Recent progress in allogeneic stem cell transplantation using alternative stem cell sources

Single cord blood transplantation in Japan; expanding the possibilities of CBT

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Abstract

Cord blood (CB) has been an alternative stem cell source for patients with a wide variety of hematological diseases. Cord blood confers the advantages of rapid availability and higher tolerance to two HLA antigen mismatches compared with unrelated donors, and this has increased opportunities for patients who do not have suitable donors or require urgent transplantation. Although the higher rate of engraftment failure remains a serious concern after cord blood transplantation (CBT), the mechanisms underlying this risk have gradually been clarified, which has helped to improve engraftment. Recent studies of CBT and other alternatives have reported comparable outcomes. Moreover, CBT shows promise even when patients are in a non-remission status, which may reflect the potent graft-versus-leukemia effect of CB. Here we compare the most recent outcomes of CBT with those of other stem cell sources and discuss the potential of CB and several outstanding issues that require resolution.

Keywords Cord blood transplantation · Engraftment failure · Early immune reactions · Graft-versus-leukemia effect

Background

Since Gluckman and colleagues achieved the first sibling human leukocyte antigen (HLA)-matched cord blood transplantation (CBT) during 1988 in a pediatric patient with Fanconi anemia [1], cord blood (CB) has gradually become an established source of hematopoietic stem cells for transplantation. Cord blood has several advantages over other stem cell sources, including widespread availability with rapid access, increased tolerance to human leukocyte antigen (HLA) mismatches and a decreased incidence of severe graft-versus-host disease (GVHD). Thus, CBT has provided opportunities for allogeneic hematopoietic stem cell transplantation (Allo-HSCT) to a far wider range of patients who lack suitable HLA-identical siblings or matched unrelated donors within reasonable time periods. Although high rates of engraftment failure and non-relapse mortality (NRM)

after CBT have been a serious concern, progress has been significant. Over 40,000 individuals worldwide have been transplanted with CB to date [2]. The annual number of CBT in Japan now exceeds 1,300 and the cumulative number of CBT reached 15,544 in 2017, accounting for almost one-third of the numbers of total CBT performed worldwide. The average body weight for a Japanese person is less than that for Europeans and North Americans, and body weight is a factor that affects the likelihood of finding a suitable CB donor. More European and North American patients have been transplanted with double units (DCBT) and the number of adult recipients of DCBT has surpassed the number of those transplanted with single units since 2005 in Europe [3], whereas almost all CBT in Japan is single CB. Furthermore, the numbers of CBT have recently decreased in Europe and in the USA [2]. This is not apparently due to comparative findings between CBT and other types of stem cell sources, but rather an increase in medical expenses associated with CBT and the expansion of haplo-identical transplants with post-transplant cyclophosphamide (Haplo-PT-CY).

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Comparison with other stem cell sources

The outcomes of CBT have never been compared with those of alternative sources in randomized prospective studies. Table 1 shows the major retrospective comparisons of CB with other sources [4–8]. Eapen et al. recently compared CBT with bone marrow transplantation (BMT) or peripheral blood progenitor cell transplantation (PBPCT) matched at the allele levels of HLA-A, -B, -C, and DRB1 from unrelated donors that are generally regarded as the gold standard [6]. A retrospective analysis of data derived from 1,525 patients with acute leukemia (single CBT $n = 165$; PBPCT $n = 888$; BMT $n = 472$) found slower neutrophil recovery in recipients of CBT than PBPCT or BMT. Rates of non-relapse mortality (NRM) were higher after CBT than after 8/8 allele-matched PBPCT (HR 1.62, $p < 0.01$) or BMT (HR 1.69, $p < 0.01$), whereas rates of leukemia relapse were similar regardless of the stem cell source. The prevalence of grades 2–4 acute and chronic GVHD was lower in recipients of CBT compared with allele-matched PBPCT (HR, 0.57; $p < 0.01$ and HR, 0.38; $p < 0.01$, respectively), whereas rates of chronic, but not acute GVHD, were lower after CBT than after allele-matched BMT. Leukemia-free survival (LFS) after CBT was comparable to 8/8 and 7/8 allele-matched PBPCT or BMT. Atsuta et al. compared data from a Japanese registry of CBT and 8/8 allele-matched unrelated BMT (UBMT), and the outcomes of patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) were separately assessed [7]. In AML patients, CBT recipients showed higher NRM rate and similar relapse rate, resulting in poorer overall and leukemia-free survival rates compared with recipients of UBMT. All these parameters were comparable between CBT and UBMT recipients who had ALL. Terakura et al. more recently analyzed the outcomes for 2,472 adults with acute leukemia who received their first myeloablative transplantation between 2000 and 2010. They compared transplantation outcomes of 8/8 and 7/8 allele-matched UBMT with those of CBT (8/8 UBMT, $n = 1,001$; 7/8 UBMT, $n = 656$; CBT, $n = 815$) [8]. Severe acute GVHD and NRM after UCBT were comparable with 8/8 UBMT, whereas those after 7/8 UBMT were significantly higher. The incidence of extensive chronic GVHD was significantly lower after CBT than 8/8 and 7/8 UBMT. The OS of patients with AML who underwent CBT and 8/8 UBMT was similar, whereas that of 7/8 UBMT was poor. Overall survival did not significantly differ among the three groups with ALL. These findings derived from registry data support the notion that CB can be transplanted into adults with acute leukemia when an HLA-matched unrelated adult donor is unavailable and when a transplant is urgently required.

A CB graft and related donor with HLA 1-antigen mismatch or haplo-match can serve as an alternative to HLA-identical siblings or matched unrelated donors, particularly when the need for transplantation is immediate. Kanda et al. compared the outcomes of a first myeloablative or reduced-intensity transplantation (2288 CBT, 525 RD/1AG-MM-GVH) with single CBT from a related donor with an HLA 1-antigen mismatch in the GVH direction (RD/1AG-MM-GVH) in a national registry of Japanese patients with leukemia or myelodysplastic syndrome (MDS) [9]. The survival rates were comparable between the CBT and RD/1AG-MM-GVH groups and better than that of the RD/1AG-MM-GVH group with an HLA-B mismatch. Neutrophil and platelet engraftment were significantly slower, whereas the incidence of acute or chronic GVHD was significantly lower in the CBT group.

In 2 parallel phase 2 trial of DCBT and Haplo-PT-CY (50 DCBT, 50 Haplo-PT-CY), in which all patients received the same reduced-intensity conditioning (RIC) regimen of fludarabine, cyclophosphamide and 2 Gy of total body irradiation (TBI) (Flu/Cy/TBI), the 1-year disease-free survival (DFS) rates were comparable at 46% and 48%, respectively. The NRM at 1 year was higher, but relapse rates were lower after DCBT than Haplo PT-CY (24% vs. 7%, and 31% vs. 45%, respectively) [10]. Patients with high risk for relapse may benefit from CBT, while assessment of NRM risk for each patients and strategies to reduce NRM should be warranted. The ongoing national US randomized comparison of DCBT vs. Haplo PT-CY in RIC patients will be the first prospective comparison of these two graft sources (BMT CTN1101).

Excellent outcomes of CBT exceeding those of other sources have been reported (Table 1). Although studies were from single institutions with relatively few patients [11, 12], the results suggest that CBT has comparable or even greater potential than other approaches and that improvements of clinical outcomes might be possible.

Factors associated with engraftment failure and clinical strategies to overcome this issue

Cell dose is one of the most important factors associated with engraftment. The median numbers of total nucleated cells (TNC) or CD 34⁺ cells infused from a single CB graft is tenfold less than those infused during adult donor stem cell grafts. Moreover, very few passively transferred T cells that would protect against graft rejection are found in CB grafts. Several studies have associated higher doses of infused TNC with better engraftment [13–16], and CB units comprising $> 2.5\text{--}3.0 \times 10^7$ TNC/kg are generally recommended to avoid risk of engraftment failure after single CBT. However, engraftment does not fail in all patients

Table 1 Comparisons of cord blood with other types of donors for transplantation

Group	Donor type	HLA mis-matches	Diagnosis	Relapse	<i>p</i>	NRM	<i>p</i>	OS or LFS/DFS	<i>p</i>
Reports from registry data									
CIBMTR and NCBP 2004 [4]	CB	1~2/6	AML, ALL, CML, MDS	26/150 (17%)	0.65 (CB vs. 1/6 BM)	95/150 (63%)	0.96 (CB vs. 1/6 BM)	OS: 26% (3 years)	0.62 (CB vs. 1/6 BM)
	Unrelated BM	0/6	AML, ALL, CML, MDS	83/367 (23%)		169/367 (46%)		OS: 35% (3 years)	
		1/6	AML, ALL, CML, MDS	12/83 (14%)		54/83 (65%)		OS: 20% (3 years)	
Eurocord and EBMT 2004 [5]	CB	0~3/6	AML, ALL	23%		44% (2 y)		OS: 36% (2 years)	
	Unrelated BM	0/6	AML, ALL	23%	0.71	38% (2 years)	0.13	OS: 42% (2 years)	0.08
JCBBN and JMDP 2009 [7]	CB	0~2/6	AML	31% (2 years)		33% (2 years)		OS: 43% (2 years)	
	Unrelated BM	0/8 (allele)	AML	24% (2 years)	0.067	22% (2 years)	0.004	OS: 60% (2 years)	<0.001
		0~2/6	ALL	31% (2 years)		24% (2 years)		OS: 49% (2 years)	
	Unrelated BM	0/8 (allele)	ALL	24% (2 years)	0.19	25% (2 years)	0.83	OS: 57% (2 years)	0.4
		0~2/6	AML, ALL	43/165 (25%)		55/165 (33%)		LFS: 98/165 (59%)	
CIBMTR and NCBP and EBMT 2010 [6]	Unrelated BM	0/8 (allele)	AML, ALL	112/332 (34%)	0.35 (vs. CB)	76/332 (22%)	0.003 (vs. CB)	LFS: 188/332 (56%)	0.25 (vs. CB)
	Unrelated PB	0/8 (allele)	AML, ALL	209/632 (33%)	0.31 (vs. CB)	149/632 (23%)	0.003 (vs. CB)	LFS: 358/632 (56%)	0.18 (vs. CB)
		1/8	AML, ALL	42/140 (30%)	0.42 (vs. CB)	46/140 (32%)	0.76 (vs. CB)	LFS: 80/140 (57%)	0.63 (vs. CB)
		1/8	AML, ALL	77/256 (30%)	0.63 (vs. CB)	93/256 (36%)	0.78 (vs. CB)	LFS: 170/256 (66%)	0.46 (vs. CB)
	CB	0~2/6	AML, ALL, CML, MDS	35% (3 years)		30% (3 years)		OS: 38% (3 years)	
JCBBN and JSHCT 2012 [9]	Related PB/ BM	1/6	AML, ALL, CML, MDS	32% (3 years)	0.041	32% (3 years)	0.47	OS: 39% (3 years)	0.115
JCBBN and JMDP 2015 [8]	CB	0~6/6 (allele)	AML	HR 0.95	0.66	RR 1.15	0.24	OS HR 1.09	0.35
	Unrelated BM	0/8 (allele)	AML	1		1		1	
		1/8 (allele)	AML	HR 0.99	0.96	RR 1.43	0.001	HR 1.30	0.006
	CB	0~6/6 (allele)	ALL	HR 1.05	0.77	RR 1.15	0.24	OS HR 1.14	0.32
	Unrelated BM	0/8 (allele)	ALL	1		1		1	
	Unrelated BM	1/8 (allele)	ALL	HR 0.95	0.75	RR 1.43	0.001	HR 1.16	0.22

Table 1 (continued)

Group	Donor type	HLA mis-matches	Diagnosis	Relapse	<i>p</i>	NRM	<i>p</i>	OS or LFS/DFS	<i>p</i>
Reports from single institutions									
IMSUT 2004 [11]	CB (<i>n</i> = 68)	0~4/6	AML, ALL, CML, MDS, ML	16% (2 years)		9% (2 years)		DFS:74% (2 years)	
	Unrelated BM (<i>n</i> = 45)	0~1/6	AML, ALL, CML, MDS	25% (2 years)	0.73	29% (2 years)	0.02	DFS:44% (2 years)	<0.01
FHCRC 2016 [12]	CB (<i>n</i> = 140)	0~2/6	AML, ALL, MDS	1*		1		1*	
	Unrelated BM/PB (<i>n</i> = 344)	0/10 (allele)	AML, ALL, MDS	HR 2.92*	0.007*	HR 0.72	0.20	OS HR 1.69*	0.08*
	Unrelated BM/PB (<i>n</i> = 98)	1/10 (allele)	AML, ALL, MDS	HR 3.01*	0.02*	HR 1.11	0.22	2.92*	0.001*

ALL acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *BM* bone marrow, *CB* cord blood, *CIBMTR* Center for International Bone and Marrow Transplant Research, *CML* chronic myelogenous leukemia, *DFS* disease-free survival, *EBMT* European Group for Blood and Marrow Transplantation, *FHCRC* Fred Hutchinson Cancer Research Center, *HR* hazard ratio, *IMSUT* Institute of Medical Science, University of Tokyo, *JCBBN* Japan Cord Blood Bank Network, *JMDP* Japanese Marrow Donor Program, *JSHCT* the Japan Society for Hematopoietic Cell Transplantation, *LFS* leukemia-free survival, *MDS* myelodysplastic syndrome, *ML* malignant lymphoma, *OS* overall survival, *NRM* non-relapse mortality, *NCBP* National Cord Blood Program, *PB* peripheral blood

*Patients with minimal residual disease

who receive CB units containing less than this “threshold”. Nakasone et al. retrospectively analyzed the outcomes of patients listed in a Japanese registry who were transplanted with a single unit of CB containing $0.5\text{--}2.5 \times 10^7$ TNC/kg [17]. They found that engraftment was more favorable for patients who were transplanted with low TNC and high CD 34⁺ cells than with high TNC and low CD 34⁺ cells. Several studies have recognized the importance of the infused dosage of CD 34⁺ cells [15, 18, 19].

The degree of HLA disparity is also important, but the significance varies among studies. Kurtzberg et al. analyzed data derived from 191 children who received a single CBT in multicenter prospective phase II study and found better neutrophil engraftment in those who received 5 or 6 of 6 matched units than 3 or 4 of 6 (Hazard ratio 1.39, *p* = 0.04 in multivariate analysis) [20]. Yet, several other studies found no relationship between HLA disparity and engraftment [14, 21]. Atsuta et al. investigated the effect of HLA disparity on the outcomes of single CBT in 498 pediatric and 1,800 adult patients in Japan [22]. They also found no correlation between HLA disparity and neutrophil engraftment in either group, although more mismatched HLA loci correlated with increased risk of NRM in the pediatric patients. Barker et al. retrospectively analyzed the combined effects of TNC and HLA match on the outcomes of 1,061 adult and pediatric CBT recipients with leukemia or myelodysplasia [23]. They found that TNC dose and HLA mismatch were independent predictors of engraftment, NRM or OS. A greater HLA

mismatch resulted in a notably greater importance of TNC to engraftment and OS. For example, units that were 4/6 HLA-matched to recipients required $\text{TNC} > 5.0 \times 10^7/\text{kg}$ to achieve a similar NRM to that of 5/6 units with $\text{TNC} > 2.5 \times 10^7/\text{kg}$. These findings suggest that a better HLA match could compensate for a lower dose of TNC.

Finding donor-specific anti-HLA antibodies (DSA) in recipients is associated with poor engraftment. A retrospective analysis of myeloablative single-unit CBT by Takanashi et al. found that engraftment was achieved in 32% and 83% of 386 patients with and without DSA, respectively [24]. The notion has been suggested that unrecognized DSA against HLA-C, -DP, -DQ or DRB3/4/5 antigens, which are not routinely typed in CB grafting, adversely affect engraftment in HLA-mismatched CBT [25]. HLA typing at these loci or cytotoxic crossmatch tests before transplantation might help to avoid transplantation with CB units that have unrecognized DSA. However, these methods have not yet been fully evaluated in the setting of CBT.

Conditioning regimens are more crucial to achieve successful engraftment in CBT compared with others sources. In a study of the outcomes of 10 adult patients who underwent DCBT with fludarabine plus a myeloablative dose of intravenous busulfan (Flu/Bu4) regimen at Duke University found that only 2 of 10 patients became engrafted with donor hematopoietic cells [26]. Considering that the FluBu4 regimen has been widely applied to allogeneic BMT or PBPC without an increase in the incidence of engraftment failure

[27, 28], more intensive immune suppression before transplantation seems crucial to ensure CB engraftment. Nakasone et al. retrospectively analyzed the impact of TBI on neutrophil engraftment in 1,464 adult recipients of CBT using registry data [29] and found that TBI significantly and favorably affected engraftment in both myeloablative conditioning (MAC) and RIC groups. Conversely, Yamamoto et al. recently investigated the novel non-TBI regimen of Flu/Bu4 + Mel regimen that was expected to increase lymphoablative and myeloablative effects. They found better engraftment and survival rates even in patients with myeloid malignancies not in remission [30].

Strategies to increase the infused cell dose and possibly improve outcomes have included DCBT. Brunstein et al. retrospectively reviewed the outcomes of 110 patients with high-risk hematological malignancies who underwent single or double CBT after the RIC regimen [31]. The targeted TNC dose was set at $3.0 \times 10^7/\text{kg}$, and patients were to receive DCBT when a single unit contained fewer cells. Overall rates of engraftment (94% vs. 91%), overall survival and NRM did not significantly differ between single vs. double. Relapse rates were notably lower in the DCBT, than the single group (30% vs. 41% $p=0.07$), whereas the incidence of acute GVHD tended to be higher in the DCBT group. Although DCBT has become popular due to the relatively larger body weight of North American and European populations, randomized single and double CBT data have never been compared in adult patients. A randomized phase III clinical comparison of single and double CBT of 224 pediatric patients found no significant differences after single and double CBT between overall rates of engraftment (89% vs. 88%), relapse rates at 1 year (12% vs. 14%), NRM at one year (19% vs. 22%), or disease-free survival (70% vs. 64%) [32]. Presently, the use of double units is justifiable only when one unit does not contain enough TNC [33].

Early immune reactions after CBT and GVHD prophylaxis

Because CB contains immature immune cells, it is thought to be less active in inducing allogeneic immune reactions than other sources of stem cells. However, several studies including ours have identified a unique clinical manifestation characterized by non-infectious high-grade fever, skin eruption, diarrhea, jaundice, and body weight gain soon after CBT [34–41] (Table 2). The onset occurred around post-transplant day 9, and it has been termed pre-engraftment immune reactions (PIR) or pre-engraftment syndrome (PES). The incidence of PIR ranged from 20 to 78% in various studies, probably because of different definitions, or different transplant procedures with respect to conditioning regimens or GVHD prophylaxis (Table 2). The clinical significance

of PIR on transplant outcomes has been controversial. In early studies from us where preparative regimen without anti-thymocyte globulin (ATG) coupled with calcineurin inhibitor (CI) alone as GVHD prophylaxis was mainly used [42, 43], PIR itself sometimes induced severe organ failure including pulmonary complications or hemophagocytic syndrome (HPS), defined as severe PIR, and it has increased the incidence of early NRM among elderly patients. HPS might be a unique and characteristic manifestation after CBT, considering that it has been regarded as a rare complication after BMT or PBPCT. Takagi et al. retrospectively analyzed the outcomes of 119 patients who underwent RIC-CBT and received less intensive GVHD prophylaxis. Twenty patients had HPS, mostly with donor-dominant chimerism, and 14 of them eventually failed engraftment due to the development of HPS [44]. We also reported that HLA disparity in the direction of GVH, but not HVG, adversely affected engraftment kinetics when single CI was administered for GVHD prophylaxis [16]. A novel mechanism of engraftment failure proposed based on these findings was that excessive immune reactions of donor cells result in engraftment failure through the development of HPS after CBT. We also found that the incidence of HPS was significantly higher among patients with severe PIR than without PIR [45]. Thus, PIR and HPS closely correlate and could be considered as post-transplant cytokine-storm syndrome, which can occur after HLA-mismatched transplantation. The mechanisms underlying PIR or HPS have not been clarified. Serum values for inflammatory cytokines such as IL-6 or IL-5, are elevated around the time of PIR onset [46, 47]. We previously showed that rapid T cell chimerism switches to the donor type, and phenotypic conversion from naïve to memory T cells are significantly associated with the development of PIR [48]. Gutman et al. also reported that CD8⁺ T cells predominately expressed effector memory or effector phenotype and produced IFN- γ in response only to non-engrafted units early after double-unit CBT, reflecting a specific immune response of the dominant, against the non-engrafted units [49]. Donor T cell activation possibly via mismatched HLA could cause inflammatory cytokine secretion and such excessive allogeneic immune reactions will activate macrophages to develop phagocytosis, which will finally cause engraftment failure. Figure 1 shows a schema of HPS development. Contrary to previous notions, CB naïve T cells have powerful proliferative potential to act against allogeneic antigens very soon after CBT, which would be the main mechanism behind early immune-related events.

Since immune cells in CB units have the conflicting features of immunological immaturity and potential for hyper-immune reactions, and since both directly impact engraftment, GVHD prophylaxis is critical to achieve engraftment. Combining CI with other immuno suppressive agents, including mycophenolate mofetil (MMF), corticosteroids,

Table 2 Selected studies of PIR or PES after CBT

Series	Patients (n)	Conditioning regimen	Graft	GVHD prophylaxis (n)	Median (range) on day of PIR onset (range)	Incidence of PIR	Risk factors PIR	Impact of PIR on transplant outcomes
Kishi et al. [34]	57	RIC	Single	CsA	Day 9 (6–13)	78%	NA	NRM → Relapse →
Narimatsu et al. [35]	77	MAC (n=31) RIC (n=46)	Single	CsA (n=23) TAC (12) CsA + sMTX (17) TAC + sMTX (23) CsA + PSL (2)	Day 6.5 (2–18)	Without MTX group: 49% With MTX group: 25%	GVHD prophylaxis without MTX	Without MTX group: OS ↓
Patel et al. [36]	52	MAC (n=36) RIC (n=16)	Double	CsA + MMF	Day 9 (5–12)	31%	Not detected	Engraftment → II–IV aGVHD → NRM → OS →
Frangoul et al. [37]	21	MAC	Single	CsA + PSL + ATG	Day 10 (5–24)	20%	TNC > 5 × 10 ⁷ /kg	Engraftment → II–IV aGVHD ↑ cGVHD ↑ OS →
Wang et al. [38]	81	MAC (n=72) RIC (n=9)	Single (n=53) double (n=28)	CsA + MMF	Day 7 (3–15)	63%	MAC vs. age (< 14 years)	Engraftment → II–IV aGVHD ↑ cGVHD → NRM → OS →
Uchida et al. [41]*	58	RIC	Single	TAC (n=29) TAC + MMF (n=9)	NA	TAC: PIR 76% vs.; severe PIR 52% TAC + MMF: PIR 69% vs.; severe PIR 16%	NA	TAC + MMF group: Engraftment ↑ Day 30 NRM ↓
Park et al. [39]	381	MAC (n=261) RIC (n=120)	Single (n=225) double (n=156)	Various including ATG (n=259) vs. MTX (34) and/or PSL (137)	Day 7 (3–41)	27%	Disease risk (low), MAC, GVHD prophylaxis without MTX or PSL, TNC > 5.43 × 10 ⁷ /kg	Engraftment ↑ II–IV aGVHD ↑ cGVHD → NRM → relapse → OS →
Kanda et al. [40]	57	MAC	Double	CsA + MMF (n=22) TAC + MMF (n=35)	Day 12 (4–22)	77%	CsA + MMF as GVHD prophylaxis	Engraftment → II–IV aGVHD → NRM → or ↑ relapse → OS →

aGVHD acute graft-versus-host diseases, cGVHD chronic graft-versus-host diseases, CsA cyclosporine, PIR pre-engraftment immune reactions, MAC myeloablative conditioning, MMF mycophenolate mofetil, Attain-t-thymocyte globulin, NA not available, NRM non relapse mortality, OS overall survival, PES preengraftment syndrome, PIR pre-engraftment immune reactions, PSL prednisolone, RIC reduced-intensity conditioning, sMTX short-term methotrexate, TAC tacrolimus, TNC total nucleated cells

*Matched pair comparison of (TAC vs. TAC + MMF)

Fig. 1 Schema of HPS development. Donor-derived CTL possibly activated by mismatched host HLA lead to release of various inflammatory cytokines that in turn induce macrophage activation in BM accompanied by downregulated CD47 expression on HSC causing them to become vulnerable to phagocytosis. Mycophenolate mofetil might suppress activated CTL and reduce incidence of HPS. *BM* bone marrow, *CTL* cytotoxic T lymphocytes, *HPS* hemophagocytic syndrome, *HSC* hematopoietic stem cells

methotrexate (MTX), sirolimus and ATG have been explored as GVHD prophylaxis in the setting of CBT. Uchida et al. conducted a matched-pair comparison of tacrolimus (TAC) + MMF vs. TAC regimens for GVHD prophylaxis, to determine the effects of MMF. Although the total incidences of any PIR were comparable between TAC + MMF and TAC, the incidences of severe PIR and HPS were significantly decreased in TAC + MMF group, resulting in lower NRM before engraftment and higher engraftment rates for TAC + MMF compared with TAC (90% vs. 66%, $p < 0.05$) [41]. Combinations of CI and MMF have been applied worldwide [50]. However, only an oral formulation is available in Japan, which raises the concern that the effect might differ among individuals due to variations in bioavailability. Therapeutic drug monitoring of MMF might further improve outcomes [51]. Adding MTX to CI reportedly reduces the incidence of PIR and severe acute GVHD [35, 52], and this has been widely applied in Japan. However, this method is accompanied by the risk of graft rejection and engraftment delay, especially in the RIC setting, possibly due to excessive immunosuppression and the cytotoxic effect of MTX. Reducing the dose of MTX in GVHD prophylaxis has recently been investigated [53]. A retrospective comparison of MMF vs. MTX by Terakura using Japanese registry data [52] showed better engraftment after GVHD prophylaxis using MMF, whereas MTX reduced the incidence or severity of acute GVHD. ATG might also be an alternative strategy for GVHD prophylaxis in terms of reduced incidence of PIR and severe GVHD [37, 54], but this strategy also has various disadvantages including delayed immune recovery, risk of infection or post transplantation lymphoproliferative disorder, and an increased incidence of relapse [55–57]. In particular, administering ATG to patients with high-risk malignancies seems highly controversial due to an increased

incidence of relapse [58]. Additionally, ATG has a long half-life and multiple factors affect its biological activities, which renders its impact on clinical outcomes highly variable according to the dose, timing of administration, and the status of patients.

Comparable or even more potent GVL effect after CBT, and CBT for patients with high-risk malignancies

Considering that CB grafts contain T cells with an immunologically naïve phenotype, concerns have been raised regarding the capacity of CB-derived lymphocytes to mediate sufficient graft-versus-leukemia (GVL) effects. However, the incidence of relapse after CBT is comparable to that after BMT or PBPCT (Table 1). Moreover, several studies have shown a decreased relapse rate with CBT despite a lower incidence of GVHD. Brunstein et al. found that recipients of myeloablative DCBT had significantly lower rates of relapse at 5 years compared with recipients of matched-related, matched-unrelated (MUD), and mismatched-unrelated (MMUD) donors (15% vs. 43, 37 and 35%, respectively) [59]. Milano et al. analyzed the outcomes of 582 patients at Fred Hutchinson Cancer Research Center who received a first myeloablative allo-HSCT using CB ($n = 140$), MUD ($n = 344$), or MMUD ($n = 98$) donors [12]. They found that the risk of relapse was significantly lower in the CB, than in the group of patients with minimal residual disease transplanted with HSC from unrelated donors. Overall survival was also significantly better for those who received CBT than after MMUD transplantation. Kanda et al. analyzed the impact of acute and chronic GVHD on outcomes in adult patients with acute leukemia or myelodysplastic syndrome

who underwent their first CBT ($n=2558$) and found that mild acute or chronic GVHD was associated not only with a low risk of relapse but also with a low risk of non-relapse mortality, and provides a survival benefit in UCBT [60]. This effect was not generally observed in other stem cell sources, indicating the unique immunological potential after CBT. The high potential of a GVL effect after CBT has not been clearly explained. The increased level of HLA mismatches or the powerful proliferative potential of CB-derived lymphocytes, as described in developing PIR, could explain the low risk of relapse associated with CBT. A recent report has described that an HLA-DPB1 mismatch induces a GVL effect without developing severe GVHD after single CBT [61].

Patients with advanced disease who need urgent transplantation frequently receive CB grafts because they are rapidly accessible and widely available. This might be associated with the high prevalence of CBT. Although the prognosis of allo-HSCT for advanced hematological malignancies is dismal due to increased risk of relapse regardless of the donor source, the outcomes of CBT with intensified conditioning regimens are encouraging (Table 3). Investigators at the Institute of Medical Science, University of Tokyo have performed single CBT using G-CSF plus high-dose

cytarabine (Ara-C) and CY/TBI and the OS at 7 years is 61.4% in patients with myeloid malignancies not in remission [62]. A Spanish group analyzed the outcomes of 49 adults with high-risk AML who underwent single CBT after a MAC regimen consisting of thiotepe, Bu, Flu, and ATG. The leukemia-free survival (LFS) rate at 2 years was 42%, although high NRM due to infections was problematic [63]. A Chinese group also reported successful outcomes in non-remission patients with DFS at 2 years of 40.3% using intensified MAC regimens [64]. A Flu/Cy/TBI 2 Gy regimen has been applied worldwide in the setting of RIC-CBT [50, 65]. However, disease relapse has been a major issue probably due to the low intensity of the regimen [66]. Ponce et al. intensified the regimen by adding thiotepe and increasing the TBI dose to 4 Gy; the 2-year DFS was 60%, although many patients with CR were included [67]. We applied a novel reduced-toxicity MAC regimen by adding Mel to the Flu/Bu4 regimen for single CBT in a relatively older population with myeloid malignancies not in remission. This non-TBI regimen provided durable engraftment and remission with acceptable NRM and 54.9% survival with DFS at 2 years [30].

Taken together, the potential GVL effect of CB combined with novel conditioning regimens and the availability of CB

Table 3 Selected studies of outcomes of CBT to treat advanced hematological malignancies

Series	Patients (<i>n</i>)	Median age years (range)	Disease	Disease status	Conditioning regimen	CI of neutrophil engraft- ment	NRM	Relapse	OS or DFS
Konuma et al. [62]	61	41 (18–55)	AML, MDS	non-CR (<i>n</i> =61)	G-CSF/HD-AraC + CY/TBI	93.40%	8.2% at 1 years	30.5% at 7 years	OS: 61.4% at 7 years
Sanz et al. [63]	88	28 (1–52)	AML, MDS, ALL, ML	CR1 (<i>n</i> =53), CR > 2 (26), non-CR (13)	TT + BU + Flu + ATG	94%	44% at 5 years	18% at 5 years	DFS: 37% at 5 years DFS: 11% at 5 years in non-CR
Yao et al. [64]	26	13 (6–32)	AML, MDS, ALL, ML	non-CR (<i>n</i> =26)	TBI + Ara-C + CY ± BCNU (<i>n</i> =14) Ara-C + BU + CY ± BCNU (<i>n</i> =11)	100%	35.2% at 2 years	28.9% at 2 years	OS: 50.5% at 2 years DFS: 40.3% at 2 years
Ponce et al. [67]	30	56 (18–69)	AML, MDS	CR1 (<i>n</i> =21) Others (<i>n</i> =9)	TT + Flu + CY + TBI	97%	28% at 2 years	11% at 2 years	OS and DFS: 60% at 2 years
Yamamoto et al. [30]	51	59 (19–70)	AML, MDS, CML	non-CR (<i>n</i> =51)	Flu + BU + Mel	90.20%	25.5% at 2 years	19.6% at 2 years	OS and DFS: 54.9% at 2 years

ALL acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *ATG* anti-thymocyte globulin, *BCNU* carmustine, *BU* busulfan, *CI* cumulative incidence, *CML* chronic myelogenous leukemia, *CR* complete remission, *CY* cyclophosphamide, *DFS* disease-free survival, *Flu* fludarabine, *HD-AraC* high-dose cytarabine, *MDS* myelodysplastic syndrome, *Mel* melphalan, *ML* malignant lymphoma, *NRM* non-relapse mortality, *OS* overall survival, *TBI* total body irradiation, *TT* thiotepe

grafts, has improved the outcomes of patients with high-risk hematological malignancies that have historically been difficult to cure.

Conclusion

During the three decades since the first CBT in France, much progress has been made, and CB has become an alternative graft chosen equally with other donors in Japan. Recently, even strong GVL effect of CB grafts despite a low incidence of severe GVHD has been suggested. Although the high engraftment failure rate and associated high NRM remain to be resolved, CB will continue to be an important stem cell source for transplantation in the future, and hopefully, there is even a chance for CB to become the graft of first priority over the others once the problems were overcome, given the obvious advantages of CB such as rapid and wide availability for many transplant candidates.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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