

Umbilical Cord Blood Transplantation

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Abstract: Umbilical cord blood transplantation (CBT) has been widely used as an alternative source of hematopoietic cell support for stem cell transplant patients. CBT offers several advantages over traditional stem cell sources, such as immediate availability, absence of risk for donors, lower risk of acute graft-versus-host disease, and a less stringent requirement for human leukocyte antigen matching. Recent studies suggest that CBT is a safe and effective strategy for adult patients lacking a suitable related or unrelated donor. However, delayed engraftment and delayed immune reconstitution are significant clinical problems. Novel strategies, such as the use of multiple donors, cotransplantation with accessory cells, ex vivo expansion of cord blood hematopoietic progenitor cells, graft manipulation to improve T-cell recovery, and pharmacologic interventions to restore early thymopoiesis, hold promise to enhance engraftment and immune reconstitution after CBT. These approaches may significantly increase the quality and availability of cord blood for transplantation.

Introduction

The field of hematopoietic cell transplantation (HCT) has come a long way since Thomas and colleagues performed the first HCT in an animal model approximately 4 decades ago.¹ It is estimated that currently there are 14.6 million individuals who have volunteered to donate hematopoietic progenitor cells (HPC).² Despite this large pool of potential donors, the probability of finding a 10/10 human leukocyte antigen (HLA) match is 35–40% for a Caucasian.³ Therefore, over the past 20 years, the suitability of umbilical cord blood (CB) as an alternate source of HPC has been explored. There is growing evidence that CB is an acceptable alternative to other sources of HPC.

Historical Perspective

The first successful cord blood transplantation (CBT) was performed by Gluckman and associates in 1988, in a patient with Fanconi anemia. The patient achieved stable engraftment of donor hematopoiesis and survived without disease relapse.⁴

Due to the concern that CB might not contain a sufficient number of cells to reliably engraft larger children or adults, initial

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Cord blood, transplantation, banking

studies were limited to children. Three simultaneous studies that extended CBT to adults were reported in 1996.⁵⁻⁷ Four adults over the age of 21 years were successfully treated with unrelated donor CBT, suggesting that CBT can be successfully used in adults.

Following these early reports, there have been a number of studies using CB for transplantation in adults. Today, it is estimated that more than 20,000 CBTs have been performed worldwide.⁸ The number of CB units (CBU) available for transplantation has increased from 44,000 in 1999 to 452,000 in 2008.⁹ In 2009, more CBTs were performed than bone marrow transplants (BMT).¹⁰

Collection of Cord Blood

The goal of CB collection is to procure a CBU with a sufficient volume of blood and CD34-positive cells. In addition, it should be free of transmissible infectious agents or maternal blood, and should not interfere with routine delivery procedures. Several pregnancy-related and ethnic factors are associated with increased cell dose.¹¹⁻¹⁵ Moreover, the perfusion of placental vessels following maximal collection from the cord results in collection of as many additional HPCs from the placenta as collected from the cord itself.¹⁶ The American College of Obstetricians and Gynecologists (ACOG)^{17,18} and the American Academy of Pediatrics (AAP)^{19,20} have cautioned against altering standard obstetric procedures to facilitate CB collections. Specific guidelines have been provided by AAP for the physicians involved in procurement of CB regarding CB collection, processing, and storage procedures.²⁰

Cord Blood Banking and Operations

The first CB Bank (CBB) was created at the Indiana University School of Medicine in the 1980s.^{4,21} The first public CBB was established in New York in 1993, and soon after, CBBs in Düsseldorf, Milan, Paris, and Sydney were established.^{22,23} At present, it is reported that there are 131 voluntary CBBs in 35 countries.⁹ According to the World Marrow Donor Association (WMDA) report, over 40% of the CBUs are exported to another country.⁹

Fewer steps are involved in procurement of a CBU than of HPCs from adult donors. This is primarily because CBUs are tested and characterized at the time of collection and storage, before being listed for searches. Studies have shown that the time to transplantation is shorter for CBT than for unrelated bone marrow.²⁴

Despite the rapidly expanding inventory of CBUs, many pitfalls remain at various steps, from collection of CB to its eventual infusion to the recipient. Standardization of CBBs remains an issue, and only 19 CBBs have been granted Foundation for Accreditation of

Cellular Therapy (FACT)-Netcord accreditation worldwide, suggesting a lack of acquisition of common international standards.²⁵ A brief overview of each step is presented here.

Donor Recruitment

A written informed consent should be obtained before the onset of labor and before CB collection. It should contain information pertaining to what tests will be performed on the CB and how the parents will be informed. If the newborn, from whom CB was collected, develops a genetic, malignant neoplastic, or immunologic disorder, the parents should notify the CBB. Such units should not be used for transplantation and should be immediately removed from the CBB inventory.

CB donation should be encouraged when CB is stored in a bank for public use, but should be discouraged when CB is to be directed for later personal or family use, unless there is a sibling in the family with a hematologic disease that would be treated with CBT. It should be disclosed that the chance an autologous unit will be used for a child or a family member is remote (approximately 1 in 2,700 individuals).¹⁸ Detailed guidelines can be found in the policy statement from AAP.²⁰

Donor Selection and Testing of CBUs

Adequate donor information is required to ensure safe and appropriate donation. Any risk factors or high-risk behavior in the donor's medical history should be identified, and the donor should undergo a thorough evaluation for potentially infectious diseases. WMDA has provided a list of diseases that CB registries and banks should screen for on the Family Medical Health History Questionnaire.⁹

CBUs undergo complete typing (ABO, rhesus, and HLA) and mandatory testing for infectious agents. Other parameters such as volume, weight, total nucleated cell (TNC) count, CD34-positive cell count, or colony forming unit (CFU) count are also characterized.²⁶

Processing and Cryopreservation of CBU

CBU processing for volume reduction and red blood cell removal allows as much as a 10-fold increase in the number of CBUs that can be stored in the same freezer space.²⁷ Moreover, a smaller volume means less dimethyl sulfoxide (DMSO) is added to the sample for cryopreservation, which might obviate the need for washing the CBU before infusion, thus avoiding washing-related TNC loss.^{28,29}

Processing, however, can lead to a significant loss of HPCs.³⁰ CBBs use different processing methods such as Sepax cell-separation technique (Biosafe), hydroxyethyl starch sedimentation (HES), semi-automated top-and-bottom (TB), AutoExpress (AXP, Thermogenesis), and

PrepaCyte-CB (BioE).^{28,31,32} With modern methods, an approximate final volume of 25 mL, TNC recovery of more than 80%, and CD34-positive recovery of more than 90% is expected.²⁸ The US Food and Drug Administration has granted clearance to AXP and Sepax.³²

Following processing, the CBUs undergo cryopreservation using a cryoprotectant (generally DMSO), at a concentration of 10%, and controlled-rate freezing (CRF), which allows for cooling at a rate of 1–2°C/minute. Devices have been developed to combine CRF with storage in the same device. This prevents the exposure of the frozen CBU to more than 200°C temperature gradients as it is transferred from one freezer to another (-196°C in the freezer, then 22°C at room temperature followed by -196°C in another freezer.)³²

CBUs should be processed and frozen as soon as possible following delivery, as the recovery of HPCs can be adversely affected by storing the CBU at room temperature for 24 hours or longer following collection. Immediately storing the units at 4°C before the CBUs are frozen has been reported to result in a better recovery of HPCs when thawed.³³

Duration of Storage

It is not known how long a CBU can be cryopreserved while maintaining adequate viability. It has been suggested that the storage duration of cryopreserved CBUs does not negatively impact the time to engraftment or lead to a higher rate of graft failure.³⁴ In a study, CB cells stored for up to 15 years showed proliferation, ex vivo expansion, and mouse engrafting ability similar to freshly procured CB cells.³⁵ Similarly, a greater than 90% recovery of HPCs has been demonstrated after storage for up to 12 years.³⁶ These data suggest that the CBU can be safely cryopreserved for a long period of time without adversely affecting the HPC recovery.

Histocompatibility Testing

WMDA has provided guidelines for HLA testing for CBUs.³⁷ At a minimum, initial HLA typing should include HLA-A and HLA-B antigens at low resolution/split antigen level. DNA-based testing methods should be used for HLA-DR typing. For new volunteer donors, DNA-based testing for HLA-A and HLA-B should replace serologic testing. Since the sample size is limited in a CBU, DNA-based HLA-typing is preferable to serologic typing.³⁸

Transportation of CBUs

The temperature of the frozen CBUs should be monitored with a continuous monitor device during shipment to ensure that the temperature is maintained at -150°C or less. Only 44 transport-related incidents (1.5% of the shipments) were reported in 2008.⁹

Limited Contiguous Segments

The contiguous segment is the sample that is stored attached to CBU. Usually, there are 1–3 contiguous segments. These samples can be used, without compromising the sterility, viability, and identity of the unit, for extended and identity typing. The likelihood that a CBU will be ordered is low once all the contiguous segments have been exhausted.⁹

Viability of CB Cells

Serious concerns about the viability of CB grafts have been raised. It has been shown that in double CBT, the unit with better viability preferentially engrafts and the units with less than 75% viability are unlikely to engraft.³⁹ In 1 series, 53% of thawed units had problems in cell enumeration, with cell yields of less than 50% in many cases.²⁵ The International Society for Hematotherapy and Graft Engineering (ISHAGE) recommendation is to enumerate CD34-positive events and use 7-actinomycin D as a viability marker. A colony forming unit (CFU) should be used to assess the stem cell function.²⁵

Clinical Results

Early Clinical Results

Due to the limited number of cells in CBUs, many concerns were initially raised, such as whether the HPC source would be sufficient for engraftment in larger children or adults; if maternal lymphocyte contamination would cause serious graft-versus-host disease (GVHD); or if immunologically-naïve cells would be able to mount a graft-versus-host disease reaction.⁴⁰ However, these concerns have largely been allayed by later studies.

In 2001, Laughlin and coauthors⁴¹ reported on the initial large series of 68 adult patients (median age, 31.4 years; median weight, 69.2 kg) undergoing CBT after myeloablative (MA) conditioning (Table 1). Of the 60 patients who survived beyond day 28, primary graft failure occurred in 5. No significant correlation between the extent of HLA matching and graft failure was seen. The probability of grade II–V acute (a) GVHD and chronic (c) GVHD in patients who engrafted and survived beyond 28 days (n=55) was 60% and 38%, respectively. Event-free survival (EFS) was 26%. CD34-positive cell count of greater than $1.2 \times 10^5/\text{kg}$ was associated with higher EFS. In addition, faster neutrophil recovery was seen with higher TNC count ($\geq 2.4 \times 10^7/\text{kg}$) before freezing.

In 2005, Cornetta and coworkers⁴² reported the results of the Cord Blood Transplantation (COBLT) study. They enrolled 34 patients with a median age of 34.5 years who received MA conditioning. CBUs with prefreeze TNC greater than $1.0 \times 10^7/\text{kg}$ were mandated. A total of 34% of patients experienced primary graft fail-

Table 1. Studies of Adult Umbilical Cord Blood Transplants With Myeloablative Conditioning

Author	Laughlin ⁴¹	Arcese ⁴⁴	van Heeckeren ⁴⁵	Ooi ⁴³	Sato ¹¹⁵
Study size	68	171	31	77	33
Disease type	ALL (15); AML (19); CML (15); CMML (1); CLL (1); NHL (1); HD (2); non-malignant (14)	AML (46); ALL (53); secondary acute leukemia (11); chronic leukemia (32); lymphomas (13); MDS(16)	AML (9); ALL (7); CML (12); others (3)	AML (77)	MDS (7); secondary AML (26)
Main conditioning regimen	TBI-based (51); Flu-based (14); all patients received ATG	TBI- or TLI-based (110); chemo-based (61); ATG, ALG, or anti-T-cell antibody (129)	TBI-based (20); Bu-based (11); all patients received ATG	TBI-based (77)	TBI-based (33)
No. of CBU infused	1	1	1	1	1
HLA match (n)	6/6 (2); 5/6 (18); 4/6 (37); 3/6 (11)	6/6 (9); 5/6 (77); 4/6 (68); 3/6 (13)	6/6 (0); 5/6 (9); 4/6 (17); 3/6 (5)	6/6 (0); 5/6 (16); 4/6 (34); 3/6 (23); 2/6 (4)	6/6 (0); 5/6 (5); 4/6 (15); 3/6 (11); 2/6 (2)
Median TNC count/kg before freezing	2.1×10^7	2.7×10^7	2.17×10^7	2.44×10^7	2.51×10^7
Median CD34+ cell count/kg	1.2×10^5	1.0×10^5	1.0×10^5	1.0×10^5	0.91×10^5
Incidence, median time to neutrophil recovery	90%*, 27 days	72 ± 3% [†] , 28 days	84%*, 28 days	94.8% [§] , 21 days	91%, 22 days
Incidence, median time to platelet recovery	NA, 58 days	46 ± 4% [‡] , 84 days	NA, 71 days	91.7% [¶] , 40 days	88%, 51 days
TRM (%)	NA	51 ± 4% at 2 years	NA	9.1% at 100 days	14% at 5 years
Survival (%)	40% (at 6 months)	33 ± 4% at 2 years	37% at 3 years	EFS 62.8% at 5 years	EFS 70% at 5 years

Note: Survival is reported as overall survival unless otherwise noted.

ALL=acute lymphoblastic leukemia; AML=acute myeloid leukemia; ATG=anti-thymocyte globulin; Bu=busulfan; CBU=cord blood unit; CLL=chronic lymphocytic leukemia; CML=chronic myeloid leukemia; CMML=chronic myelomonocytic leukemia; EFS=event-free survival; Flu=fludarabine; HD=Hodgkin's disease; HLA=human-leukocyte antigen; NHL=non-Hodgkin lymphoma; MDS=myelodysplastic syndrome; NA=not available/not applicable; TBI=total body irradiation; TLI=total lymphoid irradiation; TNC=total nucleated cell; TRM=transplant-related mortality.

*Includes estimate at 42 days.

†Includes estimate at 60 days.

‡Includes estimate at 180 days.

§Includes estimate at 50 days.

¶Includes estimate at 200 days.

ure, and the probability of survival at day 180 (primary endpoint) was 30%.

The poor EFS in the above studies could be attributed to the selection of high-risk patients. In the study by Laughlin and coworkers,⁴¹ 50 of 54 patients with hematologic malignancies were classified as intermediate or high risk, whereas in the study by Cornetta and associates,⁴² 94% of the enrolled patients were considered poor risk by National Marrow Donor Program (NMDP) criteria,

and half of the patients with leukemia (13/26) had active disease at the time of transplantation.

Results of MA Conditioning CBT in Adults

A number of trials have evaluated the feasibility of CBT after MA conditioning in adults. The transplant-related mortality (TRM) in different trials has ranged from less than 10% in a study by Ooi and colleagues⁴³ to more than 50% in a study by Arcese and coauthors.⁴⁴ Major studies

of CBT with MA conditioning regimens are summarized in Table 1. A brief description of GVHD incidence and related factors are presented here in the paragraph below.

GVHD After MA Conditioning In the study by Arcese and coauthors,⁴⁴ the cumulative incidence of grade II–IV aGVHD at 100 days and cGVHD at 2 years was 32±4% and 36±10%, respectively. In a multivariate analysis, no factor was associated with the development of acute or chronic GVHD.

In the study from Case Western Reserve University,⁴⁵ the cumulative incidence of grade II–IV aGVHD and cGVHD was 17% and 44%, respectively. HLA-DRB1 mismatch was associated with a higher rate of severe aGVHD.

More recently, in the study by Ooi and colleagues,⁴³ the cumulative incidence of grade II–IV aGVHD and cGVHD was 81.5% and 84%, respectively. HLA disparity and cell dose did not correlate with incidence of GVHD, TRM, or risk of relapse. Interestingly, TRM was relatively low, despite a high incidence of acute and chronic GVHD. The authors commented that this could be due to an institutional trend towards early discontinuation of GVHD prophylaxis. These studies suggest that the impact of HLA disparity on the incidence of acute and chronic GVHD remains to be determined in adult patients undergoing CBT after MA conditioning.

CB Versus Other Stem Cell Sources

CBT Versus Unrelated BMT/Peripheral Blood Stem Cell Transplantation Multiple comparative analyses have been performed between CBT and unrelated donor transplantation from other sources (Table 2). In most of the trials, the patients undergoing CBT had high-risk disease status and received grafts with higher HLA-disparity and a cell dose usually 10-fold less than traditional BMT. In general, CBT is associated with a lower incidence of neutrophil and platelet recovery and a significantly longer time to engraftment than seen with other stem cell sources. Rate of graft failure is higher with CBT. Moreover, the incidence of acute and chronic GVHD is lower with CBT and the survival is generally similar to that seen with BMT or peripheral blood stem cell transplantation (PBSCT).

CBT Versus Related BMT/PBSCT Takahashi and colleagues⁴⁶ reported a comparative analysis of 171 adult patients with hematologic malignancies who received unrelated CBT (n=100), BMT (n=55), or PBSCT (n=16) from related donors. All patients received total body irradiation (TBI)-based conditioning and the majority received cyclosporine and methotrexate for

GVHD prophylaxis. There were no complete HLA matches in the CBT group. The neutrophil recovery took significantly longer with CBT (22 days) versus BMT/PBSCT (17 days). However, the incidence of engraftment was similar between the groups. The cumulative incidence of grade III–IV acute and extensive chronic GVHD was significantly lower in the CBT group. On multivariate analysis, no difference in TRM or disease-free survival (DFS) was seen between the 2 groups. The 3-year probability of DFS was 70% after CBT and 60% after BMT/PBSCT.

In another study,⁴⁷ outcomes of adult patients with acute leukemia who underwent CBT (AML n=66; ALL n=73) or haploidentical (Haplo) HCT (AML n=154; ALL n=75) at European Group for Blood and Marrow Transplantation (EBMT) centers from 1998–2002 were retrospectively analyzed. Overall, delayed neutrophil recovery and higher incidence of grade II–IV aGVHD was seen in recipients of CBT compared to Haplo HCT. In patients with AML, relapse, TRM, and leukemia-free survival (LFS) were similar between recipients of CBT or Haplo HCT. However, in patients with ALL the LFS was superior (CBT, 36%; Haplo HCT, 13%) and the incidence of relapse was lower (CBT, 23%; Haplo HCT, 35%) after CBT compared to after Haplo HCT.

These data suggest that CB as a source of unrelated HCT is safe and effective and compares favorably with transplants from other related stem cell sources.

Results of Reduced Intensity Conditioning CBT in Adults

The use of reduced intensity conditioning (RIC) regimens has helped decrease the toxicity and duration of aplasia, thus extending the applicability of CBT to the older patient population. In the majority of trials, the median age ranges from the upper 40s to the upper 50s. Initial concerns regarding the insufficient conditioning leading to suboptimal engraftment have largely been unfounded. In the Minnesota group experience, in which 110 patients were treated with fludarabine, cyclophosphamide, and TBI, 92% of patients achieved neutrophil engraftment at a median of 12 days. A total of 85% of patients in this study received 2 CBUs, but 1 unit uniformly predominated.⁴⁸ GVHD prophylaxis was cyclosporine and mycophenolate mofetil (MMF), and rates of grade II–IV aGVHD and cGVHD were 59% and 23%, respectively.

Similarly, in a report by Cutler and associates,⁴⁹ in which 53 patients received double CBT after RIC conditioning consisting of fludarabine, melphalan, and anti-thymocyte globulin (ATG), 92% of patients achieved neutrophil engraftment at a median of 20 days. As a result, 100-day TRM was only 12%.

Table 2. Studies Comparing Umbilical Cord Blood Transplantation With Other Unrelated Stem Cell Sources

Author	Laughlin ¹¹⁶	Rocha ¹¹⁷	Takahashi ¹¹⁸	Eapen ¹¹⁹	Kumar ¹²⁰	Atsuta ¹²¹	Atsuta ¹²²
Study size	600 (450 BMT, 150 CBT)	682 (584 BMT, 98 CBT)	113 (45 BMT, 68 CBT)	1,240 (354 BMT, 728 PBSCT, 148 CBT)	138 (90 MRD*, 15 MUD*, 14 MMUD*, 19 CBT)	820 (533 BMT, 287 CBT)	1,614 (1,196 BMT, 418 CBT)
Disease type (n)	BMT 0-1 Ag-MM: AML (142); ALL (99); CML (182); MDS (27) CBT: AML (58); ALL (45); CML (37); MDS (10)	BMT: AML (317); ALL (267) CBT: AML (45); ALL (53)	BMT: AML (15); ALL (8); CML (18); MDS (4) CBT: AML (39); ALL (15); CML (5); MDS (7); NHL (3)	AML (707); ALL (533)	ALL (138)	BMT: AML (311); ALL (222) CBT: AML (173); ALL (114)	NA
Main conditioning regimen used (n)	BMT 0-1 Ag-MM: TBI-based (368); Bu-based (82) CBT: TBI-based (127); Bu-based (21); unknown (2)	BMT: TBI-based (426); Bu-based (158); ATG (216) CBT: TBI-based (64); Bu-based (34); ATG (75)	BMT: TBI-based (45) CBT: TBI-based (68)	NA	TBI-based (138)	BMT: TBI-based (456); non-TBI-based (77) CBT: TBI-based (264); non-TBI-based (23)	Myeloablative conditioning
No. of CBU infused	1	1	1	1	1-2	1	1
HLA-mismatch (n)	BMT: 0 (367); 1 (83) CBT: 1 (34); 2 (116)	BMT: 0 (584) CBT: 0 (6); 1 (48); 2 (37); 3 (4)	BMT: 0 (39); 1 (6) CBT: 0 (0); 1 (14); 2 (37); 3 (15); 4 (2)	BMT: 0 (243); 1 (111) PBSCT: 0 (518); 1 (210) CBT: 0-1 (38); 2 (110)	MRD & MUD: NA MMUD: 1 (14) CBT: 1 (8); 2 (11)	BMT: NA CBT: 0 (20); 1 (60); 2 (207)	BMT: Class I: 1 MM (491) Class II: 1 MM (314); 2 MM (391) CBT: 0 (25); 1 (105); 2 (288)
Median TNC count/kg	BMT 0 Ag-MM: 2.4×10^8 BMT 1 Ag-MM: 2.2×10^8 CBT: 0.2×10^8	BMT: 2.9×10^8 CBT: 0.23×10^8	BMT: 33.0×10^7 CBT: 2.4×10^7	NA	NA	NA	NA
Median CD34+ cell count/kg	NA	BMT: NA CBT: 1.1×10^5	BMT: NA CBT: 0.9×10^5	NA	NA	NA	NA
Incidence, median time to neutrophil recovery	BMT 0 Ag-MM: NA, 18 days BMT 1 Ag-MM: NA, 20 days CBT: NA, 27 days	BMT: 89%, 19 days CBT: 75%, 26 days	BMT: 100% [†] , 18 days CBT: 92% [†] , 22 days	BMT: 92%, NA PBSCT: 96%, NA CBT: 78%, NA	NA	BMT[†]: AML 94%, NA ALL 97%, NA CBT[†]: AML 77%, NA ALL 80%, NA	NA

(Table continues on following page)

Table 2. (Continued) Studies Comparing Umbilical Cord Blood Transplantation With Other Unrelated Stem Cell Sources

Author	Laughlin ¹¹⁶	Rocha ¹¹⁷	Takahashi ¹¹⁸	Eapen ¹¹⁹	Kumar ¹²⁰	Atsuta ¹²¹	Atsuta ¹²²
Incidence, median time to platelet recovery	BMT 0 Ag-MM: NA, 29 days BMT 1 Ag-MM: NA, 29 days CBT: NA, 60 days	NA	BMT: 91% [‡] , 25 days CBT: 90% [‡] , 40 days	NA	NA	BMT[§]: AML 85%, NA ALL 83%, NA CBT[§]: AML 59%, NA ALL 61%, NA	NA
TRM	TRM was similar in CBT vs mismatched BMT (HR=0.99), but was significantly lower in matched BMT vs CBT (HR=1.89) or vs mismatched BMT (HR=1.91)	BMT^{**}: 38% CBT^{**}: 44%	BMT: 29% at 1 year CBT: 9% at 1 year	BMT 0 Ag-MM: 26% BMT 1 Ag-MM: 37% PBSCT 0 Ag-MM: 27% PBSCT 1-Ag MM: 42% CBT: 41%	MRD[‡]: 47% MUD[‡]: 67% MMUD[‡]: 86% CBT[‡]: 34%	BMT^{**}: AML (22%) ALL (25%) CBT^{**}: AML (33%) ALL (24%)	NA
Survival	BMT 0-Ag MM[‡]: 35% BMT 1-Ag MM[‡]: 20% CBT[‡]: 26%	BMT^{**}: 42% CBT^{**}: 36%	BMT: 44% DFS [‡] CBT: 74% DFS [‡]	BMT 0 Ag-MM^{††}: 48% BMT 1 Ag-MM^{††}: 38% PBSCT 0 Ag-MM^{††}: 45% PBSCT 1-Ag MM^{††}: 36% CBT^{††}: 35%	MRD[‡]: 27% MUD[‡]: 13% MMUD[‡]: 14% CBT[‡]: 66%	BMT^{**}: AML (60%) ALL (57%) CBT^{**}: AML (43%) ALL (49%)	(Overall mortality) CBT vs Class II one MM BMT RR=1.0 CBT vs Class I one MM BMT RR=0.96 (<i>P</i> =.74)

Note: Survival is reported as overall survival unless otherwise noted.

Ag=antigen; ALL=acute lymphoblastic leukemia; AML=acute myeloid leukemia; CBU=cord blood unit; BMT=bone marrow transplantation; CBT=cord blood transplantation; CML=chronic myelogenous leukemia; DFS=disease-free survival; LFS=leukemia-free survival; MDS=myelodysplastic syndromes; MM=mismatch; MMUD=mismatched-unrelated donor; MRD=matched-related donor; MUD=matched-unrelated donor; NA=not applicable; NHL=non-Hodgkin lymphoma; PBSCT=peripheral blood stem cell transplantation; RR=relative risk; TBI=total body irradiation; TNC=total nucleated cell; TRM=transplant-related mortality.

*The source stem cells in all unrelated donors was bone marrow (BM). In the MRD group, 80% were BM and 20% were peripheral blood stem cells.

[‡]Estimate at 42 days.

[‡]Estimate at 100 days.

[§]Estimate at 4 months.

[‡]Estimate at 3 years.

^{**}Estimate at 2 years.

^{††}Median follow-up is 2 years.

More recently, Société Française de Greffe de Moelle-Thérapie Cellulaire (SFGM-TC)/Eurocord presented results of 155 patients, with a median age of 47 years, who received RIC CBT with conditioning regimens consisting of fludarabine, cyclophosphamide, and TBI

and GVHD prophylaxis with cyclosporine and MMF.⁵⁰ A total of 38% of patients received 2 CBUs. Cumulative incidence of neutrophil engraftment by day 60 was 80% at a median of 20 days posttransplant. Cumulative incidence of acute and chronic GVHD was 37% and 39%,

respectively. TRM and overall survival (OS) at 18 months were 18% and 62%, respectively.

Several other groups have also reported an early TRM of less than 20% and a survival comparable to that seen in recipients of transplants from other stem cell sources.^{48,49,51-53} Key studies are summarized in Table 3.

Strategies for Improving Engraftment

CBUs contain a relatively low number of HPCs compared to other stem cell sources. The engraftment failure has been an issue, with the rate of nonengraftment ranging from 10–20%. In addition, CBT is associated with a longer time to engraftment and delayed immune reconstitution, thus exposing the patients to a relatively longer immunocompromised state. A number of strategies have been explored to increase the engraftment potential of CB cells.

Expansion of CB Cells

It has been shown that CB cells can be expanded *ex vivo*; however, the concern is the preferential expansion of short-term populating hematopoietic stem cells (HSC) at the expense of long-term reconstituting HSC.⁵⁴ Conversely, some studies have suggested that *ex vivo*-expanded CB cells retain their long-term engraftment potential and homing capabilities.^{55,56} In attempts to overcome the loss of long-term hematopoietic reconstitution, the *ex vivo*-expanded CBU has been cotransplanted with an unmanipulated CBU. The clinical data suggest that the *ex vivo*-expanded unit provides early, short-term hematopoiesis, whereas the unmanipulated unit provides long-term, durable hematopoiesis.⁵⁷

Currently, there are several different strategies for *ex vivo* expansion. In different studies, an up to 660-fold increase in TNC and a 160-fold increase in CD34-positive cells have been observed.⁵⁸ Early-phase trials using tetraethylenepentamine (TEPA), reported by de Lima and coworkers, or Notch-ligand Delta 1, reported by Delaney and colleagues, to induce *ex vivo* expansion have shown promising results.⁵⁹⁻⁶² *Ex vivo* expansion using the most reported techniques requires that the early hematopoietic progenitor cells (eg, CD34-positive or CD133-positive cells) first be selected from the CB mononuclear cell (MNC) fraction. This may be a consequence of accessory cells present in the CB MNC, which may inhibit *ex vivo* expansion.⁶³ Such selection procedures incur significant cell losses (median CD34-positive cell recovery of 35%; range, 4–70%), which markedly impact the level of *ex vivo* expansion that can be achieved. An *ex vivo* expansion strategy that does not require selection (thereby avoiding cell losses) would therefore be of benefit. Such a strategy has been developed at M.D. Anderson Cancer Center. Nonselected CB MNC are cocultured with

a component of the hematopoietic microenvironment (mesenchymal stem cells [MSC]). The cellular interaction between hematopoietic cells and their surrounding hematopoietic microenvironment is critically important, and is mediated by cellular and extracellular components (growth factors, cell adhesion molecules, and extracellular matrix molecules).⁶⁴⁻⁶⁷ These components provide complex molecular cues, which direct hematopoietic stem cell self-renewal and proliferation and ultimately regulate the differentiation and maturation of hematopoietic progeny.⁶⁶ As a consequence, the significant shortcoming of any cytokine-driven suspension culture system is the absence of these critical microenvironmental cues. MSCs are a key cellular component of the hematopoietic stem cell niche. They can be isolated as adherent cells from bone marrow aspirates and expanded *ex vivo* to generate sufficient numbers to allow their use in *ex vivo* coculture systems with CB MNCs, where they support *ex vivo* expansion.⁶⁸⁻⁷⁰ Since MSCs do not express HLA-DR, they are immunologically privileged, thereby reducing any immunologic complications that might arise with CB MNC coculture. They also allow the use of third-party “off-the-shelf” sources of cGMP MSC to speed up the *ex vivo* expansion process, which is particularly important in patients with rapidly progressing disease. The combination of CB MNC/MSc coculture and exogenous growth factors markedly increases the TNC and CD34+ cell number generated in the *ex vivo* expansion product. In a double cord blood transplant setting in combination with unmanipulated CB, this enables cell doses greater than those ever transplanted to date to be achieved, with significant improvements observed in time to neutrophil and time to platelet engraftment.⁷¹ Clinical trials employing different expansion methods are summarized in Table 4.

Enhancing Homing of CB Cells

Outside of cell numbers, aberrant and/or inadequate homing of the *ex vivo*-expanded CB cells may ultimately limit the efficacy of CBT. The recruitment of primitive hematopoietic progenitors to the marrow is governed by a cascade of molecular interactions between members of the selectin, integrin, and CD44 superfamilies of adhesion molecules and their receptors.⁷²⁻⁷⁹ A key initial step associated with homing is the rolling of primitive hematopoietic cells on E-selectin expressed by the marrow vasculature; however, effective rolling of the hematopoietic progenitors depends upon appropriate carbohydrate modification (fucosylation) of E-selectin counter receptors. CB progenitors are poorly fucosylated in comparison to marrow and PB progenitor cells and this, at least in part, is believed to be responsible for the delayed engraftment associated with CBT. The low levels of CB progenitor cell fucosylation can be markedly increased by *ex vivo*

Table 3. Studies of Umbilical Cord Blood Transplantation With Reduced Intensity Conditioning

Author	Rizzieri ¹²³	Chao ⁵³	Miyakoshi ¹²⁴	Kishi ¹²⁵	Yuji ¹²⁶	Misawa ¹²⁷	Rocha ¹²⁸
Study size	2	13	30	57	20	12	65
Disease type (n)	MCL (1); NHL (1)	*	AML (14); ALL (3); AA (4); other (9)	ALL (8); AML (21); MDS (3); other (25)	Advanced lymphoma (20)	AML (3); ALL (1); NHL (2); MDS (4); other (2)	ALL (10); AML (37); lymphoma (10); MDS (4); CML (3); myeloma (1)
Main conditioning regimen (n)	Flu, Cy, ATG	Flu, Cy, ATG	Flu, Mel, TBI	Flu, Bu, TBI (6) Flu, Mel, TBI (51)	Flu, Mel, TBI	Flu, Cy, TBI	Multiple
No. of CBU infused (n)	1	1	1	1	1	1	1
HLA-mismatch (n)	2 (2)	1 (3); 2 (10)	1 (6); 2 (24)	0 (1); 1 (8); 2 (48)	0 (1); 2 (19)	0 (1); 1 (3); 2 (8)	0 (3); 1 (15); 2 (37); 3 (10)
Median TNC count/kg	6.5×10^7 2.9×10^7	2.07×10^7	3.1×10^7	NA	2.75×10^7	2.55×10^7	2.4×10^7
Median CD34+ cell count/kg	3.7×10^5 1.0×10^5	1.3×10^5	0.74×10^5	2.9×10^5	NA	0.91×10^5	NA
Incidence, median time to neutrophil recovery	NA, 10 and 30 days	12 days	87%, 17.5 days	79%, 19 days	75%, 20 days	91%, 17 days	87% and 65% [†] , 20 days
Incidence, median time to platelet recovery	NA	NA, 14 days	40%, 39 days	NA	75%, 39 days	42%, 32 days	NA, 35 days
TRM	0	NA	27% at 100 days	62% at 180 days	41% at 100 days	41.7% at 100 days	45% at 1 year
Survival	100% at 1 year	43% at 1 year	32.7% at 1 year	NA	50% at 1 year	41.7% at 1 year	DFS at 1 year HLA 5-6/6 (42%) HLA 4/6 (27%) HLA 3/6 (0%)

(Table continues on following page)

treatment with a ^{1,3}fucosyltransferase (FT-VI) prior to transplant.⁸⁰⁻⁸² Preclinical studies in immunocompromised mice have revealed that the ex vivo fucosylation of CB progenitors prior to transplant is correlated with more rapid and greater magnitude engraftment.⁸³ The efficacy of this as a strategy to improve the rate and magnitude of engraftment in the clinic in patients receiving double CB transplantation is currently under evaluation at the M. D. Anderson Cancer Center. Under consideration are fucosylation of the smaller of the 2 unmanipulated CB units and fucosylation of the ex vivo-expanded product in patients receiving 1 unmanipulated and 1 ex vivo-expanded CB unit.

Another strategy currently under evaluation, which may improve engraftment, is the inhibition of CD26, a surface serine dipeptidyl peptidase IV (DPPIV) respon-

sible for cleavage of many cytokines including stromal-derived factor-1 (SDF-1/CXCL12), which is an important player in HSC homing, engraftment, and mobilization. Campbell and coauthors⁸⁴ showed that pretreatment of CD34-positive human CB cells with diprotin A (a DPPIV inhibitor) significantly enhanced engraftment in non-obese diabetic/severe combined immunodeficiency mice. As our knowledge of intracellular pathways increases, more sophisticated ways to enhance homing of CB cells are likely to emerge.

Direct Intra-BM Injections

A significant number of HPC/HSC do not ultimately home to the BM after intravenous (IV) infusion.⁸⁵ In an attempt to minimize cell loss during transplantation, some investigators have evaluated intra-BM injections

Table 3. (Continued) Studies of Umbilical Cord Blood Transplantation With Reduced Intensity Conditioning

Author	Brunstein ⁴⁸	Miyakoshi ⁵¹	Komatsu ⁵²	Uchida ¹²⁹	Cutler ⁴⁹	Kindwall-Keller ⁹⁶
Study size	110	34	17	70	51	37
Disease type (n)	Acute leukemia (41); CML (7); MDS (17); NHL (33); other (12)	AML(13); MDS (3); ALL (3); lymphoma (5); other (10)	Multiple	AML(28); MDS (3); ALL (11); NHL (8); CML (4); other (16)	NA	MDS/AML (28); other (9)
Main conditioning regimen (n)	Flu, Cy, TBI	Flu, Mel, TBI	Bu, Flu	Flu, Mel, TBI (65); Bu, Flu, TBI (4); Other (1)	Flu, Mel, ATG	Flu, Cy, TBI, ATG
No. of CBU infused (n)	0 (17); 1 (93)	1	1	1	2	1 (27); 2 (10)
HLA-mismatch (n)	NA	NA	1 (1); 2 (16)	1 (9); 2 (61)	NA	≤2 (36); 3 (1)
Median TNC count/kg	1 Unit: 3.3×10^7 2 Units: 3.7×10^7	2.4×10^7	2.6×10^7	2.8×10^7	4.4×10^6	NA
Median CD34+ cell count/kg	1 Unit: 3.8×10^5 2 Units: 4.9×10^5	NA	0.74×10^5	0.84×10^5	1.9×10^5	NA
Incidence, median time to neutrophil recovery	92%, 12 days	91%, 20 days	53%, 18 days	92%, 18 days	21 days	1 unit: 24.5 days 2 units: 25 days
Incidence, median time to platelet recovery	65%, 49 days	79%, 38 days	NA	63%, 35 days	42 days	1 unit: 38.5 days 2 units: 63.5 days
TRM	19% at 180 days	12% at 100 days	NA	42.8% at 100 days	12% at 100 days	1 unit: 3/10 patients 2 units: 2/27 patients
Survival	45% at 3 years	70% at 1 year	6 of 17 alive with median follow up 13.1 months	23% at 2 years	74% at 1 year	(At 4 years) 1 unit: 35.6% 2 units: 33.3%

Note: Survival is reported as overall survival unless otherwise noted.

AA=aplastic anemia; ALL=acute lymphoblastic leukemia; AML=acute myeloid leukemia; ATG=anti-thymocyte globulin; BMT=bone marrow transplantation; Bu=busulfan; CBU=cord blood unit; CML=chronic myelogenous leukemia; Cy=cyclophosphamide; DFS=disease-free survival; Flu=fludarabine; HLA=human leukocyte antigen; HR=hazard ratio; MCL=mantle cell lymphoma; MDS=myelodysplastic syndrome; Mel=melphalan; NA=not applicable; NHL=non-Hodgkin lymphoma; TBI=total body irradiation; TNC=total nucleated cell; TRM=transplant-related mortality.

*12 patients had relapsed hematologic malignancies; 1 patient had metastatic melanoma.

[†]Incidence of platelet engraftment was different in recipients of different preparative regimens.

(IBMI) of HPC/HSC. Frassoni and colleagues⁸⁶ studied the role of IBMI in 32 patients with acute leukemia who received single-unit unrelated CBT after MA conditioning. With a median infused TNC of 2.6×10^7 /kg, the median times to neutrophil and platelet recovery were 23 and 34 days, respectively. Grade II–IV aGVHD was seen only in 4 patients, and only 1 patient experienced graft failure. More recently, Brunstein and associates⁸⁷ reported results of 10 patients with hematologic malignancies who underwent double CBT with IBMI and IV infusion. MA conditioning was used and

both units were partially ($\geq 4/6$) matched with each other and the recipient. The IBMI unit was always infused first. The median TNC dose was 3.7×10^7 /kg with no difference between IBMI and IV units. The median times to neutrophil and platelet (>50,000) recovery were 21 and 69 days, respectively. IBMI was safe, but compared to historical controls, it offered no clinical benefit, and the study was terminated early.

The clinical experience with this technique is limited, and questions remain regarding the optimal speed and volume of infusion. It is also possible that there is no

Table 4. Summary of Clinical Trials Using Ex Vivo Expanded Cord Blood Cells

Expansion Type	Liquid Suspension			Stromal Culture	Bioreactor	
Study	Shpall ¹³⁰	de Lima ⁶¹	Delaney ⁵⁹	de Lima ¹⁰⁰	Jaroscak ¹³¹	Pecora ⁵⁷
Size	37	10	6	6	28*	2
Disease (n)	AML (10); ALL (10); CML (3); CLL (3); NHL (5); HD (3); other (3)	AML (2); ALL (5); HD (2); NHL (1)	AML (5); Bi-phenotypic leukemia (1)	NA	Malignant conditions (19); non-malignant conditions (9)	CML (2)
Conditioning regimen (n)	TBI, Mel (20); Bu, Mel (5); TBI, Cy, Ara-C (12) [†] , ATG (37)	Mel, Thio, Flu (8) Bu, Flu (2)	Flu, Cy, TBI	Flu, Mel, Thio, ATG (6)	TBI, Mel, ATG Bu, Mel, ATG [‡] Bu, Cy, ATG [§]	Bu, Cy, ATG (1) Cy, TBI, ATG (1)
Cytokines	SCF, TPO, G-CSF	SCF, FL, IL-6, TPO, G-CSF	Notch ligand Delta 1, SCF, FL, IL-6, IL-3	SCF, TPO, G-CSF	PIXY321, FL, EPO	PIXY321, FL, EPO
Days in culture	10	21	16	14	12	12
Fold expansion TNC, CD34+	56, 4	219, 6	660, 160	12, 12	2.4, 0.5	2.6, 1.6, and 1.8 [¶]
Median TNC/kg infused	0.99×10^7	1.8×10^7	$2.9 \times 10^{7**}$ $4.6 \times 10^{7**}$	NA	2.05×10^7	344×10^6 and 232×10^6
# Days to ANC >500	28	30	14	14.5	22	33 and 34
# Days to platelets >20,000	106	48	NA	30	71	52 and 60
Incidence of aGVHD (grade II–IV)	67%	44%	NA	33%	36%	0%
Survival	35% alive at 30 months	90% at 100 days	83% alive at 277 days	5 of 6 patients alive at 1 year	43% alive at 47 months	100% alive at 19 and 8 months

ALL=acute lymphoblastic leukemia; AML=acute myeloid leukemia; ANC=absolute neutrophil count; Ara-C=cytosine arabinose; ATG=anti-thymocyte globulin; BMT=bone marrow transplantation; Bu=busulfan; CBU=cord blood unit; CLL=chronic lymphocytic leukemia; CML=chronic myelogenous leukemia; Cy=cyclophosphamide; EPO=erythropoietin; FL=FLT-3 ligand; Flu=fludarabine; G-CSF=granulocyte-colony stimulating factor; GVHD=graft-versus-host disease; HD=hematologic disease; Mel=melphalan; NA=not applicable; NHL=non-Hodgkin lymphoma; RR=relative risk; SCF=stem cell factor; TBI=total body irradiation; Thio=thiotepa; TNC=total nucleated cell; TPO=thrombopoietin.

*One patient received cord blood unit from a related donor.

[†]In pediatric patients only.

[‡]Patients with hematologic malignancies who were not candidates for TBI.

[§]Patients with inherited disorders.

[¶]CD34+ cells decreased from 0.544×10^6 to 0.023×10^6 .

**For cultured and non-cultured units.

homing advantage to IBMI, and the cells enter systemic circulation.⁸⁸ The low incidence of aGVHD in the study by Frassoni and colleagues⁸⁶ is intriguing, but further studies are needed to determine whether the IBMI of CB is more effective than IV infusion.

Cell Dose and HLA-Matching

Cell dose and HLA disparity are the independent prognostic factors in CBT. Best available HLA match and a higher cell dose are preferred. It has been suggested that a higher cell dose can overcome, to some extent, the deleteri-

ous affect of HLA disparity. Some have speculated that for each HLA disparity, the TNC dose should be increased by $1 \times 10^7/\text{kg}$.⁸⁹ Recently, Barker and coworkers⁹⁰ reported the outcomes of 1,061 patients treated with single CBT after MA conditioning. The TNC dose and HLA match each affected survival by their impact on TRM. The control group was selected as having 1 HLA mismatch (MM) with TNC $2.5\text{--}4.9 \times 10^7/\text{kg}$. The authors observed that the TNC dose was positively associated with neutrophil and platelet engraftment, but had no relationship with GVHD or relapse, and that the best engraftment and survival outcomes were seen in recipients of 0 MM units, regardless of the TNC dose. They also noted that the second best survival outcomes were seen in recipients of 1 MM plus TNC greater than or equal to $2.5 \times 10^7/\text{kg}$ or 2 MM plus TNC greater than or equal to $5.0 \times 10^7/\text{kg}$; that there was no survival difference between recipients of 1 MM plus TNC greater than or equal to $2.5 \times 10^7/\text{kg}$ or 2 MM plus TNC greater than or equal to $5.0 \times 10^7/\text{kg}$; and that the recipients of 3 MM units and those with 1–2 MM plus a TNC dose less than $2.5 \times 10^7/\text{kg}$ did substantially worse. This study suggested that a better HLA match could compensate for a lower TNC dose and vice versa. In addition to TNC dose and HLA parity of CBUs, the recipient's pretransplant anti-HLA antibody status is important, since the presence of such antibodies can adversely affect the neutrophil and platelet engraftment.⁹¹ The Eurocord-Netcord registry and EBMT have made recommendations regarding criteria for CBU selection.⁹² A summary of these recommendations is presented in Table 5.

Double CBT

In order to overcome the limitation posed by relatively smaller cell dose in CBUs, double CBT was initially developed at the University of Minnesota.⁹³ Chimerism studies reveal that cells from only 1 unit provide long-term engraftment.⁹⁴ The underlying mechanisms leading to the dominance of one unit over the other are largely unknown. Gutman and associates⁹⁵ recently showed that 1 unit fails to engraft due to the immune rejection mediated by effector CD8-positive cells that develop after CBT from naïve precursors.

The data for MA and RIC suggest that transplantation with 2 partially matched units can successfully achieve engraftment, with outcomes comparable to that seen with other stem cell sources.^{48,94,96} In one study, however, successful engraftment was seen in only 2 of 10 patients who underwent double CBT after a busulfan/fludarabine myeloablative conditioning regimen.⁹⁷ Moreover, it has been shown that the recipients of 2 CBUs have a lower risk of disease relapse versus the recipients of 1 CBU.⁹⁸ In a recent study from the Minnesota group, 177

Table 5. Eurocord-Netcord and EBMT Recommendations for Selection of Cord Blood Units⁹²

- 1. At selection, the presence of HLA antibodies in the recipient should be evaluated.**
- 2. For a single CBT in patients with malignant disorders and a CBU with 6/6 or 5/6 HLA match:**
 - Minimum TNC count at freezing and after thawing should be $2.5\text{--}3.0 \times 10^7/\text{kg}$ and $2.0\text{--}2.5 \times 10^7/\text{kg}$, respectively.
 - Minimum CD34+ cell dose at freezing or after thawing should be approximately $1.2\text{--}1.7 \times 10^5/\text{kg}$.
- 3. For a single CBT in patients with malignant disorders and a CBU 4/6 HLA match:**
 - Minimum TNC dose at freezing and after thawing should be $3.5 \times 10^7/\text{kg}$ and $3.0 \times 10^7/\text{kg}$, respectively.
 - Minimum CD34+ cell dose at freezing or after thawing should be approximately $>1.7 \times 10^5/\text{kg}$.
- 4. For a single CBT in patients with nonmalignant disorders and a CBU with 6/6 or 5/6 HLA match:**
 - HLA match should be selected. HLA-DRB1 mismatching should be avoided.
 - Same TNC and CD34-positive cell dose as for malignant disorders.
- 5. For a single CBT in patients with nonmalignant disorders and a CBU with 4/6 HLA match:**
 - Minimum TNC count at freezing and after thawing should be $4.0\text{--}5.0 \times 10^7/\text{kg}$ and $3.5 \times 10^7/\text{kg}$, respectively.
 - Minimum CD34-positive cell dose at freezing or after thawing should be approximately $>2.0\text{--}2.5 \times 10^5/\text{kg}$.
- 6. CBU with 3/6 HLA match should be avoided except in extremely severe cases for patients with malignant disorders.**
- 7. If the above criteria for a single CBT are not met, a double CBT should be considered in prospective trials.**

CBT=cord blood transplantation; CBU=cord blood unit; HLA=human-leukocyte antigen; TNC=total-nucleated cell.

patients with acute leukemia received either 1 (47%) or 2 (53%) partially matched CBUs after MA conditioning. The time to neutrophil and platelet recovery was similar in both groups. The incidence of grade II–IV aGVHD was significantly higher in recipients of 2 units (48% vs 29%; $P<.01$). TRM was however similar, and the risk of relapse for patients in first or second remission was significantly lower in recipients of 2 units versus recipients of 1 unit (16% vs 31%; $P=.03$).⁹⁹

The experience with double CBT has also provided the basis for studying newer strategies, such as ex vivo expansion.^{59,87} At M.D. Anderson Cancer Center, a clinical trial is under way combining an unmanipulated CBU with a CBU that has been ex vivo expanded on a layer of related donor MSC (minimum 2/6 HLA match). For the 6 patients who received double CBT after MA conditioning, the median time to neutrophil and platelet engraftment was 14.5 and 30 days, respectively. Only 2 patients developed aGVHD that resolved with steroids.¹⁰⁰

Cotransplantation of CBU With CD34-positive Cells From Haploidentical Family Donors

Fernandez and colleagues¹⁰¹ performed CBT with coinfusion of a limited number of highly purified mobilized hematopoietic stem cells from an HLA unrestricted third party donor. The transplanted CBU had 0–3 HLA mismatches with a median TNC of $2.39 \times 10^7/\text{kg}$. The cumulative incidence of neutrophil engraftment was 0.96. It was 100% in patients receiving highly purified mobilized hematopoietic stem cells from nonmaternal third party donors. Interestingly, none of the 4 patients who had their mothers as third party donors achieved engraftment. The cumulative incidence of final full CB chimerism was higher than 90% at 100 days. The incidence of serious infections was remarkably low, owing to the early recovery of absolute neutrophil count from the third party donors acting as a bridge to final CB engraftment. The cumulative incidence of aGVHD was 28%, and survival was comparable to HLA identical sibling transplants.¹⁰¹ Similarly, encouraging results were seen in the study by Bautista and colleagues¹⁰²; however, the risk of infections was higher in their study. Overall, this strategy appears promising but more experience is needed.

Cotransplantation of CBU With Ex Vivo–Expanded Parental Haploidentical MSC

Macmillan and coauthors¹⁰³ reported the results of a single institution phase I–II clinical trial in which 8 pediatric patients with high-risk acute leukemia received culture-expanded MSC from haploidentical parental donors at the time of CBT. All evaluable patients achieved neutrophil engraftment at a median of 19 days, with a 75% probability of platelet engraftment at a median of 53 days. No serious adverse events were noticed with any MSC infusion. Five patients remain alive and disease free at a median follow-up of 6.8 years.

In another study, ex vivo–expanded BM MSCs were infused at the time of CBT or in the case of refractory aGVHD.¹⁰⁴ Nine patients received MSCs immediately after CB and third party donor highly purified mobilized hematopoietic stem cells. There were no immediate

adverse affects. No significant differences in engraftment or incidence of aGVHD were observed. Interestingly, in 2 patients who developed steroid-refractory grade II aGVHD, MSC infusion led to complete remission.

These initial results suggest that infusion of ex vivo–expanded haploidentical MSC with unrelated CBT can be safely performed. Further studies are likely to evaluate other clinical outcomes with this strategy.

Immune Reconstitution After CBT

Infections are a major cause of early death after CBT. Some studies have suggested that more than 50% of deaths in the first 100 days post CBT are due to infections.¹⁰⁵ In a study of 100 CBTs performed at M.D. Anderson Cancer Center, the incidence of infections was 2.4 times higher in adult versus pediatric CBT recipients.¹⁰⁶ Moreover, the incidence of hemorrhagic cystitis and several viral infections such as varicella zoster, cytomegalovirus, and human herpes virus 6 is reported to be higher after CBT.^{107–110} In a prospective study of immune reconstitution at M.D. Anderson Cancer Center, infection was a major cause of death in 31% of patients after CBT. Marked CD4-positive and CD8-positive lymphopenia was observed, along with the absence of thymopoietic function in adult CBT recipients, relative to the other HCT population.

Several strategies to improve immune recovery after CBT are being explored. Some of these include ex vivo expansion of CB progenitor cells, induction of memory T-cell responses by ex vivo stimulation of naïve cord blood T cells, regulatory T-cell therapy to target alloreactive T cells in vivo, and the enhancement of thymic function by keratinocyte growth factor, interleukin-7, or androgen ablation.^{111–114}

Conclusion

The use of CBT has dramatically increased in the last 2 decades. Engraftment and other clinical outcomes continue to improve as we advance our understanding of how to better identify donors (cell dose and HLA matching), improve patient care, and gain more experience with CBT. The delayed immune reconstitution remains a concern, but a number of new approaches are being explored that have the potential of significantly improving outcomes.

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