

CONCISE REVIEW

Umbilical cord blood transplantation: Still growing and improving

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Abstract

Umbilical cord blood transplantation (UCBT) has been performed in the clinic for over 30 years. The biological and immunological characteristics of umbilical cord blood (UCB) have been re-recognized in recent years. UCB, previously considered medical waste, is rich in hematopoietic stem cells (HSCs), which are naïve and more energetic and more easily expanded than other stem cells. UCB has been identified as a reliable source of HSCs for allogeneic hematopoietic stem cell transplantation (allo-HSCT). UCBT has several advantages over other methods, including no harm to mothers and donors, an off-the-shelf product for urgent use, less stringent HLA match, lower incidence and severity of chronic graft-vs-host disease (GVHD), and probably a stronger graft-vs-leukemia effect, especially for minimal residual disease-positive patients before transplant. Recent studies have shown that the outcome of UCBT has been improved and is comparable to other types of allo-HSCT. Currently, UCBT is widely used in malignant, nonmalignant, hematological, congenital and metabolic diseases. The number of UCB banks and transplantation procedures increased exponentially before 2013. However, the number of UCBTs increased steadily in Asia and China but decreased in the United States and Europe year-on-year from 2013 to 2019. In this review, we focus on the development of UCBT over the past 30 years, the challenges it faces and the strategies for future improvement, including increasing UCB numbers, cord blood unit selection, conditioning regimens and GVHD prophylaxis for UCBT, and management of complications of UCBT.

KEYWORDS

graft-vs-host disease, graft-vs-leukemia, stem cell, transplantation, umbilical cord blood

Significance statement

This article focuses on the development of umbilical cord blood transplantation (UCBT) over the past 30 years, the challenges faced, and the strategies for future improvement. Optimal cord blood unit selection for transplantation is discussed. With the development of HLA matching, cord blood unit selection, modified conditioning regimens, and effective management of complications, UCBT has achieved comparable overall survival and better graft-versus-host disease-free and relapse-free

Xiaoyu Zhu and Baolin Tang contributed equally to this work and should be considered as co-first authors.

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survival than other allo-HSCT types. Future directions should focus on innovative research on the basic biology of UCB stem cells, novel randomized controlled clinical trials, and perfect quality control of UCB banking, making UCBT more popular for more patients.

1 | DEVELOPMENT OF UMBILICAL CORD BLOOD TRANSPLANTATION IN THE PAST 30 YEARS

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains an effective and curative therapy for malignant, nonmalignant, hematological, congenital, and metabolic diseases.^{1,2} Unfortunately, fully matched related donors, which is preferred, are not always available for the majority (approximately 70%) of patients³ and are even less available in China because of the once one-child policy. Finding matched unrelated donors through the registry is also difficult and time consuming. Since the first successful umbilical cord blood transplantation (UCBT) was performed on a 5-year-old boy with Fanconi anemia in 1988 at Hospital Saint-Louis in Paris, France,⁴ umbilical cord blood (UCB) has become an available graft source of allo-HSCT for over 30 years, with the advantages of rapid availability, no harm to mothers and donors, low immunogenicity, decreased chronic graft-vs-host disease (GVHD), and low relapse rate in minimal residual disease (MRD).^{5,6}

Unrelated donor cord blood transplantation (CBT) is an effective and reliable alternative to peripheral blood (PB) or bone marrow (BM) transplant and has emerged as a widely accepted treatment for a wide variety of hematologic diseases such as: acute lymphoblastic leukemia (ALL),⁷⁻⁹ acute myeloid leukemia (AML),¹⁰⁻¹² myelodysplastic syndrome (MDS),¹³⁻¹⁵ and aplastic anemia (AA)¹⁶⁻¹⁸ (Table 1).

UCB, previously considered medical waste, was suggested as a potential source of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) by Hal Broxmeyer in a private meeting with the late Edward A. Boyse and Judith Bard in 1982. This conference led to the creation of a UCB company named Biocyte Corporation and a series of studies on the biology and cryopreservation of UCB cells.^{19,20} These studies identified the possibility of using UCB as an available source of HSCs and HPCs, leading to the first HLA-identical sibling UCBT⁴ and subsequent UCBTs, including the first HLA-identical sibling UCBT in a patient with juvenile chronic myelogenous leukemia (JCML) at Johns Hopkins University in 1992,²¹ the first unrelated UCBTs in children reported by Joanne Kurtzberg et al in 1996²² and the initial unrelated UCBT experience with adults in 1996.²³

The first public UCB bank was established at the New York Blood Center in 1993,²⁴ and the Eurocord Netcord network was created by Gluckman et al in 1997,²⁵ and there are currently more than 100 UCB banks in Asia, Europe, Oceania, North America, and South America.²⁶ According to the World Marrow Donor Association (WMDA), over 778 000 cord blood units are available worldwide to be used for any patient in need, and approximately 35 000 UCBTs have been performed up to the end of 2019. The number of UCB banks and transplantation increased exponentially before 2013. However, according

to data from the WMDA and China Bone Marrow Transplantation Registry (CBMTR), with the widespread application of haploidentical transplants, the number of UCBTs decreased in the United States and Europe year-on-year from 2013 to 2019, although it increased steadily in Asia (from 1397 to 1645) and China (from 106 to 514) (Figure 1). Patients who undergo UCBT have had to face the challenges of delayed engraftment, risk of graft failure, increased transplant-related mortality (TRM) and infection. Many strategies have been attempted to address these issues to further improve UCBT as a feasible and more attractive option for allo-HSCT.

2 | ADVANTAGES OF UCBT

2.1 | Biology of UCB

Research on the biology and cryopreservation of UCB cells showed that UCB from a single donor could be used as a source of autologous or major histocompatibility complex-matched allogeneic transplantable hematopoietic repopulating cells. The process of cryopreservation of UCB cells should not require the need to discard any type of cells prior to freezing, and the cells should not be washed or otherwise handled after thawing, as all of these procedures would result in severe loss of HPCs.¹⁹ The above-mentioned process allows UCB units to be rapidly available for patients in urgent need of transplantation. In addition, a UCB unit could be collected at birth without any harm to the newborn or mother. These scientific findings paved the way for UCB as a potential source of transplantable HSCs/HPCs. Since then, our understanding of the biological characteristics of UCB has increased, emphasizing the advantages of UCBT.

UCB units usually contain one log less total nucleated cell (TNC) and CD34⁺ cells than a unit of bone marrow or peripheral blood, accompanied by delayed engraftment of neutrophils and platelets or higher incidence of graft failure.^{27,28} Many studies have revealed the proportions of hematopoietic progenitor cells such as primitive HPCs and multipotent colony-forming cells in UCB are significantly higher than those of BM CD34⁺ cells and peripheral blood stem cells. Moreover, UCB CD34⁺ progenitors have higher proliferation and multiple cell division potential.²⁹⁻³¹ Furthermore, the *in vivo* hematopoietic reconstitution capacity of UCB-derived HSCs in a nonobese diabetic/severe combined immunodeficiency (NOD/SCID) repopulation assay is superior to that of BM CD34⁺ cells.^{32,33} Several unique characteristics of UCB HSCs may lead to the above observations, including longer telomeres, a higher self-renewal capacity due to overrepresentation of transcription factor such as NF- κ B, and autocrine production of certain cytokines such as granulocyte-macrophage colony-stimulating factor and IL-3.^{34,35}

TABLE 1 Umbilical cord blood transplantation for acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and aplastic anemia (AA)

References	Diagnosis	Patients (n)	Graft*	Median age, years (range)	Neutrophil engraftment	Platelet engraftment ($\geq 20 \times 10^9/L$)	Relapse	TRM	Grade II-IV Acute GVHD	Chronic GVHD	Overall survival	Disease-free survival
Matsumura et al ⁷	ALL	256	Single unit	40 (1.6-74)	78% at day 100	64% at day 100	43% at 2 years	35%	37%	24% at 2 years	42% at 2 years	36% at 2 years
Page et al ⁸	ALL	640	Single unit	6.4 (0.5-17.9)	/	/	26%	/	40.4%	17% at 5 years	51.3% at 5 years	47.4% at 5 years
Piñana et al ⁹	PH + ALL	45	Single unit	31 (3-47)	96% at 44 days	73% at 183 days	31% at 5 years	31%(18%-45%) at 5 years	31%	53% (36-70%) at 5 years	44% (28-60%) at 5 years	36% at 5 years
Sanz et al ¹⁰	High-risk AML	49	Single unit	34 (1.6-52)	96% at 57 days	73% at 250 days	19% at 2 years	2-year NRM: 39%	26%	46% at 2 years	42% at 2 years	37% at 4 years
Baron et al ¹¹	AML	1068	Single unit: 567, double units: 501	45.5 (18-73)	77%	/	32% at 2 years	38% at 2 years	31%	42% at 2 years	32% at 2 years	30% at 2 years
Yanada et al ¹²	AML	1355	Single unit	52 (1.6-85)	/	/	18.2% at 3 years	29.5% at 3 years	/	/	55.1% at 3 years	/
Madureira et al ¹³	MDS	70	Single unit	6 (<1-17)	76% at day 60	57% at day 180	13 of 70	53% prior to 2001 (n = 30); 31% after 2001 (n = 40)	30%	23% at 3 years	42% at 3 years	39% at 3 years
Robin et al ¹⁴	MDS	129	Single unit: 49, Double units: 80	57 (20-72)	78%	/	30%	42% at 2 years	31%	23%	30%	28% ± 4%
Gerds et al ¹⁵	MDS	176	Single unit: 36, double units: 140	56 (18-73)	/	/	32% at 3 years	40% at 3 years	38% at 3 years	28% at 3 years	31% at 3 years	28% at 3 years
Kuwatsuka et al ¹⁶	SAA	69	Single unit	49 (17-73)	71% at day 42	/	/	/	32%	21% at 3 years	69% at 3 years	/
de Latour et al ¹⁷	SAA	26	Single unit: 16, double units: 10	16 (9-23)	/	/	/	11.5% at 1 year	45.8%	36% at 1 year	88.5% at 1 year	/
Yoshimi et al ¹⁸	SAA	31	Single unit	27.9 (0.8-72.7)	54.8	72.2%	/	/	17.1%	19.7%	41.1% at 2 years	/

Furthermore, *in vitro* and *in vivo* studies showed that HSPCs derived from UCB have higher proliferation and expansion potential than their adult BM cells, which may be because UCB cells exit the G0/G1 phase of the cell cycle more rapidly than adult BM progenitors and have longer telomeres than BM cells.³⁶ It should be noted that in addition to being a rich source of HSCs and HPCs, UCB contains an abundance of B cells with immunoregulatory functions. In patients who underwent UCBT, the recovery frequencies and absolute numbers of IL-10-producing Bregs were higher than those of healthy donors or patients before transplant. The reconstituting Bregs showed a strong inhibitory effect against allogeneic CD4⁺ T cells *in vitro* but were deficient in patients with chronic GVHD.³⁷ IL-10-producing B cells may protect against chronic GVHD after UCBT. In addition, T cells from UCB mediated enhanced antitumor responses compared with peripheral blood (PB) T cells in a murine model of B-cell lymphoma. The antitumor activity was correlated with increased tumor-homing of CCR7^{high} UCB CD8⁺ T cells and rapid gain of cytotoxic and T-helper (Th) 1 function,³⁸ which may be related to the advantage of a lower relapse rate in MRD positive patients before UCBT.

As for Tregs, the expression of CD4⁺ CD25⁺ T cells and Foxp3 between UCB and APB remains controversial.³⁹⁻⁴¹ Many *in vivo* studies have demonstrated that donor or host Tregs are able to prevent GVHD in allogeneic transplantation mouse models.⁴²⁻⁴⁴ The infusion of UCB Tregs in humans who received UCBT was safe and effective for reducing the incidence of GVHD.^{45,46}

2.2 | Comparison of UCBT to other graft sources

A series of clinical cohort studies comparing UCBT to other graft sources have further confirmed the advantages of UCBT: a lower incidence of chronic GVHD and stronger graft-vs-leukemia (GVL) effects for MRD-positive patients.

Chronic GVHD is usually accompanied by severe morbidity and impairment of quality of life (QoL). The Blood and Marrow Transplant Clinical Trials Network defined a novel composite GVHD-free endpoint, namely, relapse-free survival (GRFS), which represents a better QoL and ideal recovery after HCT.⁴⁷ UCBT has indicated comparable overall survival and a very low incidence of chronic GVHD with favorable GRFS vs matched related or unrelated transplantation.⁴⁸⁻⁵⁰

In a retrospective study performed by our transplantation center, the First Affiliated Hospital of the University of Science and Technology of China (USTC), unrelated UCBT was compared with HLA-matched sibling donor (MSD) transplants using a myeloablative regimen in AML patients. A total of 162 consecutive AML patients receiving a single unit of unrelated UCBT (n = 107) or MSD transplant (n = 55) were investigated. No differences were seen in grade II-IV or III-IV acute GVHD and TRM between the two transplant types. A lower incidence of chronic GVHD and extensive chronic GVHD, and a lower relapse rate and better GVHD-free and relapse-free survival (GRFS) were observed in the UCBT arm.⁴⁸ Another retrospective study reported by Sharma et al⁴⁹ compared outcomes among adult MSD transplants (n = 123) and adult patients undergoing double-unit UCBT (dUCBT) (n = 190). Overall survival (OS) was comparable, and GRFS was significantly improved among UCBT patients (P = .0056), primarily because of decreased moderate to severe chronic GVHD following CBT (P < .0001).

When UCBT was compared with HLA-matched or mismatched unrelated donor transplants in patients with acute leukemia or MDS using myeloablative conditioning, the relative risks of death and relapse appeared to vary according to the presence of MRD status before transplantation. Among patients with MRD, the probability of OS after UCBT was at least as favorable as that after an HLA-matched unrelated donor transplant and was significantly higher than the probability of OS after an HLA-mismatched unrelated donor transplant. Furthermore, the relapse rate was lower in the UCBT group than in the other groups.⁵ In a multicenter retrospective study, 79 acute

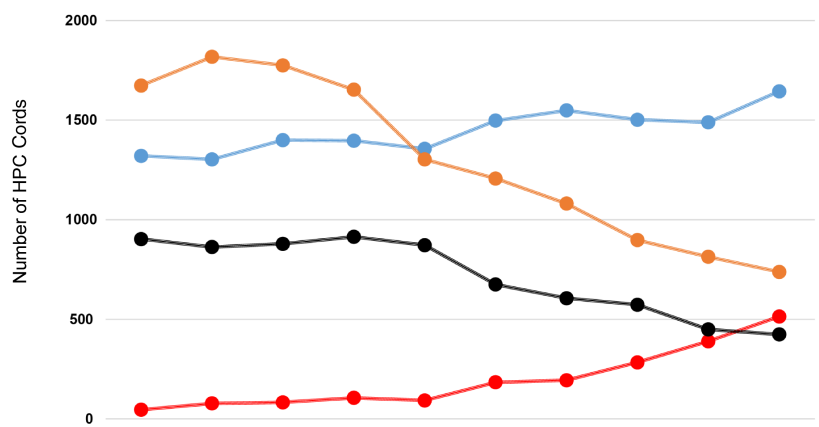


FIGURE 1 Shipments of HPC cords provided by the continents from WMDA and the number of UCBTs in China from CBMTR from 2010-2019

	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Asia	1321	1303	1400	1397	1356	1498	1549	1502	1489	1645
China	46	78	83	106	93	184	194	284	390	514
Europe	903	863	879	914	872	675	606	573	449	424
North America	1674	1818	1775	1653	1302	1207	1081	898	814	738

leukemia (AL) patients who underwent UCBT and 96 AL patients who underwent unrelated peripheral blood stem cell transplantation (UPBSCT) with myeloablative conditioning were compared. Acute GVHD, TRM, OS, and leukemia-free survival (LFS) were similar between the two transplant types. Less chronic GVHD, less moderate and severe chronic GVHD and lower incidences of Epstein-Barr virus viremia and posttransplantation lymphoproliferative disease were found in the UCBT group. UCBT recipients had higher Karnofsky performance scores for activity and 3-year GRFS than the UPBSCT group.⁵⁰

Haploidentical donors are one of the three alternative donor options, whereas the other two options are UCB and mismatched unrelated donors. In 2014, the number of haploidentical transplants surpassed the total number of UCB transplants performed in the United States. This increasing trend has continued, with these transplants representing 21% of transplants, and the number of UCB transplants is nearing the number of MSD transplants, which represented 25% of allo-HSCTs in the United States in 2018 (CIBMTR Summary Slides, 2019, available at <https://www.cibmtr.org>). A pilot study was conducted by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) to compare the results of two parallel multicenter phase 2 trials about reduced intensity conditioning (RIC) dUCBT (BMT CTN 0604) and HLA-haploidentical-related donor BM (haplo-marrow) transplant (BMT CTN 0603) for patients with leukemia or lymphoma at 27 transplantation centers in the United States.⁵¹ Lower TRM but a higher relapse rate was seen for haplo-marrow transplant, which ultimately resulted in similar OS and progression-free survival (PFS), 62% and 48%, respectively, after haplo-marrow transplantation ($n = 50$), and 54% and 46%, respectively, after dUCBT ($n = 50$). These multicenter studies set the stage for the development of a multicenter randomized phase III clinical trial BMT CTN 1101.⁵² No differences were seen in cumulative incidences of platelet recovery, grade II to IV and grade III to IV acute GVHD, chronic GVHD or the relapse rate. Although the results did not show a statistically significant difference in 2-year PFS between the donor sources, delayed neutrophil recovery, increased TRM, and decreased OS were observed in the UCBT cohort. In a retrospective study from our transplant center and Peking University People's Hospital, the therapeutic effects of single UCBT and unmanipulated haplo-HSCT in high-risk ALL children were compared.⁵³ The incidences of grade II to IV and III to IV acute GVHD, chronic GVHD, and moderate and severe chronic GVHD were lower for UCBT patients with decreased DFS than patients who underwent haplo-HSCT. Thus, UCBT is a reasonable option with these advantages, especially for those patients who do not have a donor available and are in urgent need of transplantation.

3 | CURRENT CHALLENGES

Although UCBT is immediately available and associated with a lower incidence of chronic GVHD, limited numbers of total nucleated cells and CD34⁺ cell doses in UCB units are still the main deficiency, which result in delayed hematopoietic recovery and increased rates of graft

failure, thus increasing the risks of infection and TRM. Moreover, antithymocyte globulin (ATG) is commonly used in UCB transplant recipients, especially in Europe. T-cell depletion in vivo may reduce the risk of GVHD, which in turn increases graft failure and relapse of the primary disease.⁶ Relapse remains the major cause of death after transplant.²⁶ To overcome these challenges, many investigators and clinicians have explored different ways to improve the efficacy of UCBT.

4 | MAJOR STRATEGIES FOR IMPROVEMENT

4.1 | Double-unit UCBT

The first double-unit UCBT (dUCBT) was performed in Europe in 1999. Both recipients had signs of donor engraftment but unfortunately died of relapse and hemorrhage 3 months post dUCBT (Eurocord, unpublished data, 2010).⁵⁴ In 2001, the first 2 units of UCB from male infant donors into a 53-year-old, 84-kg woman with accelerated-phase chronic myelogenous leukemia (CML) was performed by Barker et al,⁵⁵ and each unit contributed to hematopoiesis for at least 60 days after transplantation. Although this patient died of disseminated *Aspergillus* infection 68 days after transplantation, these findings prompted further investigation of UCBT from two partially HLA-matched donors as a method of increasing cell dose, especially for adult recipients. Since then, dUCBT has become a strategy for patients with insufficient units. According to Eurocord, since 2005, the number of adult patients receiving dUCBT has exceeded that of adults with single-unit UCBT (sUCBT).⁵⁶ Generally, only one unit can persist for a long time after dUCBT, which indicates that the 2 units might react against each other and have an impact on the efficacy of transplant. The effects of sUCBT and dUCBT were compared through several studies (Table 2).⁵⁷⁻⁶² An open-label, phase 3, multicenter, randomized trial reported by Wagner et al⁵⁸ determined the effect of the graft composition (double-unit vs single-unit) on 1-year survival among patients who received the same conditioning and GVHD prophylaxis regimen. The results showed that recipients of dUCBT had no engraftment or survival benefit compared with those receiving a sufficient dose of sUCBT. In addition, poorer platelet recovery and higher rates of grade III to IV acute and extensive chronic GVHD were observed after dUCBT. Michel et al⁵⁹ also found that the incidence of extensive chronic GVHD in dUCBT was higher than that in sUCBT. However, in MRD-positive patients who had not received ATG during their conditioning regimen, the relapse rate was lower in the dUCBT group than that in the sUCBT group, leading to a higher 3-year OS. We retrospectively analyzed 79 patients with hematological malignancies who received UCBT between November 2005 and December 2013 in our single transplant center. Patients who had dUCBT had a lower myeloid and platelet engraftment rate, higher TRM, and reduced OS, DFS and GRFS than patients who had sUCBT with a sufficient cell dose.⁶²

4.2 | Ex vivo expansion of UCB cells

Several investigators have explored a variety of approaches to expand functional UCB cells (HSCs and HPCs) *in vitro*, including recombinant hematopoietic cytokines, growth factors, stromal cells, and different small molecules. Recombinant hematopoietic cytokines were initially used to expand primitive hematopoietic cells from UCB, which was beneficial for self-renewal.^{63,64} Based on the favorable effect of cytokines on the *ex vivo* expansion of UCB, various growth factors, including FLT3 ligand, stem cell factor, erythropoietin, and thrombopoietin, were extensively tested. Although the number of HPCs increased significantly, no positive effects were observed in myeloid, erythroid, or platelet engraftment when UCB cells expanded with these growth factors were infused into patients.^{65,66}

Stromal cells were considered to be effective in the expansion of HSCs, since the maintenance of HSCs *in vivo* is closely related to special microenvironments, termed niches.^{67,68} As a part of the hematopoietic microenvironment, mesenchymal stem cells (MSCs) can be isolated from a variety of fetal and adult tissues.^{69,70} Research has shown that CB coculture with MSCs results in superior *ex vivo* expansion of total nucleated cells (TNCs) and HPCs.⁷¹ A clinical trial to assess the safety and efficacy of transplantation of CB expanded with an MSC coculture strategy was conducted (Funded by the National Cancer Institute and others; ClinicalTrials.gov number, NCT00498316). The results demonstrated that this approach appeared to be safe and effective, significantly promoting neutrophil and platelet engraftment time from 24 and 49 days, respectively, in historical controls to 15 and 42 days, respectively, in the recipients of expanded CB ($P < .001$; $P = .03$). The 26-day cumulative incidence of neutrophil engraftment was 88% with expansion vs 53% without expansion ($P < .001$); the 60-day cumulative incidence of platelet engraftment was 71% and 31%, respectively ($P < .001$).⁷²

More recently, different small molecules, including but not limited to, diethylaminobenzaldehyde.

(DEAB), copper chelator (StemEx), Notch ligand, StemRegenin 1 (SR1), nicotinamide, and UM171, have been reported as agonists for experimental *ex vivo* expansion of human HSCs and HPCs.⁷³⁻⁷⁸ Clinical trials of some small molecules have been reported and are summarized in Table 3.^{72,79-86} Delaney et al⁷⁹ infused *ex vivo* expansion CB in the presence of Notch ligand Delta 1 in a clinical setting for stem cell transplantation, and the time to neutrophil recovery was substantially shortened to 16 days. The Nicord product, first used in the dUCBT setting with the expansion of a single CB unit before infusion, showed 13-day neutrophil engraftment and 1-year OS and PFS rates of 82% and 73%, respectively.⁸⁰ Then, a phase I/II clinical study of sUCBT expanded *ex vivo* in the presence of nicotinamide was performed, which shortening median neutrophil recovery to 11.5 days and median platelet recovery to 34 days.⁸¹ This trial established the feasibility, safety, and efficacy of an *ex vivo* expanded UCB unit as a stand-alone graft. SR1 and UM171 are effective amplification agents for HSCs and are also used as stand-alone grafts. SR-1 produced a 330-fold increase in CB CD34⁺ cells and led to 100% engraftment at a median of 15 days for neutrophils and 49 days for platelets.⁸⁴ A

recent phase I/II clinical study of single UM171-expanded cord blood transplantation explored 100% engraftment, and was feasible, safe, and allowed for the use of small single cords without compromising engraftment.⁸⁵ Although these *ex vivo* expansion results are exciting, due to the limited sample size, there is still much work to be done in this area, and more mechanisms of HSC amplification need to be further explored.

4.3 | Cord blood unit selection

Choosing the most suitable CB unit is the first step of successful UCBT. Optimal unit selection requires consideration of HLA match, unit quality and cell dose. Conventionally, HLA typing for UCBT relies on low-resolution typing for HLA-A and HLA-B (antigen-level) and allele-level typing for DRB1 and does not consider HLA-C locus matching. In 2011, Eapen et al⁸⁷ found that HLA-C antigen matching should be included to minimize mortality risks for units that were matched at HLA-A, HLA-B, or HLA-DRB1 or in the presence of a single locus mismatch at HLA-A, HLA-B, or HLA-DRB1. Later reports support the importance of high-resolution typing and the degree of HLA mismatch at HLA-A, HLA-B, HLA-C, and HLA-DRB1 when selecting UCB units for transplantation both for malignant and nonmalignant diseases.^{88,89} Additionally, the role of donor-specific anti-HLA antibodies (DSA) and other immunogenetic factors, such as killer-cell immunoglobulin-like receptors (KIRs) and non-inherited maternal antigen (NIMA) compatibility, are currently under investigation.⁹⁰ A retrospective analysis using the database of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) showed that pretransplant DSA with a mean fluorescence intensity (MFI) ≥ 1000 was associated with an increased risk of graft failure (GF) in sUCBT. In an MD Anderson Cancer Center (MDACC) analysis of 110 sUCBT patients, patients homozygous for HLA-C2 group alleles had a higher 1-year relapse rate and worse survival than HLA-C1/C1 or HLA-C1/C2 (HLA-C1/x) patients.⁹¹ Patients lacking a KIR ligand of HLA group C1 or C2 had a better outcome after UCBT.⁹² However, Tanaka et al⁹³ found no effects of KIR ligand incompatibility in the GVH direction on sUCBT outcomes for AL patients without ATG use. Different results indicate that the impact of KIR alloreactivity on UCBT outcomes may depend on the preconditioning regimen and GVHD prophylaxis. Some studies showed that HLA-mismatched UCBT in which the mismatched antigen in the patient matched the NIMA of the UCB donor (NIMA-matched transplantation) was associated with greater neutrophil recovery and better transplant outcomes.^{94,95} However, since the frequency of NIMA matching is below 10%, whether this should be considered in CB selection needs to be further investigated.

In the setting of UCBT, non-HLA factors are as critical as HLA matching in CB unit selection. Unit quality mainly depends on the qualification of UCB banks and is highly related to unit efficacy, including the viability and recovery of CD34⁺ cells after thawing.⁹⁶ Eurocord criteria recommend that cord blood units meet ≤ 2 HLA disparities and $> 3 \times 10^7$ NC/kg or $\geq 2 \times 10^5$ CD34⁺ cells/kg before freezing in malignant diseases, and the cell dose should be increased

TABLE 2 Comparison of transplant effects between single-unit and double-unit UCBT

Ref.	Transplant type	Patient no., conditioning	Neutrophil engraftment rate	Median neutrophil engraftment time	Grade III to IV acute GVHD	Chronic GVHD	Relapse rate	TRM	OS	LFS
Kindwall-Keller et al ⁵⁷	sUCBT	27, RIC	85%	25	19.2%	21.7%	59.3%	11.1%	35.9%	28.6%
	dUCBT	23, RIC	91%	23	17.4%	26.3%	30.4%	17.4%	39.1%	39.1%
	P value	/	.99	.99	.87	.73	.045	.48	.86	.71
Wagner et al ⁵⁸	sUCBT	113, MAC	89%	21	13%	9% ^a	12%	19%	73%	70%
	dUCBT	111, MAC	88%	23	23%	15% ^a	14%	22%	65%	64%
	P value	/	.29	>.05	.02	.05 ^a	.12	.43	.17	.11
Michel et al ⁵⁹	sUCBT	68, MAC	92.6%	24.8	25.0%	14.7% ^a	23.5%	5.9%	68.8%	67.6%
	dUCBT	69, MAC	94.2%	23.5	18.8%	31.9% ^a	17.4%	11.6%	74.8%	68.1%
	P value	/	>.05	>.05	.40	.02 ^a	.31	.25	.56	.74
Baron et al ⁶⁰	sUCBT	172, RIC	77%	19	11%	28%	32%	22%	41%	46%
	dUCBT	362, RIC	83%	24	13%	36%	35%	29%	51%	36%
	P value	/	.40	<.001	.6	.2	.5	.2	.03	.06
Balligand et al ⁶¹	sUCBT	56, MAC (MRD+)	N.R.	N.R.	N.R.	N.R.	41.7%	19%	53.6%	53%
	dUCBT	59, MAC (MRD+)	N.R.	N.R.	N.R.	N.R.	10.5%	22%	82.6%	82.6%
	P value	/	N.R.	N.R.	N.R.	N.R.	.025	.43	.031	.028
Zheng et al ⁶²	sUCBT	60, MAC	96.7%	19	12.1%	24.4%	11.7%	33.3%	56.7%	55.0%
	dUCBT	37, MAC	89.2%	22	8.7%	28.4%	13.5%	54.1%	37.8%	32.4%
	P value	/	.026	.079	.59	.72	.82	.026	.037	.017

^aExtensive chronic GVHD.

Abbreviations: dUCBT, double-unit UCBT; GVHD, graft-vs-host disease; LFS, leukemia free survival; MAC, myeloablative conditioning; MRD, minimal residual disease; N.R., not reported; OS, overall survival; RIC, reduced intensified conditioning; sUCBT, single-unit UCBT; TRM, transplant-related mortality; UCBT, umbilical cord blood transplantation.

TABLE 3 Clinical trials for ex vivo manipulation of UCB to transplant

	Expansion approach	Clinical trial staging	Expanded TNC, Median (range), ×10 ⁷ /kg	Expanded CD34 ⁺ , Median (range), ×10 ⁵ /kg	Neutrophil engraftment rate	Neutrophil time, median (range), days	Platelet engraftment time median (range), days
Delaney et al ⁷⁹	Notch ligand	Phase I (n = 10)	4.6 (0.6-9.1)	60 (9.3-130)	N.R.	16 (7-34)	N.R.
Horwitz et al ⁸⁰	NICord dUCBT	Phase I (n = 11)	2.5 (1.7-3.8)	35 (9-183)	42d 90.9%	13 (7-26)	33 (26-49)
Horwitz et al ⁸¹	NICord sUCBT	Phase I/II (n = 36)	4.9 (2.0-13.3)	63 (14-149)	42d 94%	11.5 (9-14) ^a	34 (32-42) ^a
de Lima et al ⁸²	Copper chelator (StemEx)	Phase I/II (n = 10)	0.9 (0.1-29.8)	1.2 (0.16-45.04)	46d 90%	30 (16-46)	48 (35-105)
Stiff et al ⁸³	Copper chelator (StemEx)	N.R. (n = 101)	2.2 (N.R.)	9.26 (0.83-117.56)	91.9%	21 (18.4-23.5) ^a	54 (43.3-61.9) ^a
Wagner et al ⁸⁴	StemRegenin-1 (SR1)	Phase I/II (n = 17)	5 (1-12)	175 (14-483)	100%	15 (6-30)	49 (28-136)
Cohen et al ⁸⁵	UM171	Phase I/II (n = 22)	N.R.	28.75 (7.89-54.58)	100%	18 (12.5-20.0) ^b	42 (35-47) ^b
de Lima et al ⁷²	MSC	N.R. (n = 24)	5.84 (0.03-14.37)	9.5 (16-93.4)	42d 96%	15 (9-42)	42 (15-62)
Mehta et al ⁸⁶	MPC	N.R. (n = 27)	5.7 (1.35-11.8)	16 (0.4-53)	26d 78%	12 (1-28)	31 (9-52)

^a95% confidence interval.

^binterquartile range.

Abbreviations: dUCBT, double-unit UCBT; MPC, mesenchymal precursor cell; MSC, mesenchymal stromal cell; N.R., not reported; sUCBT, single-unit UCBT; TNC, total nucleated cell; UCB, umbilical cord blood.

to >3.5 × 10⁷ NC/kg and ≤1 HLA disparities in nonmalignant diseases.⁹⁷ The UK consensus guidelines state different requirements for HLA matching and cell dose.⁹⁸ Less stringent criteria consisting of a TNC dose ≥2.0 × 10⁷/kg and 4/6 or better matching for HLA-A, HLA-B, and HLA-DR, all at the antigen level, are acceptable for the Japanese patient population.¹² Recent unrelated CB unit selection guidelines from the National Marrow Donor Program and the Center for International Blood and Marrow Transplant Research (NMDP/CIBMTR) recommend a minimum of 8 high-resolution (HLA-A, HLA-B, HLA-C, and HLA-DRB1) for both patients and CB units, ≥ 4/6 HLA-A and HLA-B antigen, HLA-DRB1 high-resolution (traditional match), ≥ 4/8 high-resolution match (some centers are investigating the use of 4/6 and 3/8 units if there is an adequate dose), TNC ≥2.5 × 10⁷/kg, and CD34⁺ cells ≥1.5 × 10⁵/kg (some centers recommend a higher CD34⁺ dose as minimum) in sUCBT.⁹⁹ Overall, suitable UCB unit selection should use the following principles: (a) optimal allele-level HLA matching at HLA-A, HLA-B, HLA-C, and HLA-DRB1; DSA should be avoided; (b) adequate unit quality; and (c) minimum required TNC and CD34 cell doses.

4.4 | Pitfalls of the ATG-containing conditioning regimen in UCBT

ATG, with its well-documented effect on T-cell depletion, has been used to improve engraftment and reduce the risk of GVHD after allogeneic HSCT from related sibling, haplo-identical and unrelated donors. However, the use of ATG as part of the conditioning regimen in UCBT is still under debate. Sanz et al¹⁰ from Hospital Universitario La Fe in Spain routinely used ATG as part of the conditioning regimen prior to UCBT. This conditioning regimen consists of thiotepea, busulfan, cyclophosphamide and horse ATG (Lymphoglobuline, 15 mg/kg per day on days -5, -4, -3, and -2). The cumulative incidence of neutrophil and platelet engraftment was 96% and 73% at median times of 20 and 62 days, respectively. The cumulative incidences of grade II ~ IV and III ~ IV acute GVHD and extensive chronic GVHD were 26%, 15%, and 30%, respectively. LFS, NRM, and relapse at 2 years were 42%, 39%, and 19%, respectively. Patients transplanted in first complete remission (CR1) receiving TNC >2 × 10⁷/kg had a 4-year LFS of 75%.

Controversially, ATG has a long half-life and contributes to viral reactivation and the development of lymphoproliferative disease. On the other hand, the CD8⁺ T-cell dose influences neutrophil engraftment time when CD34⁺ cells are lower. In vivo depletion of donor graft-facilitating CD8⁺ lymphocytes by ATG may affect CB engraftment in the setting of UCBT. Pascal et al¹⁰⁰ investigated the role of ATG in UCBT with RIC consisting of low-dose total body irradiation (TBI), cyclophosphamide, and fludarabine (Cy/Flu/TBI 200). In multivariate analyses, the use of ATG was associated with a decreased incidence of acute GVHD (*P* < .0001), a higher incidence of NRM (*P* = .0009), and decreased OS (*P* = .003). Therefore, these results suggested that the use of ATG could be detrimental, especially if it was administered too close to the graft infusion in adults undergoing

UCBT following the Cy/Flu/TBI 200 regimen. A retrospective analysis evaluated the effect of ATG on patient outcomes in 207 children with high-risk or advanced hematological malignancies at our transplant center and the other seven child blood disease centers in China. The results demonstrated that patients who received conditioning that omitted ATG had a faster platelet recovery, a comparable GVHD and TRM, a significantly lower relapse risk, and improved long-term survival compared with those patients who received ATG during conditioning.¹⁰¹ A retrospective (development) and a prospective (validation) study in our single transplant center confirmed the superiority of modified myeloablative conditioning without ATG to myeloablative conditioning with ATG in UCBT for hematological malignancies.¹⁰² The use of ATG in UCBT conditioning regimens remains controversial. A randomized study is required to determine whether omitting ATG confers a survival advantage for patients undergoing UCBT.

4.5 | Pre-engraftment syndrome

Pre-engraftment syndrome (PES) was initially described as a pre-engraftment immune reaction (PIR) in 2005 and was first proposed by Professor Young-Ho Lee.^{103,104} Although a uniform definition is lacking, PES has overlapping features with hyperacute GVHD and engraftment syndrome (ES), which are commonly characterized by noninfectious fever, erythematous rash, diarrhea, jaundice, and capillary leak syndrome (CLS), including noncardiogenic fluid retention or pulmonary manifestations such as tachypnea, hypoxemia, and pulmonary edema, before neutrophil engraftment.¹⁰³⁻¹⁰⁵ The pathogenesis and severity classification are still not clear; PES may be caused by cytokine storms associated with toxicities of the conditioning regimen, GVHD prophylaxis drugs, DMSO, G-CSF, or mismatched antigens by donor T cells. However, heterogeneity of the conditioning regimens and specific GVHD prophylaxis strategies may account for the different ranges (20-78%) of the reported incidences of PES.¹⁰⁶⁻¹⁰⁹

Generally, PES may lead to a higher incidence of grade II-IV acute GVHD,^{20,21} but data are conflicting as to whether PES may benefit cord blood engraftment and whether PES influences TRM, relapse and survival.^{103,104,110-113} PES may be self-limited and require no therapy in some mild patients, and most patients are responsive to methylprednisolone (MP). Tocilizumab-targeted anticytokine therapy may be an effective adjuvant treatment in steroid-resistant cases or in severe patients who have clinically significant manifestations of CLS, especially hypoxemia and pulmonary edema.

5 | CYTOMEGALOVIRUS REACTIVATION

CMV reactivation is a significant complication in UCBT patients, associated with increased transplant-related morbidity and mortality.^{114,115} Because of the difference in condition regimens and GVHD prophylactics, the infection rates of CMV post UCBT varies

substantially in many studies. Recipient CMV serostatus was the most important risk factor that predicted the reactivation of CMV viremia or disease, while CMV serologies of cord blood donor infants and their mothers may not improve the risk of CMV reactivation.^{116,117} The association between CMV reactivation and the clinical outcome of cord blood transplantation is still controversial. In a recent large-scale study, 3147 eligible UCBT patients older than 16 years showed a favorable effect of CMV reactivation on relapse and OS was observed in high-risk AML and MDS.¹¹⁸ While a Korean study revealed that CMV reactivation did not impact leukemia relapse or survival, and CMV disease can result in higher TRM and lower survival.¹¹⁹ The results were not consistent in different studies, this might be due to discrepancies in patient characteristics such as age and the use of antithymocyte globulin.¹¹⁸⁻¹²⁰

6 | CONCLUSION

UCB remains a viable donor option for allo-HSCT. Rapid availability and easy transport, especially during the COVID-19 pandemic, lower immunogenicity of UCB, lower incidence and severity of chronic GVHD, and a stronger GVL effect on recipients with MRD are advantages of UCBT. However, delayed engraftment and GF, increased infection risks and TRM remain important challenges for UCBT. To overcome these challenges, new strategies are constantly being explored, including dUCBT and ex vivo expansion of UCB cells, but no significant improvement in transplant outcomes has been demonstrated. With the development of HLA matching, CB unit selection, modified conditioning regimens and effective management of complications, UCBT has achieved comparable OS and better GRFS than other allo-HSCT types. Future directions should focus on innovative research on the basic biology of UCB stem cells, novel randomized controlled clinical trials, and perfect quality control of UCB banking, making UCBT more popular for more patients.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

X.Z., B.T.: conception and design of the study as well as writing of the manuscript; Z.S.: conception and design of the study, final approval of the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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