree of HLA mismatch (including at HLA-C) (Crocchiolo et al. 2009; Petersdorf et al. 1998; Olsson et al. 2013), use of unrelated grafts (Satwani et al. 2013; Olsson et al. 2013), graft manipulation (e.g., T-cell depletion) (Marmont et al. 1991), low cell dose (Sanz et al. 2010), use of reduced-intensity conditioning (RIC) (Olsson et al. 2013; Le Blanc et al. 2004), allosensitization and presence of donor-specific antibodies (DSA) (Ruggeri et al. 2013; Takanashi et al. 2008), diseases with altered bone marrow microenvironment and immune system (e.g., severe aplastic anemia, myelofibrosis) (Stucki et al. 1998), nonmalignant disorders or chemo-naïve patients (Satwani et al. 2013), and major ABO mismatch (Remberger et al. 2007; Badros et al. 2002). Additional factors may affect hematopoietic recovery or stem cell function even after engraftment, such as infections in the recipient (Mattsson et al. 2008) and drug toxicity and rejection, which is thought to be recipient immune mediated, involving T lymphocytes (Raff et al. 1986; Cudkowicz and Bennett 1971), natural killer (NK) cells (Kiessling et al. 1977; Murphy et al. 1987a, b), and donor-specific antibodies (Nordlander et al. 2008; Warren et al. 1981; Taylor et al. 2007).

GF is a relatively rare occurrence in adult allogeneic transplantation (e.g., matched related and matched unrelated HCT), occurring in <5% of transplants (Hansen et al. 1998; Kernan et al. 1993; Davies et al. 1994; Wolff 2002), but is most commonly described in the setting of aplastic anemia and/or with the use of T-cell-depleted grafts (Storb et al. 1983). However, increasing unrelated and alternative

Table 15.2 Risk factors for UCB graft failure (Sanz et al. 2010; Satwani et al. 2013; Rocha et al. 2004; Chan et al. 2008)

Greater degree of HLA mismatch
Lower cell dose (TNC and CD34/kg)
RIC/NMA conditioning (Le Blanc et al. 2004)
Allosensitization (Olsson et al. 2013)

Abbreviations: HLA human leukocyte antigen, *TNC* total nucleated cells, *RIC* reduced-intensity conditioning, *NMA* nonmyeloablative

donor transplantation, along with increasing use of reduced-intensity conditioning regimens, has led to increased graft failure (Kernan et al. 1993). UCBT specifically has been associated with increased risk of graft failure (Rocha et al. 2001; Satwani et al. 2013; Rocha and Gluckman 2009; Brunstein et al. 2011a; Ruggeri et al. 2011) (Table 15.2). This has been described both in the MA and RIC settings (Laughlin et al. 2001; Narimatsu et al. 2006) but is not universally significant in all UCBT reports (Olsson et al. 2013). Reported rates of cord graft failure (CGF) in UCB transplantation range from 10% to 30%, with risk factors of greater HLA mismatch, lower cell dose (TNC and CD34/kg), and reduced-intensity conditioning (Sanz et al. 2010; Satwani et al. 2013; Rocha et al. 2004; Chan et al. 2008). HLA-sensitized patients (by blood transfusions or pregnancy) also have an increased risk of graft failure, due to production of memory T-cells and donor-specific antibodies (DSA) in response to childbirth or transfusions that subsequently recognize major and minor histocompatibility antigens on donor cells (Olsson et al. 2013). Leukodepletion and irradiation of transfusion products can minimize the risk of sensitization in those requiring numerous transfusions (Storb et al. 1977; Bean et al. 1991, 1994). Additional strategies being tested for those with no other available donors include those used to reduce DSA titers in solid organ transplants.

With many transplant-requiring diseases, i.e., AML and MDS occurring at ages of 60 and above, the incorporation of RIC or nonmyeloablative conditioning (NMAC) has been crucial in extending HCT to older and less fit patients who would otherwise not tolerate fully myeloablative regimens. However, the lower-intensity regimens may allow persistence of host immune function, resulting in increased risk of graft rejection (Le Blanc et al. 2004). Thus, theoretically, the use of RIC/NMAC may compound the increased risk of GF inherent in UCBT; however, this is not clearly demonstrated in an analysis of multiple earlier RIC/NMAC UCBT experiences, which showed comparable overall survival and disease-free survival with that of younger, fitter patients transplanted with myeloablative transplantation (Schoemans et al. 2006).

However the use of RIC and NMAC in contrast to myeloablative regimens results in higher incidence of recovered autologous reconstitution after CGF. One group reported median survival of 2.9 months compared to 13.7 months in those without and with autologous reconstitution, respectively, though the 5-year overall survival was not significantly different (19% and 11%, respectively) (Rondon et al.

2008). Causes of death were graft failure (18%), infection (27%), and original malignancy (41%). In brief, CGF with autologous reconstitution may result in longer short-term survival due to count recovery, though most will still die from their original disease.

Early identification of graft failure in UCBT may facilitate successful intervention. Donor chimerism <5% in the unfractionated compartment at day 28 (Tsai et al. 2016), day 35 (Cimino et al. 1999), or at any point (Chan et al. 2008) has been reported to be associated with CGF. Additionally, mixed chimerism before day 35 or significant fall in chimerism has also been reported to predict for CGF and a high probability of leukemic relapse (Cimino et al. 1999). When there is concern for impending GF, aggressive reassessment of the patient is crucial, including a review of all medications, eliminating any potentially stem cell toxic drugs, assessing for any infections especially viruses (CMV, HHV-6, parvovirus, etc.), institution of growth factor support, and planning for a near-future intervention, such as additional cell therapy.

Management of GF is typically dictated by degree of hematopoietic function or disease relapse/progression, as well as by individual transplant center preferences and expertise, and might include infusion of a previously collected autologous unit, a second UCBT, a haploidentical transplant, use of growth factors, and an increase in recipient immunosuppression. Some advocate for collection of an autologous unit prior to transplant in those at high risk for GF. In one study, infusion of an autologous unit after GF from differing allogeneic sources resulted in neutrophil recovery in 7/10 patients with a median survival of 21 months (Rondon et al. 2008). However, this is not always feasible or practical due to poor mobilization and reserve, high risk of contamination by cancer cells, relapse after engraftment, and perceived high cost-benefit ratio. A majority of non-US transplant centers store a secondary hematopoietic stem cell aliquot for MUD HCT and 50% for MRD HCT (Pottinger et al. 2002; Tan et al. 2011).

Second transplants after GF usually are largely unsuccessful with an early mortality rate exceeding 50% and poor long-term survival (Davies et al. 1994; Narimatsu et al. 2006; Guardiola et al. 2000; Grandage et al. 1998). This is due to the combined prolonged cytopenias in the recipient, diminished recipient performance status, and organ damage from previous cytoreductive therapies. Five-year overall survival rates at 1, 2, and 5 years have been reported as low as 31%, 24%, and 15%, respectively, in 68 graft failures out of 1726 patients who underwent allogeneic transplant between 1990 and 2000 at a single institution (Rondon et al. 2008). The only factor significantly associated with OS was a diagnosis of acute leukemia (myeloblastic or lymphoblastic). Age, sex, disease risk, conditioning regimen (TBI containing, intensity, etc.), and donor type (MRD, MUD, MMRD, MMUD) were not associated. In spite of achieving hematopoietic recovery after second allogeneic transplant for GF, outcomes remain poor. A less cytoreductive and more immunosuppressive regimen may suffice, to reduce toxicities (Narimatsu et al. 2006; Chan et al. 2008). The optimal graft source for a second transplant is not clear. Haploidentical and UCB grafts are readily obtained in a majority of people, and both have had reported

successes in salvage transplant after graft failure (Yoshihara et al. 2012; Tang et al. 2015; Salit et al. 2016; Ishiyama et al. 2016).

Infectious complications account for most early deaths after second transplant including viral infection even after myeloid recovery. This is likely due to the relatively slow immune recovery, at least in part due to additional immunosuppressive conditioning given, coupled with a lack of adoptive transfer of donor immunity, specifically in second UCBT (Barker et al. 2005; Sashihara et al. 2002; Parody et al. 2006). Special attention should be paid toward monitoring for the more common posttransplant viral infection after second transplant, such as EBV and CMV. Chronic GvHD after second UCB transplant also seems to be increased relative to first UCBT and is associated with not only defective immune reconstitution and infection complications but also diminished quality of life and overall health (Chan et al. 2008; Ohwada et al. 2004; Fernandes et al. 2007).

Ideally graft failure should be avoided given dismal outcomes of salvage therapies after GF. To reduce the risk of CGF, attention should be paid to a high titer of DSA, conditioning regimen, cell/stem cell/CFU cell dose, and the immunosuppression protocol should be carefully selected. Continued efforts to better understand these aspects of transplantation will hopefully reduce future rates of graft failure.

15.1.3 Relapse

The efficacy of allogeneic Hematopoietic cell transplant (HCT) relies on the provision of a new immune system to the recipient to produce a graft-*versus*-tumor (GvT) effect, mediated primarily by donor lymphocytes. Long-term disease control or cure may subsequently be achieved by the GvT effect. However, allogeneic transplantation is not nearly as successful as one would hope. Relapse remains the leading cause of transplant failure after allogeneic Hematopoietic cell transplant (HCT), including umbilical cord blood transplantation (UCBT). Common strategies after relapse include withdrawal of immunosuppression, further chemotherapy either to facilitate a graft-*versus*-tumor effect or in preparation for a second allogeneic transplant, clinical trials, additional cellular therapy when available, or best supportive care. Such strategies are also possible after relapse after UCBT, except for additional cellular therapies, as the donor source (the fetus) is no longer available for additional cell collection. Outcomes after relapse posttransplant remain suboptimal, as even those undergoing intensive treatment who achieve a second remission remain at high risk of death from relapse, with a 1-year survival reported as 22% by one group (Bejanyan et al. 2014).

Factors associated with leukemia relapse after adult UCBT (Table 15.3) include advanced disease stage (Ooi et al. 2009; Michel et al. 2003; Arcese et al. 2006), poor-risk cytogenetics (Ooi et al. 2009), recipient cytomegalovirus seronegativitiy (Iori et al. 2004), HLA match between UCB and recipient (Gluckman et al. 2004), and cell dose (Verneris et al. 2009; Parkman et al. 2006). In a registry study, factors associated with lower mortality after relapse include longer time from HCT to

Table 15.3 Risk factors of relapse after UCBT

Advanced disease stage (Ooi et al. 2009; Michel et al. 2003; Arcese et al. 2006)
Poor-risk cytogenetics (Ooi et al. 2009)
Recipient CMV seronegativity (Iori et al. 2004)
Higher degree of HLA match between UCB and recipient (Gluckman et al. 2004)
Lower cell dose (Verneris et al. 2009; Parkman et al. 2006)
RIC/NMA conditioning

Abbreviations: UCBT umbilical cord blood transplant, *CMV* cytomegalovirus, *HLA* human leukocyte antigen, *TNC* total nucleated cells, *RIC* reduced-intensity conditioning, *NMA* nonmyeloablative

relapse and first HCT using reduced-intensity conditioning. Factors associated with worse outcomes after relapse include age >40 years, active acute GvHD at time or relapse, adverse cytogenetics, and use of mismatched unrelated or UCB donors in the first HCT (Bejanyan et al. 2014, 2015).

Initially, there was concern about diminished graft-*versus*-tumor (GvT) effect in UCB due to fivefold fewer lymphocytes, compared to adult bone marrow grafts, coupled with the naïvety of the fetal lymphocytes. However, these concerns were not born out with multiple UCBT experiences reporting similar relapse rates, compared to adult allograft transplants (Eapen et al. 2007, 2010; Laughlin et al. 2004; Rocha et al. 2004; Peffault de Latour et al. 2013). In certain cases, relapse rate was lower than with other adult allografts, including in the double UCB (Eapen et al. 2007; Verneris et al. 2009; Brunstein et al. 2010) setting. Remarkably, this is without increased rates of graft-*versus*-host disease (GvHD) or in some cases with decreased GvHD, both in the single (Eapen et al. 2010; Takahashi et al. 2007; Chen et al. 2013) and double (Gutman et al. 2016) UCB setting, though there is possible increased acute or extensive chronic GvHD in the double *versus* single UCB setting (Michel et al. 2016; Brunstein et al. 2007).

Some have attributed decreased relapse to increased HLA mismatch, reporting lower relapse in 4/6 matched UCB units (Eapen et al. 2007; Sanz et al. 2014a; Rocha and Gluckman 2009). However, this would imply that haploidentical transplant, with higher degree of HLA mismatch than a 4/6 or greater matched UCB, would also demonstrate lower relapse rates. This has not been the case, including in two parallel, nearly identically designed single arm trials using haploidentical or double UCB transplantation (Luznik et al. 2008; Brunstein et al. 2011b). Killer cell immunoglobulin-like receptor (KIR)-ligand mismatch has also been implicated (Willemze et al. 2009). But since KIR mismatch also occurs in adult allograft transplant, there must be other factors in play. Another hypothesis suspects that decreased relapse may be related to maternal cells sensitized against inherited

paternal antigens (IPA) by the fetus, which circulate in the maternal-fetal circulation in utero. These sensitized maternal cells may cross into the fetal circulation to be collected in the UCB unit as remaining maternal microchimerism. In a large retrospective review of the New York Blood Center National Cord Blood Program, including 1403 UCB transplants, they found that recipients who shared one or more HLA-A, -B, or DRB1 antigens with their UCB donor's IPAs had significantly decreased relapse, compared to those without shared IPAs (HR = 0.38, $p < 0.001$) (van Rood et al. 2012). Importantly, this was without significantly increased GvHD. UCB clearly represents an immunologically complex graft, differing markedly from that in adult allografts. Further understanding of its immunobiology may facilitate optimal application in the future.

15.2 Unique/Augmented Complications of Umbilical Cord Blood Transplantation

Non-relapse-related mortality (NRM) is a major cause of transplant failure and is a big consideration prior to deciding whether one should proceed with allogeneic hematopoietic stem cell transplantation. Typical causes of NRM in allografts include infections (bacterial, viral, and fungal), graft-*versus*-host disease (GvHD), graft failure, organ damage/failure, and other causes. UCBT, in comparison to adult allografts, is associated with differing if not increased NRM due to lower cell doses, which is associated with delayed engraftment and infections, slower immune reconstitution, and higher rates of graft failure (Eapen et al. 2007, 2010). However, many other groups have reported comparable rates of NRM with that of adult allograft transplants (Sanz et al. 2010; Peffault de Latour et al. 2013; Takahashi et al. 2007). Even when NRM has been shown to be higher than living donor allografts, overall survival and leukemia-free survival is often comparable due to decreased rates of GvHD or relapse.

Potentially lethal complications occur in the peri-engraftment period and include neutropenic infections and thrombocytopenic bleeding and mucosal and other direct toxicities related to the preparative regimen and engraftment syndrome and later caused by pulmonary edema syndromes, which can occur due to iatrogenic fluid overload, pulmonary infections and cardiac dysfunction, infectious complications, hepatic veno-occlusive disease (VOD), and acute graft-*versus*-host disease (GvHD).

Unrelated UCB has been associated with some unique complications that are not typically seen or are seen in higher percentages as compared to other graft sources. These unique complications include pre-engraftment and engraftment syndromes, cord colitis syndrome, and infectious complications that are more commonly seen with UCB transplant recipients compared to those who received grafts from a living matched or unmatched sibling or unrelated donor. Table 15.4 outlines the unique pre-engraftment and cord colitis syndromes.

Table 15.4 Unique complications to UCBT

Complication	Etiology	Clinical presentation	Incidence	Onset	Risk factors	Treatment
Pre-engraftment syndrome (Park et al. 2013; Hong et al. 2013; Kanda et al. 2013; Patel et al. 2010; Brownback et al. 2014; Morita-Hoshi et al. 2010; Saliba et al. 2007)	Poorly understood Likely interaction between cytokine release triggered by neutrophil regeneration and SC expansion early post-UCBT Possible role of graft-*versus*-host reaction by UCB T-cells	*Typically occurring >5 days prior to engraftment* °Noninfectious fever Skin rash Diarrhea Noncardiogenic pulmonary edema, tachypnea, and/or hypoxemia Weight gain Jaundice	21–77%	11–20 days before neutrophil engraftment 9–13 days post-UCB infusion	dUCBT TBI-based conditioning MA conditioning Higher counts of CD34/kg and TNC/kg CsA use	IV methylprednisolone 0.5–1 mg/kg/day x 3–5 days without taper *Note: mild cases might be self-limiting*
Cord colitis (Herrera et al. 2011; Magenau and Reddy 2014; van Bekkum et al. 2011; Matuchansky 2011; Bhatt et al. 2013; Gorkiewicz et al. 2013)	Poorly understood; suggested mechanisms include: Delayed or atypical GvHD TRAGI T. whipplei infection *B. enterica* infection Overgrowth of *B. fragilis*	*Symptoms*: Persistent diarrhea (typically >7 days) Weight loss Fever *Colonoscopy*: Erythematous mucosa with or without ulceration, no pseudomembranes *Pathology*: Chronic active colitis, basal plasmacytosis, architectural distortion, surface epithelia injury, neutrophilic granulomas, negative microbial cultures *Imaging*: Nonspecific, focal, or diffuse colonic wall thickening	Approx. 10%	Median of 131 days after UCBT	UCBT is the only known risk factors *No clear association between incidence and any other allo-HCT variables*	2-week course of antibacterial agents with adequate gut coverage (Ex. Fluoroquinolone + metronidazole) *May repeat course if symptoms persist or relapse*

Abbreviations: *UCB* umbilical cord blood, *UCBT* UCB transplant, *dUCBT* double UCBT, *TBI* total body irradiation, *MA* myeloablative, *TNC* total nucleated cells, *CsA* cyclosporine-A, *GvHD* graft-*versus*-host disease, *TRAGI* transfusion-related acute gut injury, *T. whipplei* Tropheryma whipplei, *B. enterica Bradyrhizobium enterica*, *B. fragilis Bacteroides fragilis*

15.2.1 Pre-Engraftment Syndrome

Posttransplant immune reactions seen at the time of neutrophil engraftment can be subdivided into pre-engraftment syndrome, engraftment syndrome, and acute GvHD.

Engraftment syndrome (ES) is a post-HCT complication that manifests with a constellation of symptoms including fever, erythematous skin rash, diarrhea, and noncardiogenic pulmonary edema that occurs in the absence of an infectious etiology. Multiple diagnostic criteria have been proposed to define ES which makes interpretation of its true incidence across the different studies challenging. The most widely accepted definition is the Spitzer's criteria (Spitzer 2001) which include three major criteria (noninfectious temperature of 38.3 °C, erythematous skin rash, and noncardiogenic pulmonary edema with hypoxia) and four minor criteria (hepatic dysfunction, renal insufficiency, weight gain, and transient encephalopathy). Diagnosis is established by the presence of all major criteria and one or more minor criteria within 96 h of neutrophil engraftment. The pathogenesis of ES is not clearly understood, but it is thought to be multifactorial and involve cellular interactions of T-cells, monocytes, complement activation and pro-inflammatory cytokine release, and activation in correlation with neutrophil regeneration during the posttransplant period (Spitzer 2001; Takatsuka et al. 2000).

Pre-engraftment syndrome (PES), a syndrome similar to ES in terms of clinical picture yet unique in the timing of its occurrence, has been reported more frequently in UCB transplant recipients as compared to other types of allogeneic transplant. PES was first described in 2005 by Kishi et al. in patients receiving reduced-intensity UCB transplant (Kishi et al. 2005). The incidence of PES following UCB transplant has been reported as 20–70% across the different studies (Kishi et al. 2005; Narimatsu et al. 2007). PES is more frequently reported in UCB transplant recipients; however, Lee et al. (2008) have reported that PES can still occur with other graft sources. As in ES, a uniform definition for PES is lacking, and the clinical significance and outcomes are limited by the variable definitions of the syndrome and the limited number of patients in each study.

15.2.1.1 Clinical Presentation

The term is commonly used to describe a clinical syndrome of *pre-engraftment* noninfectious fever with either one or more of the following: skin rash, diarrhea, noncardiogenic pulmonary edema, weight gain, and jaundice occurring >5 days prior to engraftment (Narimatsu et al. 2007; Lee et al. 2008; Park et al. 2013; Hong et al. 2013; Kanda et al. 2013; Patel et al. 2010). Pulmonary manifestations of PES are variable. Tachypnea, hypoxemia, and pulmonary edema have been reported in 13–69% of patients with PES (Park et al. 2013; Kanda et al. 2013; Patel et al. 2010), with hypoxemia reported in nearly 50% of patients (Brownback et al. 2014).

15.2.1.2 Pathophysiology

The pathogenesis of PES is poorly understood and has been suggested to develop by the interaction of two simultaneous mechanisms: one related to cytokine release triggered by neutrophil regeneration and stem cell expansion in the early

posttransplant period along with graft-*versus*-host reaction against major and minor mismatched antigens by donor T-cells (Kanda et al. 2013; Morita-Hoshi et al. 2010; Saliba et al. 2007).

The support for the first mechanism comes from Park et al. (2013) who noted that PES also developed in some patients who had not achieved engraftment, suggesting that the mechanism of PES differs from that of engraftment syndrome or GvHD. Furthermore, there was no significant difference in the median time to neutrophil engraftment between patients who had PES *versus* those who did not. This observation was reported by other studies as well (Takahashi et al. 2010; Szabolcs 2010); one possible explanation is that PES includes induction of a cytokine storm by mediators that already existed in the donor cord blood cells independent of any interaction with the recipient's systems.

15.2.1.3 Incidence and Onset

The incidence of PES varies across studies as a result of different criteria used to define the syndrome. It ranges from 21.4% up to 77%, noted to be highest in transplants using double UCB (dUCB) units and in those who receive conditioning regimens containing total body irradiation (TBI) (Lee et al. 2008; Park et al. 2013; Kanda et al. 2013; Patel et al. 2010; Brownback et al. 2014). The median time of onset of PES is around 11–20 days before neutrophil engraftment which is approximately 9–13 days post-UCB infusion (Park et al. 2013; Kanda et al. 2013; Patel et al. 2010; Brownback et al. 2014).

15.2.1.4 Risk Factors

Multiple studies agree that the use of myeloablative conditioning regimens as well as TBI-based conditioning regimens is associated with increased risk of developing PES (Park et al. 2013; Hong et al. 2013; Brownback et al. 2014). Transplantation of dUCB units is also associated with higher PES risk (Hong et al. 2013; Kanda et al. 2013). This may be explained by the biological nature of graft-to-graft interaction followed by single-unit dominance, where graft-*versus*-graft immune reaction involves a complex process that includes a cascade of pro-inflammatory mediators (Hong et al. 2013).

Interestingly, some have reported that high numbers of $CD34^+$ cells/kg and TNC/kg were significantly associated with PES (Park et al. 2013). However, this association remains questionable as it was also found that, when seen with transplants from other graft sources (matched unrelated, matched sibling, haploidentical, and autologous), no significant differences were found between patients with regard to gender, age, body weight, ABO incompatibility, HLA disparity, infused cell dose, and starting date of GCSF treatment (Lee et al. 2008; Patel et al. 2010).

The choice of GvHD prophylaxis regimen is thought to affect the incidence of PES (Park et al. 2013; Kanda et al. 2013; Iguchi et al. 2016). Methotrexate-based prophylaxis was reported to be associated with lower risk to develop PES (Park et al. 2013; Kanda et al. 2013; Iguchi et al. 2016), whereas the use of cyclosporine-A rather than tacrolimus is associated with higher risk (Kanda et al. 2013). This latter observation might be, in fact, a reflection of the stronger immunosuppressive effect of tacrolimus compared to cyclosporine-A, especially in the initial

posttransplant period (Nash et al. 2000; Hiraoka et al. 2001; Yanada et al. 2004). Recently, the addition of mycophenolate mofetil to tacrolimus for GvHD prophylaxis in single UCB transplantation was associated with a lower incidence of PES and acute GvHD compared to tacrolimus alone (Uchida et al. 2011). In addition, the incidence of PES was only 20% in a study where antithymocyte globulin (ATG) and high-dose methylprednisolone were used for GvHD prophylaxis (Frangoul et al. 2009). Intensification of immunosuppression following transplantation may, therefore, alter the cascade leading to immune reactions like PES and GvHD.

15.2.1.5 Treatment

Like engraftment syndrome, almost all patients respond to treatment with IV methylprednisolone 0.5–1 mg/kg/day given for three to five consecutive days without taper; however, the optimal therapy and duration are not yet known, and some mild cases are thought to be self-limiting. Caution should be followed if higher doses or longer courses of steroids are required as Park et al. reported increased risk of infections in patients exposed to steroids for prolonged periods of time in the initial posttransplant phase (Park et al. 2013). A search for biomarkers may provide clues to etiology and guide the choice of patients who require therapy *versus* those who can be carefully monitored without intervention (Schots et al. 2003).

15.2.1.6 Effects on Transplant Outcomes

Engraftment The effect of PES on rate and speed of UCB engraftment has varied. While some reported no difference in sustained donor engraftment between patients with and without PES (Patel et al. 2010; Brownback et al. 2014), there is some evidence that points toward more rapid engraftment by a median of 2.5 days for UCB recipients who developed PES (Lee et al. 2008). The cumulative incidence of engraftment in patients with PES ranges between 84 and 92% compared with 77% in those without PES (Frangoul et al. 2009; Wang et al. 2012). This interesting observation may reflect the complex interaction between the bone marrow microenvironment and the cytokines released by the different cells involved in the process of PES, which may, in turn, trigger neutrophil regeneration during the early posttransplantation period (Spitzer 2001; Park et al. 2013; Ravoet et al. 1996). Furthermore, the absence of PES was reported to be a significant risk factor for graft failure (RR 5.50 and $p < .01$) (Park et al. 2013).

Graft-*Versus*-Host Disease (GvHD) While most studies reported no impact of PES on the development of acute or chronic GvHD at subsequent posttransplantation phases (Lee et al. 2008; Kanda et al. 2013; Patel et al. 2010; Brownback et al. 2014; Hong et al. 2013), one study reported that steroid-refractory GvHD was noted only in patients who developed PES at an earlier phase (0% *vs.* 40%, $p = 0.04$) (Kanda et al. 2013). On the other hand, Park et al. reported incidence of grade II–IV GvHD by day 100 56% in patients with PES *versus* 34% without PES ($p < .01$), however, with no association with the incidence of chronic GvHD. This association was proven to be true in a multivariate analysis after adjusting for age, sex, weight, conditioning regimen, HLA match, number of donors, and total cell counts. Of note, among the patients with PES, there was a negative correlation between the day of

PES development and the incidence of acute GvHD. That is, those in whom PES occurred earlier had a higher incidence of grade II to grade IV acute GvHD (Park et al. 2013).

Overall Survival (OS), Treatment-Related Mortality (TRM), and Relapse Despite being a rather common complication in UCB transplant recipients, PES was not associated with any statistically significant adverse effects on OS, TRM, or disease relapse (Lee et al. 2008; Park et al. 2013; Kanda et al. 2013; Patel et al. 2010; Brownback et al. 2014). This benign course may be attributed to the fact that PES can be adequately managed with short courses of steroids. Alternatively, reduced graft lymphocyte count and, therefore, limited response to recipient alloantigens may also contribute to the favorable outcomes (Liu et al. 2004). One study noted a nonsignificant trend toward reduced survival in those who developed hypoxemia with PES (Brownback et al. 2014), while another reported that early complete chimerism achievers developed PES more frequently (Hong et al. 2013).

15.2.2 Cord Colitis Syndrome

Diarrhea is a frequent symptom after all types of allogeneic Hematopoietic cell transplant (HCT). The etiology varies according to the posttransplant phase, with regimen-related toxicity and infections being the most common in the pre-engraftment phase, while infections and acute GvHD predominate in the post-engraftment period. Cord colitis, as the name implies, is a unique type of diarrhea that is seen in UCB transplant recipients and was first described in 2011 by Herrera et al. (2011). It is a syndrome of culture-negative, antibiotic-sensitive non-bloody but watery diarrhea that is described as being persistent (typically >7 days), starting typically when chronic GvHD occurs, at a median of 131 days after UCB infusion. The initial report by Herrera et al. (2011) was based on a retrospective review of 104 UCB transplant patients who underwent UCB transplant at a single institution and had these symptoms at a rate of nearly 10%. Given the similarities in symptoms and time frame of presentation between cord colitis and GvHD, it is very important to make the distinction between the two entities.

15.2.2.1 Presentation

Symptoms of cord colitis that are most commonly associated with the diarrhea include weight loss (91%) and fever (64%). Colonoscopy, often done to rule out other etiologies, typically shows inflamed erythematous mucosa with or without ulcerations and absence of pseudomembranes. Colonic biopsies often show findings of chronic active colitis with basal plasmacytosis, architectural distortion, surface epithelial injury, and neutrophilic granulomas with negative microbial cultures. Paneth cell metaplasia is typically noted, suggesting chronicity. The latter, along with paucity of crypt apoptosis, are the distinct histological features thought to distinguish cord colitis syndrome from classic acute gut GvHD (Magenau and Reddy 2014). Radiographic findings are usually nonspecific and show focal or diffuse colonic wall thickening consistent with other forms of colitis (Herrera et al. 2011).

15.2.2.2 Etiology

Several hypotheses have been suggested to explain the etiology of cord colitis syndrome including delayed or atypical GvHD, transfusion-related acute gut injury (TRAGI), Tropheryma whipplei infection, and/or a chronic interferon-γ response against nondominant cord unit in cases of double UCB transplant (van Bekkum et al. 2011, Matuchansky 2011).

In an attempt to investigate whether cord colitis syndrome represents an infectious etiology, shotgun DNA sequencing was performed on paraffin-embedded colon biopsy specimens from patients with cord colitis syndrome. This has led to the discovery of nonhuman DNA sequences that have similarities with *Bradyrhizobium japonicum* bacteria. This newly discovered bacterium was called *Bradyrhizobium enterica* and is proposed to be an opportunistic human pathogen responsible for the development of cord colitis. Polymerase chain reaction (PCR) studies detected sequences from this new genome in samples from three additional patients with cord colitis and in no samples from healthy controls or patients with graft-*versus*-host disease (Bhatt et al. 2013).

In contrast to these findings, Gorkiewicz et al. were unable to identify sequences of *B. enterica* using 16S rDNA-based microbial community profiling with the use of high-throughput pyrosequencing. However, they reported overgrowth of *Bacteroides fragilis* in the samples tested, which disappeared with successful therapy (Gorkiewicz et al. 2013). This data together may suggest that the cord colitis syndrome is not caused by a single microbe but rather reflects overgrowth of a variety of infectious organisms.

15.2.2.3 Treatment

The fever and diarrhea in most cases are responsive to a 2-week course of antibacterial agents with adequate gut coverage (usually metronidazole and a fluoroquinolone). Approximately 50% of the patients respond to a single course of treatment; however, the other half may experience relapse after discontinuation of antibiotics but do respond to re-initiation of antibiotic therapy (Herrera et al. 2011).

Interestingly, it has been suggested that metronidazole may have immunomodulatory properties that help explain how it alters the course of cord colitis syndrome and/or mild forms of GvHD (Magenau and Reddy 2014). This is based on the observations that prophylaxis with ciprofloxacin and metronidazole has been shown to reduce the incidence of GvHD in matched related donors (Beelen et al. 1999), and in cases of inflammatory bowel disease, it appears to have modest activity irrespective of the presence of infectious processes (Steinhart et al. 2002).

15.2.2.4 Controversies

While Herrera et al. reported cord colitis as a unique syndrome to UCB transplantation, some argue that it represents a delayed GvHD reaction (van Bekkum et al. 2011), especially given that the histopathologic findings described in cord colitis resemble those noted in chronic GvHD in monkeys and mice (van Bekkum et al. 2011). Furthermore, in a blind histological review of 153 colon biopsy specimens from 45 UCB transplant recipients and 45 matched allograft controls obtained between day +70 and +365 posttransplantation, Milano et al. reported

no histological differences between UCB transplant recipients and controls with diarrhea or between the entire cohort of UCB transplant recipients and their controls in a patient group in whom chronic active colitis was noted in 58% of UCB recipients and 62% of controls. They concluded that colitis occurring after day +70 in allografted controls was related to acute GvHD, independent of the source of donor cells (Milano et al. 2014). Additionally, in a retrospective review of over 200 patients, Shimoji et al. reported that the presence of chronic active colitis with granulomas and/or Paneth cell metaplasia should not be considered diagnostic of cord colitis syndrome as they were also reported in patient with GvHD and CMV colitis regardless of the stem cell source (Shimoji et al. 2013). It was suggested that the discrepancies between these studies are due to different conditioning regimens (viz., myeloablative regimens), varying immune prophylaxis and different trends in the use of corticosteroid treatments *versus* antibiotics for empiric treatment of diarrhea in the posttransplant setting (Magenau and Reddy 2014).

15.2.3 Infectious Complications

Infections are a major cause of morbidity and mortality following UCB transplantation. The frequency of specific infections in UCB transplant patients is higher than other transplants, although overall these are the same infections seen in other allograft patients. Based on registry data, the rate of infectious deaths in this population is higher than other allografts, with most studies demonstrating an approximate rate of infection at death of 30–40% among UCB transplant recipients (van Burik and Brunstein 2007). In an early UCB transplant study by Saavedra et al., of adult UCB recipients who were treated with an ablative regimen, the incidence of infectious episodes was strikingly high reaching 100% with 55% incidence of bacteremia, 58% incidence of CMV viremia, and 11% incidence of fungal infections (Saavedra et al. 2002). Furthermore, day 100 TRM was 37% with 80% of deaths attributed to infectious complications. While prolonged neutropenia plays a role in the higher incidence of infections following UCB transplantation, the delay in lymphocyte subset recovery seems to play a crucial role and explains the continued high rates of infections after myeloid recovery (Saavedra et al. 2002). Interestingly in this series, more than half of infections occurred after myeloid engraftment.

Most recently, Ballen et al. evaluated rates of bacterial, viral, and fungal infections in 1781 adults with acute leukemia among different donor graft sources, including single and double UCB transplants, matched unrelated donor transplants (*MUD*), as well as one antigen-mismatched unrelated donor (*MMUD*) transplants. The incidence of bacterial and viral infections was significantly higher in UCB recipients reaching up to 72% and 68%, respectively, compared to lowest incidence of 59% and 45% in MUD transplants. In multivariate analysis, bacterial and viral, but not fungal, infections were more common after UCB than MMUD transplants (Ballen et al. 2016). Table 15.5 summarizes the unique trends of infectious complications in UCBT patients.

Table 15.5 Infectious trends unique to UCBT

Type of infection		Trends
Bacterial (Barker et al. 2005; Parody et al. 2006; van Burik and Brunstein 2007; Hosokawa et al. 2014; Miyazaki et al. 2003)		More frequent in UCBT compared to other types of allo-HCT Risk factors: delayed engraftment, higher incidence of CGF, slow immune reconstitution Pre-engraftment period: Coagulase-negative staphylococcus and Gram-negative bacilli are the predominant infectious agents C. diff colitis: similar incidence and recovery to other allo-HCT types
Fungal (Barker et al. 2005; van Burik and Brunstein 2007; Miyakoshi et al. 2007; Fukuda et al. 2003; Kojima et al. 2005; Martino et al. 2006)		Incidence: up to 40% Risk factors: delayed engraftment, higher incidence of CGF, slow immune reconstitution, prolonged/intensive GvHD prophylaxis, prolonged use of antibacterials, MA conditioning, use of steroids in the early post-UCBT period Candida species are the most common fungal agents encountered Aspergillus is the most common mold infection (50–70% of all IFI), median onset around day +20 Adult patients seem to be at a higher risk, particularly in the early posttransplant phase
Viral		Higher risk continues till immune reconstitution recovery of native thymopoiesis (Brown et al. 2010) Most viral infections in the post-UCBT period are due to Herpesviridae family
	CMV (Tomonari et al. 2003; Beck et al. 2010; Matsumura et al. 2007)	Incidence 20–100%, higher risk in MA conditioning regimens Associated with higher TRM and lower overall survival UCBT patients have higher risk of requiring >1 course of ganciclovir to treat recurrent CMV viremia compared to other allograft patients In RIC-UCBT, reactivation happens earlier compared to other RIC transplants, and the risk of progression to CMV disease is higher
	HHV-6 (Takahashi et al. 2007; van Burik and Brunstein 2007; Chevallier et al. 2010; Scheurer et al. 2013; Dulery et al. 2012; Cheng et al. 2010; Mori et al. 2010)	Higher incidence in UCBT, HHV-6B subtype is the most common Onset: typically 2–4 weeks post-UCBT Clinical presentation: fever, rash, CNS dysfunction, delayed engraftment, variable cytopenias Early plasma monitoring is recommended, starting 7–10 days post-UCBT and at least once a month through day 100 Preemptive therapy with foscarnet or ganciclovir is recommended if blood viral load >1000 copies/ml
	EBV/PTLD (Ballen et al. 2010)	Increased risk in UCBT due to higher degree of HLA mismatch Risk factors: RIC-UCBT when ATG is used (incidence up to 21%) and in HL patients (incidence 26%) receiving UCBT Aggressive course; frequent extranodal and CNS involvement, short interval between EBV detection and progression to PTLD, with poor response to therapy EBV monitoring by PCR starting with engraftment and at least biweekly is recommended Preemptive therapy with single agent rituximab is recommended if viral load >1000 copies/ml

(continued)

Table 15.5 (continued)

Type of infection		Trends
	BKV (Penn and Porat 1995; Micallef et al. 1998)	Some evidence of higher risk in UCBT especially in the setting of MA conditioning and HLA mismatch No clear data regarding exact frequency and severity of symptomatic BKV in UCBT settings
	Adenovirus (Keddis et al. 2012; Awosika et al. 2013; Robin et al. 2007)	In UCBT, infection can be acquired as a primary infection *vs.* latent reactivation Few case reports noted more severe adenoviral infections in UCBT
	Respiratory viruses (Parody et al. 2006; Butnor and Sporn 2003)	Serious infections due to respiratory viruses with 4% mortality after UCB transplant were reported, namely, parainfluenza

Abbreviations: *HCT* hematopoietic cell transplant, *CGF* cord graft failure, *C. diff Clostridium difficile*, *GvHD* graft-*versus*-host disease, *MA* myeloablative, *CMV* cytomegalovirus, *HHV-6* human herpes virus-6, *EBV* Epstein-Barr virus, *PTLD* posttransplant lymphoproliferative disorder, *BKV* BK virus, *TRM* treatment-related mortality, *ATG* antithymocyte globulin, *PCR* polymerase chain reaction

15.2.3.1 Immune Reconstitution

Studies investigating immune reconstitution after UCB transplantation reported an association between increasing CD34 cell dose and favorable transplant outcomes in terms of engraftment, infection risk, TRM, and survival rates (Barker et al. 2010). Another study reported 0% incidence of infection-related mortality in UCB transplant recipients achieving sustained engraftment (Cahu et al. 2009). The heightened infection risk seems to be related, at least in part, to intrinsic properties of UCB having more phenotypically naïve T-cells which underwent slower expansion to antigenic stimulation and demonstrated less effective cytotoxicity when compared to adult donor T-cells (Risdon et al. 1994). In addition, delayed thymic production of new T-cells in adult recipient of UCB transplantation contributes to the risk especially after myeloablative conditioning regimens.

Double UCB (dUCB) transplant has been used to evade cell dose limitation of single UCB transplant. Nonetheless, this strategy is still associated with delayed immune reconstitution and increased risk of infection. In a study of 35 consecutive dUCB transplant recipients, decreased T-cell and B-cell counts with expansion of natural killer cells were noted until 9 months posttransplantation (Ruggeri 2011).

The persistent high rate of infections after myeloid recovery in UCB transplant recipient has shed light on the role of lymphocyte recovery in modulating infection risk. When compared to bone marrow allografts, UCB transplantation is associated by an average of 5 months delay in the recovery of $CD8^+$ T lymphocyte (7.7 months *versus* 2.8 months), however, with similar rates of $CD4^+$ T lymphocyte reconstitution (Renard et al. 2011).

Nonmyeloablative (NMA) conditioning regimens in bone marrow and peripheral blood allograft recipients have been associated with faster reconstitution of T-cell repertoire (Morecki et al. 2001). There is body of evidence supporting the same observation in UCB transplants using NMA regimens, but the number of patients studied is small, and this observation is yet to be confirmed. However recently an NMA *vs.* MA study demonstrated for the NMA treats patients a shorter lymphocyte recovery time, a markedly more diverse and robust T-cell repertoire at similar points posttransplant, higher numbers of $CD45RA^+$ (phenotypically naïve) T-cells at 1-year posttransplant with a rapidly expanding population, and more rapid detection of T-cell receptor excision circle (TREC) suggestive of accelerated thymic recovery (Chao et al. 2002). One possible explanation for this accelerated recovery after NMA regimens is decreased damage and/or preservation of peripheral "niches" in which T-cells can proliferate. Alternatively, the lower incidence of early acute GvHD in NMA UCB transplantation may also play an important role in the preservation of the peripheral and central niches for T-cell development (Chao et al. 2002).

15.2.3.2 Specific Infections Seen More Frequently in UCB Transplant Patients

Bacterial Infections Bacterial infections are noted more frequently in the pre-engraftment period in UCB transplants as expected, due to a delay in neutrophil engraftment, with an increased incidence among patients with graft failure (van Burik and Brunstein 2007). Otherwise the types of infections are similar to other forms of transplantation. Coagulase-negative staphylococcus and Gram-negative bacilli are the predominant infectious agents encountered in the pre-engraftment period (Parody et al. 2006; van Burik and Brunstein 2007). Although reported in UCB transplant recipients, infection with mycobacterium species does not seem to be seen in an increased frequency in UCB transplant compared to other graft sources (Barker et al. 2005; Parody et al. 2006; Maeda et al. 2005). Clostridium difficile infection was reported at a similar incidence and recovery in UCB transplant compared to transplants from other graft sources (Hosokawa et al. 2014). Only a few cases have been reported due to uncommon bacterial species following UCB transplant like *Clostridium subterminale*, *Chryseobacterium meningosepticum*, *Ralstonia pickettii*, and Fournier's gangrene; however, it is difficult to draw any conclusions regarding the true rates/risks of these infections in UCB transplant recipients given the limited number of patients reported (Miyazaki et al. 2003; Adachi et al. 2004).

Fungal Infections Also partially due to slower neutrophil engraftment, a high incidence of fungal infections has been reported in UCB transplant recipients, up to 40%, with Candida species being the most common among yeasts and Aspergillus at the top of the list of mold infections scoring 50–70% of all invasive fungal infections in some reports (Barker et al. 2005; Parody et al. 2006; van Burik and Brunstein 2007). Adult patients seem to be at a higher risk, particularly in the early

posttransplant phase (Parody et al. 2006). Slow neutrophil engraftment, delayed immune recovery and thymopoiesis, development of GvHD, prolonged/intensive immunosuppression for GvHD prophylaxis, and the use of antibacterials are all factors that contribute to development of fungal infections post-UCB transplant (van Burik and Brunstein 2007).

Most of these data come from studies in patients who received myeloablative conditioning prior to their UCB transplant and include both adults and pediatrics age groups. Little is known regarding any differences in these trends with the use of reduced-intensity conditioning (RIC) regimens. In a study by Miyakoshi et al., 128 patients undergoing RIC-UCB transplant with a uniform GvHD regimen were reviewed. Invasive fungal infections (IFI) were diagnosed in 14 patients with a median onset around day 20 posttransplant (range 1–28 days). Interestingly, no patients developed IFI after day 100 and only one patient diagnosed after day 30. Such early development of IFI may suggest that these cases represent a reactivation of a latent infection during the period of neutrophil depletion and immunosuppression (Miyakoshi et al. 2007). In univariate and multivariate analysis, prior use of steroids in the early posttransplant phase was significantly associated with increased risk of IFI (Miyakoshi et al. 2007). Of notice, this trend is different from what was reported in RIC transplants using bone marrow or peripheral blood stem cell allografts, where IFI were noted more frequently at later stages posttransplant (Fukuda et al. 2003; Kojima et al. 2005; Martino et al. 2006).

Viral Infections Viral infections and reactivations are common after UCB transplantation and are typically noted during the period between engraftment and immune reconstitution (van Burik and Brunstein 2007). The type of conditioning regimen (MA *vs.* NMA) and type and duration of GvHD prophylaxis and its therapy if needed as patients' viral serostatus pretransplant all play a role in the development of viral infections after UCB transplantation. Most viral infections in the post-UCB transplant period are attributed to members of the Herpesviridae family; however, a number of cases exist reporting serious infections due to respiratory viruses with 4% mortality after UCB transplant (Parody et al. 2006; Butnor and Sporn 2003), namely, parainfluenza viruses, as well as BK virus causing hemorrhagic cystitis (Benketira et al. 2005) and adenovirus causing respiratory, GI, and GU infections.

Cytomegalovirus (CMV) Infection/Reactivation CMV reactivation is a major cause of morbidity and mortality after allogeneic Hematopoietic cell transplant (HCT) and has been reported with all types of grafts and with both MA and RIC transplants. In UCB transplant, CMV reactivation is almost exclusively a result of reactivation of endogenous virus in the host as CMV infection in newborns is fairly rare and infected UCB units are not banked or used clinically.

Among UCB transplant recipients, CMV reactivation is fairly common and incidence ranged between 21% and 100% in different series compared to 43–69% in MRD and 30–79% in MUD transplants (Parody et al. 2006; Tomonari et al. 2003; Takami et al. 2005). One study evaluated the impact of pretransplant CMV-seropositive status of the patient on transplant outcomes in 332 patients undergoing

myeloablative and RIC-UCB transplant using either single or double UCB units (Beck et al. 2010). When compared to CMV-seronegative patients, those with pre-transplant seropositive status had similar TRM, GvHD, relapse, and survival rates. CMV reactivation did not affect transplant outcomes; however, patients who developed CMV disease had higher TRM and lower overall survival (Beck et al. 2010). Furthermore, UCB transplant patients were noted to have a higher risk of requiring two or more courses of ganciclovir to treat recurrent CMV viremia compared to patients receiving other allograft sources (Tomonari et al. 2003). Interestingly, CMV clearance and survival after CMV reactivation have been associated with recovery of native thymopoiesis (Brown et al. 2010), which is not fully achieved until later stages following UCB transplant especially when myeloablative conditioning is used.

In a study that looked exclusively at RIC-UCB transplants, Matsumura et al. reported the incidence of CMV reactivation to be 55%, which is consistent with the incidence reported with other types of RIC transplants (Matsumura et al. 2007). Two unique findings were noted in this study: first, the timing of CMV reactivation after RIC-UCB transplant was earlier than after RIC transplant using different allograft sources and, second, the risk of progression from CMV reactivation to CMV disease was higher in RIC-UCB transplants, likely a function of more intense immunosuppression and delayed immune recovery (Matsumura et al. 2007).

Human Herpes Virus-6 (HHV-6) Infections HHV-6 is frequently encountered in the posttransplant setting, with a higher incidence in UCB transplant recipients as compared to other types of transplants (Chevallier et al. 2010; Mori et al. 2010; Scheurer et al. 2013). The majority of the infections is due to the HHV-6B subtype and occurs between 2 and 4 weeks posttransplant. Proposed explanations for this observation include delayed immune reconstitution of T-cell repertoire after UCB transplant, and, being antigen naïve, the UCB graft lacks HHV-6-specific mature/memory T-cell needed to contain and limit viral reactivation following Hematopoietic cell transplant (HCT) (Scheurer et al. 2013; Dulery et al. 2012; Takahashi et al. 2007; Cheng et al. 2010). Additionally, CD46 a surface marker thought to be important for HHV-6 viral entry point into the host cell is more abundant on UCB compared to GCSF-mobilized stem cells, which likely contributes to the higher risk of HHV-6 reactivation after UCB transplant compared to other allograft types (Chevallier et al. 2010; Scheurer et al. 2013; Thulke et al. 2006).

Early reactivation of HHV-6 is often noted in UCB transplant patients and is particularly an important consideration for patients with encephalitis, delayed engraftment, variable cytopenias, and skin rash (van Burik and Brunstein 2007). HHV-6 encephalitis is defined as the presence of central nervous system (CNS) dysfunction due to HHV-6 infection confirmed by positive cerebrospinal fluid polymerase chain reaction (HHV-6 DNA CSF PCR), in the absence of any other cause of CNS dysfunction (Scheurer et al. 2013). Mori et al. suggested that the diagnosis can be made even if CSF PCR cannot be confirmed, provided that the patient has confirmed HHV-6 in the serum and evidence of limbic encephalopathy selectively affecting the temporal lobe on magnetic resonance imaging (2010). Ogata et al.

reported a correlation between rising IL-6 level in serum and CSF of patients with HHV-6 reactivation and development of encephalitis (2010). Treatment is with either foscarnet or ganciclovir, with the choice usually dictated by renal function or engraftment, with a switch to the alternative agent if weekly plasma viral loads do not decrease.

With a high incidence and the potentially devastating clinical effects, early plasma monitoring is becoming commonplace starting as early as 7–10 days post-transplant and continuing through day 100 on at least a monthly frequency. Preemptive therapy with foscarnet or ganciclovir is also being increasing utilized for a rising blood viral load that reaches 1000 copies/ml of plasma.

Epstein-Barr Virus (EBV) Infections and Posttransplant Lymphoproliferative Disorder (PTLD) EBV reactivation/infection most commonly occurs in the late post-engraftment phase, typically in the first 6 months posttransplant (Lucas et al. 1996). Unlike solid organ transplants, EBV reactivation and PTLD in the allogeneic HCT setting are mostly donor derived as confirmed by chimerism studies (Ballen et al. 2010; Sanz et al. 2014b). However, ablation of the recipient's immune system and cytotoxic T-cells prior to transplantation may allow for preferential proliferation of previously EBV-infected transformed B-cells and explains the cases of recipient-derived EBV reactivation and subsequent PTLD (Brunstein et al. 2006).

PTLD can manifest as fever, lymphadenopathy, and/or extranodal lymphomatous proliferation in various organs. Known risk factors for PTLD after allogeneic HCT include EBV reactivation, degree of HLA mismatch, chronic GvHD, and the use of pretransplant T-cell-depleting agents like ATG and alemtuzumab (Ballen et al. 2010; Brunstein et al. 2006). In UCB transplants, HLA mismatch at multiple loci is common, which poses an inherent higher risk for PTLD compared to other types of allogeneic HCT (Ballen et al. 2010). A particularly high incidence was noted after RIC dUCB transplants when ATG was used as part of the conditioning regimen, with an incidence as high as 21% (Brunstein et al. 2006). A recent report showed a relatively low incidence of PTLD after ATG-containing MA, rather than RIC, regimens (Sanz et al. 2014b).

The clinical course of EBV-PTLD after UCB transplant in adults with hematological disease is very aggressive with a characteristic presentation that includes extranodal involvement and frequent CNS, spleen and liver infiltration, and a high frequency of monomorphic PTLD. A short interval between EBV detection and PTLD progression was noted recently in a large cohort study, and response to therapy was generally poor (Sanz et al. 2014b). CNS involvement affected one third of the patients in this study, contrary to previous reports of low incidence of CNS disease in allogeneic HCT recipients (Penn and Porat 1995; Micallef et al. 1998). Of note, all patients in this study received ATG as part of the conditioning regimen at a dose of 6–8 mg/kg. Patients with Hodgkin lymphoma (HL) were also noted to have an unexpectedly high risk for development of PTLD post-UCB transplant, with incidence up to 26% (Pinana et al. 2016). Aggressive clinical course and ominous prognosis were reported in these patients, with PTLD resulting in more than one

third of transplant-related deaths (Pinana et al. 2016). Active HL at time of transplant is thought to be a contributing factor since EBV has an established role in the pathophysiology of HL. One possible explanation is selection and expansion of the EBV-infected clone in active HL cells following the conditioning regimen especially when ATG is used.

Strategies for EBV monitoring by PCR starting with engraftment and occurring at least biweekly with preemptive therapy with single agent rituximab when the viral load reaches 1000 copies/ml are becoming commonplace and seem to be decreasing the progression of viremia to actual PTLD in the UCB transplant setting.

BK Virus (BKV) BK virus, a polyomavirus that typically lies latent in the uroepithelial cells with the potential to reactivate during periods of immunosuppression, is commonly implicated in the pathogenesis of hemorrhagic cystitis in the post-allogeneic HCT period. The symptomatic infection typically occurs within the first 60–100 days after allo-HCT, and it was more frequently observed after neutrophil and platelet engraftment suggesting a possible inflammatory component in the pathogenesis of hemorrhagic cystitis triggered by the conditioning regimen and BKV presence (Silva Lde et al. 2010; Giraud et al. 2008). The symptomatic infection clinically manifests as hemorrhagic cystitis that may range from microscopic hematuria and dysuria to life-threatening bleeding and urinary tract clots that may lead to urinary outflow obstruction and acute kidney injury (Giraud et al. 2008). The combination of myeloablative conditioning and HLA mismatch, a common scenario in the UCB transplant setting, seems to be associated with a higher risk of BKV-associated hemorrhagic cystitis (Silva Lde et al. 2010). In a study by MD Anderson Cancer Center Group, it was noted that recipients of myeloablative haploidentical or UCB transplants who had a positive urine BK PCR pretransplant had a significantly higher risk of developing hemorrhagic cystitis compared to recipients of other graft sources and/or reduced-intensity conditioning (58% *vs.* 7%) (Silva Lde et al. 2010). This likely reflects the complex interaction between BKV infection, the intensity of the conditioning regimen, and the donor type. To date, this is the largest study that investigated BKV infection with reports about a slightly different trend in UCB transplant recipient. One limitation of this study is that it included both UCB and haploidentical transplants in one group when reporting the abovementioned trend. Biologically, however, the two groups are likely different in that the risk is higher in haploidentical HCT likely due to delayed immune reconstitution, whereas in the UCB transplant setting, the higher risk is likely due to a combination of delayed immune reconstitution and to the UCB, given its immunologically naïve nature, inherently lacking immunity to BKV.

The role of GvHD in the pathogenesis of BKV-associated hemorrhagic cystitis is not clearly established (Silva Lde et al. 2010). Additionally, there is paucity of data regarding the exact frequency of symptomatic BKV infection in UCB transplant recipient and whether severity is different compared to BKV infections encountered in other allo-HCT settings.

Adenovirus Infection Adenovirus is a non-enveloped double-stranded DNA virus that infects epithelial cell lines. Adenoviral infection is well characterized after allo-HCT and can clinically manifest in a wide variety of ways including pneumonia, colitis, hepatitis, hemorrhagic cystitis, tubulointerstitial nephritis, encephalitis, and disseminated disease that is sometimes accompanied by multi-organ failure (Ison 2006; Baldwin et al. 2000; Vyas and Marasco 2012). In the UCB transplant setting, adenoviral infection can be acquired either by primary infection or latent reactivation. To the best of our knowledge, there have been no case reports of adenoviral transmission through UCB grafts, a mechanism that can lead to adenoviral infection in other allo-HCT settings. Given the immunologically naïve nature of UCB and the delayed immune reconstitution in UCB transplant setting, one would expect an increased incidence of adenoviral infections in this population; however, there is paucity of data investigating this hypothesis. Few case reports have been published linking adenoviral infection in UCB transplant recipients with more severe clinical syndromes like interstitial nephritis (Keddis et al. 2012) and fatal encephalomyeloradiculitis (Awosika et al. 2013). In a report by Robin et al., UCB transplant was associated with higher risk of disseminated adenoviral infection ($p = 0.029$), but this observation is limited by the retrospective nature of this study and the limited number of patients included (Robin et al. 2007).

15.2.4 Improving Outcomes

UCBT remains an area of active research. Due to this graft source's unique immunologic properties, it remains an important alternative donor course. Improving its shortcomings as well as its outcomes will lend to its increasing use. Efforts to increase cell dose and subsequently time to engraftment as explained elsewhere in this volume include using two UCB units, ex vivo expansion techniques (de Lima et al. 2012; Delaney et al. 2005), enhanced homing such as with intra-bone injection of UCB cells (Frassoni et al. 2008, 2010; Brunstein et al. 2009), and co-infusion of other cells, such as a haploidentical graft (Liu et al. 2011; Tsai et al. 2016). Approaches are also being investigated to optimize graft-*versus*-tumor effect and immune reconstitution while minimizing incidence of graft-*versus*-host disease. With improved antimicrobial agents and engineered T0Cells to combat many of the now lethal resistant viral infections seen in this population, the outcome should improve. This should occur without decreasing the graft-*versus*-tumor effect that may ultimately make UCBT transplant preferable over haploidentical transplants.

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Future Prospects of Umbilical Cord Blood Transplantation

16

Mitchell E. Horwitz

The information provided in the preceding chapters of this book demonstrate the tremendous progress that the field of cord blood transplantation has experienced in recent years. While improvements in outcome have been observed for all ages of recipients, the most dramatic changes have been in adult umbilical cord blood transplantation. With the development of the dual cord blood approach, many adult recipients previously felt to be too large for cord blood transplantation are now viable candidates. Improved graft quality as a result of FDA licensure and a more refined understanding of cell dose thresholds have reduced the graft failure rate and have helped define appropriate candidates for this approach.

Despite the considerable progress, many questions remain unanswered in the field of umbilical cord blood transplantation (Table 16.1). It would be reassuring to state that ongoing, or upcoming, clinical trials will soon clarify the most pressing questions such as how to prioritize matched unrelated, haploidentical, and umbilical cord blood graft sources. But given patient scarcity and patient heterogeneity with respect to age, disease, and disease risk status, the prospect of practice changing clinical trials will be a long time coming. Future prospects for the use of umbilical cord blood are hard to predict. Provided below are the predictions by this author of what can be expected in the coming years.

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_16

Table 16.1 Clinical Need and Unresolved Questions Surrounding the Clinical Application of Umblical Cord Blood

Clinical need	Open questions		
	Adult	Pediatric	Ongoing prospective trial
Graft source prioritization (standard-risk hematologic malignancies)	1. Matched sibling 2. Matched unrelated donor or haploidentical or UCB	1. Matched sibling 2. Matched unrelated or UCB or haploidentical	1. Haploidentical *vs.* UCB/ phase III (NCT01597778)
Graft source prioritization (high-risk hematologic malignancies)	Is there differential graft *vs.* tumor potential among graft sources?		None
Cerebral palsy		Regenerative potential of autologous or allogeneic UCB	1. Autologous cord blood infusion/phase I (NCT01072370, NCT02551003, NCT02256618, NCT02612155)
Cord blood expansion/ manipulation	1. Can UCB expansion/ manipulation speed engraftment and reduce graft failure		1. NiCord *vs.* standard UCB transplantation/phase III 2. Expansion with UM171/ phase I/II (NCT02668315) 3. Expansion with Notch-1 ligand/phase III (NCT01690520) 4. Ex vivo UCB manipulation with fucosylation/phase II (NCT01471067)
Hypoplastic left heart syndrome		Regenerative potential of autologous UCB	1. Autologous UCB infusion for HLHS/phase I (NCT01883076)
Ischemic stroke	1. Regenerative potential of allogeneic UCB		1. Infusion of allogeneic UCB following ischemic stroke (NCT02433509)

16.1 Future Prospects of Pediatric Umbilical Cord Blood Transplantation

The use of umbilical cord blood as a graft source for pediatric hematopoietic stem cell transplantation appears solid and likely to increase in the coming years. The most obvious reason for this positive projection is the fact that cell dose constraints are not as critical an issue for pediatric compared to adult recipients. Although there are no published randomized studies comparing umbilical cord blood transplantation to other graft sources, the published outcomes for the treatment of hematologic

malignancies are excellent. Based on results of two randomized studies, we now know that one unit of adequate size (containing a minimum of 2.5×10^7 nucleated cells/kg recipient weight) performs as well as a double-unit graft (Wagner et al. 2014; Michel et al. 2016).

In the coming years, increased effort will be focused on the use of umbilical cord blood transplantation for the treatment of nonmalignant conditions. Sickle cell disease and aplastic anemia are of particular interest. Patients with these disorders have historically been difficult to transplant with umbilical cord blood, leaving those patients without an available matched donor with few curative options. In the case of sickle cell disease, the use of an ex vivo expanded umbilical cord blood graft may help to overcome the historically high graft failure rate (NCT01590628, NCT02504619). Encouraging results from small, single-center studies of umbilical cord blood transplantation for patients with relapsed or refractory aplastic anemia have prompted renewed interest in this treatment modality (Chan et al. 2008; Yoshimi et al. 2008). The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) will soon launch a multicenter phase II study targeting such patients without an available matched stem cell donor. The transplant center will choose to transplant these patients with either a haploidentical or umbilical cord blood graft that is preceded by non-myeloablative conditioning. The coming years will also see increased effort toward determining the reparative capacity of umbilical cord blood for neurologic disorders (see chapter entitled "Cord Blood for Non-hematopoietic Disorders").

16.2 Future Prospects of Adult Cord Blood Transplantation

The emergence of haploidentical transplantation using posttransplantation cyclophosphamide for graft *versus* host disease prevention is beginning to impact the usage of umbilical cord blood transplantation for adult recipients. A few centers are even prioritizing related haploidentical donor grafts over those from HLA-matched unrelated donors. Transplant centers and physicians are drawn to the relative ease of both donor selection and graft acquisition. Furthermore, the cost of graft acquisition is lower.

The question of the optimal alternative donor graft source will come with the completion and analysis of BMTCTN 1101, a prospective randomized study of non-myeloablative haploidentical *versus* umbilical cord blood transplantation for the treatment acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). The currently available phase II data surrounding these two approaches suggests equipoise, so until BMTCTN 1101 is complete, the decision as to which graft to use will remain with the treating physician. However, this study will only answer this question for older, less fit patients with AML or MDS who are not candidates for myeloablative conditioning regimens. The ideal alternative graft source for younger, fitter allogeneic hematopoietic cell transplant candidates who are candidates for myeloablative conditioning remains to be studied in a prospective fashion.

Probably the most innovative advances in umbilical cord blood transplantation are the various ex vivo manipulation strategies that are designed to improve

engraftment (see chapter by Mehta and Shpall). This, of course, is most significant for adult recipients who suffer the most from slow engraftment and increased risk for engraftment failure. While the short-term data look promising, unanswered is the long-term safety of these techniques. Long-term follow-up of study participants is ongoing and will be highly informative. The viability of ex vivo cord blood manipulation strategies will also depend on pharmacoeconomic considerations. They will, no doubt, increase the upfront cost of the transplant procedure, but further study will be needed to determine if the upfront costs are negated by improved patient outcomes.

16.3 Summary

Through research both in the laboratory and at the bedside, the clinical uses of umbilical cord blood in the treatment of human disease continue to expand. The unique properties of umbilical cord blood mean that many questions remain unanswered. We are already seeing cord blood used to treat metabolic disorders, and the prospect for its utility in other non-hematopoietic disorders is quite bright. Fortunately, this is a readily available resource, and the effort of the international cord blood banking community will ensure that basic and clinical innovation will continue to emerge.

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