Biol Blood Marrow Transplant 25 (2019) e55–e59

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Brief Articles

Umbilical Cord Blood Transplantation Using Reduced-Intensity Conditioning without Antithymocyte Globulin in Adult Patients with Severe Aplastic Anemia

Tetsuro Ochi, Yasushi Onishi*, Kentaro Nasu, Koichi Onodera, Masahiro Kobayashi, Satoshi Ichikawa, Tohru Fujiwara, Noriko Fukuhara, Minami Yamada-Fujiwara, Hideo Harigae

Department of Hematology and Rheumatology, Tohoku University Hospital, Miyagi, Japan

Article history:
Received 16 August 2018
Accepted 27 September 2018

Key Words:
Aplastic anemia
Umbilical cord blood transplantation
Reduced-intensity conditioning
Total body irradiation
Antithymocyte globulin

A B S T R A C T

Umbilical cord blood transplantation (UCBT) is a possible option for patients with aplastic anemia (AA) without a related or unrelated HLA-matched donor, particularly if immunosuppressive therapy (IST) has failed or transplantation is urgently needed. However, a higher rate of graft failure after UCBT remains a major problem, and the optimal conditioning regimen for stable engraftment after UCBT has not been established. Here we investigated 6 adult patients with AA who underwent UCBT using a reduced-intensity conditioning (RIC) regimen comprising fludarabine 125 mg/m^2, cyclophosphamide 120 mg/kg, and 4 Gy of total body irradiation (Flu/CY/TBI4Gy) without antithymocyte globulin (ATG). Five patients underwent UCBT after IST failure, and 1 patient underwent UCBT as a first-line treatment due to a fulminant clinical finding of a neutrophil count of 0, despite granulocyte colony-stimulating factor administration. Regarding graft-versus-host disease (GVHD) prophylaxis, 2 patients received tacrolimus plus short-term methotrexate and 4 patients received tacrolimus plus mycophenolate mofetil, and all patients achieved sustained engraftment of both neutrophils and platelets, at a median of 17.5 days (range, 14 to 37 days) and 38.5 days (range, 31 to 86 days), respectively, with complete donor chimerism confirmed in all patients at a median of 14 days (range, 14 to 32 days). Three patients developed grade II acute GVHD (aGVHD), but grade III/IV aGVHD was not observed, whereas 4 patients developed chronic GVHD involving only skin. At the time of this report, all 6 patients were alive without the need for blood transfusion, at a median follow-up of 16 months (range, 12 to 131 months). Although further study is needed, our findings suggest that conditioning with Flu/CY/TBI4Gy without ATG might allow stable engraftment in UCBT for adults with AA.

INTRODUCTION

Immunosuppressive therapy (IST) consisting of antithymocyte globulin (ATG) and cyclosporine A is provided as a first-line treatment in patients with aplastic anemia (AA) without an HLA-matched sibling donor, or when a patient age is >40 years [1–3]. The reported response rate to IST is between ~50% and 70%, with relapse after IST occasionally observed [4,5]. Patients with AA in whom IST failed ideally require allogeneic hematopoietic stem cell transplantation (HSCT) because of poor outcomes associated with repeated IST [6]. Although bone marrow from an HLA-matched donor is the preferred stem cell source for HSCT in patients with AA, patients without an HLA-matched donor need to undergo allogeneic HSCT with an alternative donor.

Outcomes of umbilical cord blood transplantation (UCBT) comparable to those of unrelated donor HSCT are observed in patients with acute leukemia [7,8]; however, opportunities for UCBT to treat AA have been limited owing to a high rate of graft failure (GF) after UCBT in these patients [1,9]. Previous studies have evaluated the use of several types of conditioning regimens in UCBT for patients with AA [10–14], and a reduced-intensity conditioning (RIC) regimen comprising fludarabine (Flu), melphalan (Mel), and low-dose total body irradiation (TBI) showed promising results in 12 adult patients with AA [15]. It is possible that the exclusion of ATG from their regimen might be associated with the lower rate of GF.

In a recent retrospective study, survival rates associated with UCBT were similar to those for unrelated bone marrow transplantation (BMT) in patients age <40 years but tended to be lower in patients age >40 years [16]. However, GF remained a problem after UCBT, with a cumulative incidence of

Financial disclosure: See Acknowledgments on page e59.

* Correspondence and reprint requests: Yasushi Onishi, MD, Department of Hematology and Rheumatology, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Miyagi, Japan.
E-mail address: yonishi@med.tohoku.ac.jp (Y. Onishi).

https://doi.org/10.1016/j.bbmt.2018.09.039
1083-8791/

neutrophil recovery at day 42 of 71% (95% confidence interval [CI], 59% to 80%). Although UCBT has become a promising salvage treatment for patients with AA, particularly after IST failure, a more effective conditioning regimen is warranted to reduce the risk of GF. Toward this end, we investigated the outcomes of 6 consecutive patients who underwent UCBT for AA using a non-ATG regimen comprising Flu, intermediate-dose cyclophosphamide (CY), and low-dose TBI.

METHODS

Patients

We retrospectively reviewed the medical records of 6 consecutive patients with AA who underwent UCBT at Tohoku University Hospital (Miyagi, Japan) between August 2007 and September 2017. The diagnosis of AA and the assessment of disease severity were established according to published criteria [1]. The study was approved by the Ethics Committee of Tohoku University Graduate School of Medicine.

Cord Blood Units

Cord blood units were provided by the Japanese Cord Blood Bank, and all patients in this study received a single unit of cord blood. HLA-A, -B, -C, and -DRB1 were typed at the allele level for both the cord blood and the recipient. We used a cord blood unit within 2-loci mismatches among HLA-A, -B, and -DR loci at the antigen level and at least 1 allele-matched at HLA-DRB1. A cord blood unit with higher total nucleated cell (TNC) and $CD34^+$ cell counts was preferentially selected among at least 2.0×10^7/kg recipient weight of TNCs. If possible, a unit with $>1.0 \times 10^5$/kg $CD34^+$ cells was selected. We performed screening tests for anti-HLA antibodies in all patients to exclude a unit of cord blood with HLA antigens potentially reacting to specific antibodies detected before transplantation.

Engraftment and Graft-versus-Host Disease

Neutrophil engraftment was defined as the first day of an absolute neutrophil count of $>500/\mu L$ for 3 consecutive days. Platelet recovery was defined as a platelet count of $\geq 20{,}000/\mu L$ independent of transfusions for 7 days. Donor chimerism was measured using multiplex polymerase chain reaction for short tandem repeats, and fluorescence in situ hybridization for sex chromosomes was performed in cases of sex-mismatched transplantation. Acute graft-versus-host disease (aGVHD) and chronic GVHD (cGVHD) were graded according to established criteria [17,18]. The cutoff date for analysis was July 31, 2018.

RESULTS

The median age of patients at the time of UCBT was 38.5 years (range, 32 to 45 years). Five of 6 patients were diagnosed with idiopathic AA, and 1 patient had hepatitis-associated AA. Five patients received IST containing ATG and cyclosporine A as the first-line therapy, which subsequently resulted in failure to sustain hematologic recovery. The patient with hepatitis-associated AA (case 1) had received 3 cycles of steroid-pulse therapy for severe hepatitis before receiving ATG plus cyclosporine A. One patient with idiopathic AA (case 3) presented with a fulminant clinical phenotype that showed a $0/\mu L$ neutrophil count despite granulocyte-colony stimulating factor administration and high-grade fever. Because this patient was considered unresponsive to IST, UCBT was urgently performed as a first-line treatment to recover hematopoiesis. The median interval from diagnosis of AA to UCBT was 158 days (range, 21 to 7177 days). Five patients experienced infectious complication before UCBT (2 with febrile neutropenia, 2 with bacteremia, and 1 with infectious colitis). In all patients, the infectious complications were successfully treated with antibiotics before UCBT. Baseline characteristics are summarized in Table 1.

The median number of prefrozen and post-thawed TNCs from a cord blood unit was 2.9×10^7/kg (range, 2.2 to 4.6×10^7/kg) and 2.9×10^7/kg (range, 2.4 to 4.3×10^7/kg), respectively. The median number of prefrozen and post-thawed $CD34^+$ cells was 1.4×10^5/kg (range, 0.62 to 1.7×10^5/kg) and 1.2×10^5/kg (range, 0.46 to 1.6×10^5/kg), respectively. The number of HLA mismatches among 8 loci of HLA-A, -B, -C, and DRB1 ranged from 1 to 5 for the host-versus-graft direction and from 2 to 5 for the graft-versus-host direction (Table 2). Anti-HLA antibodies were detected in 2 patients (cases 4 and 5), but none of the patients were reactive to donor antigen.

All patients received preparative conditioning comprising 30 mg/m^2 Flu daily for 5 days (days −6 to −2), 60 mg/kg CY daily for 2 days (days −6 and −5), and 4 Gy TBI in 2 fractions on the same day (day −1). GVHD prophylaxis included tacrolimus (Tac) plus short-term methotrexate (MTX) (10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6) in 2 patients and with mycophenolate mofetil (MMF) in the other 4 patients. MMF was administered orally at a dose of 30 mg/kg/day from day -1 to day 35 and tapered thereafter. Data on the transplantation are described in Table 2.

All patients were alive at the time of analysis. Neutrophil and platelet engraftment were achieved in all cases, at a median of 17.5 days (range, 14 to 37 days) and 38.5 days (range, 31 to 86 days), respectively. The first evaluation of donor chimerism was performed in peripheral blood mononuclear cells (case 1), bone marrow (case 2), and whole blood (cases 3–6). Complete donor chimerism was confirmed in all patients at a median of 14 days (range, 14 to 32 days), with no secondary GF observed. Three patients developed grade II aGVHD, but grade III or IV aGVHD was not observed. Four patients developed cGVHD involving only skin in all patients (3 limited, 1 extensive). Patients with aGVHD and/or cGVHD were successfully treated with a topical steroid and/or low-dose (10 mg/day) prednisolone. At last follow-up, 1 patient (Case 3) received low-dose prednisolone (4 mg/day), which was gradually decreased, and the other 5 patients did not receive systemic steroid treatment. Tapering of Tac was started at a median of 167 days (range, 115 to 266 days) among 5 patients whose GVHD was completely resolved. At 1 year after UCBT, the median dose of Tac was 0.85 mg (range, 0.5 to 1.6) in all patients. Tac was discontinued in case 1 on day 828 and in case 2 on day 536.

Table 1
Baseline Patient Characteristics

Case	Age at UCBT, yr	Previous Treatment	Interval from Diagnosis to UCBT, d	Transfusions before UCBT (RBCs/Platelets)	Severity of AA at UCBT	Infectious Complications before UCBT (Interval to UCBT)
1	37	ATG + CsA	134	10-19/>20	Very severe	Bacteremia; *Streptococcus mitis* (2 mo)
2	36	ATG + CsA	7177	>20/1-9	Very severe	Infectious colitis (2 mo)
3	40	None	21	1-9/1-9	Very severe (fulminant)	FN (3 wk)
4	32	ATG + CsA	182	>20/>20	Very severe	FN (1 mo)
5	45	ATG + CsA	533	>20/>20	Moderate	None
6	40	ATG + CsA	119	>20/>20	Very severe	Bacteremia; *Streptococcus epidermidis* (1 mo)

CsA indicates cyclosporine A; FN, febrile neutropenia.

Table 2
Transplantation Characteristics

Case	Sex, R/D	ABO Type, R/D	Number of Mismatched HLA Alleles (HVG Direction)					Number of Mismatched HLA Alleles (GVH Direction)					TNCs, $\times 10^7$/Recipient kg		$CD34^+$ Cells, $\times 10^5$/ Recipient kg		GVHD Prophylaxis
			A	B	C	DRB1	Total	A	B	C	DRB1	Total	Prefrozen	Post-Thawed	Prefrozen	Post-Thawed	
1	F/F	O/O	0	0	1	0	1/8	0	0	1	1	2/8	2.2	2.4	.62	0.46	Tac + sMTX
2	M/F	AB/A	1	1	1	0	3/8	1	1	1	0	3/8	2.5	3.0	1.5	1.5	Tac + sMTX
3	M/M	O/O	1	0	0	0	1/8	1	0	0	1	2/8	4.6	4.3	1.3	1.0	Tac + MMF
4	F/M	A/O	0	1	1	1	3/8	0	1	1	1	3/8	4.3	4.0	1.7	1.6	Tac + MMF
5	F/F	B/O	2	1	1	1	5/8	2	1	1	1	5/8	3.2	2.8	1.6	1.3	Tac + MMF
6	F/M	O/A	1	0	1	1	3/8	1	0	1	1	3/8	2.6	2.7	.84	0.88	Tac + MMF

R indicates recipient; D, donor; F, female; M, male; HVG, host-versus-graft; GVH, graft-versus-host; sMTX, short-term methotrexate.

In one patient (case 1), cryptogenic organizing pneumonia occurred on day +116 and was treated with systemic steroid administration (1 mg/kg prednisolone). No obstructive change was observed according to a pulmonary function test. As a severe infectious complication, 1 patient (case 3) developed pulmonary zygomycosis (*Rhizopus* sp) on day +7 that was successfully treated with long-term administration of liposomal amphotericin B and lobectomy. A documented bloodstream infection involved *Staphylococcus haemolyticus* in 1 patient (Case 2), and cytomegalovirus (CMV) antigenemia occurred in 5 of 6 patients, although none progressed to CMV disease owing to preemptive antiviral therapy. At 6 years after UCBT, 1 patient (Case 1) developed chronic renal failure necessitating hemodialysis, and it is possible that long-term foscarnet and gancyclovir treatment for the CMV infection might have affected her renal dysfunction.

No post-transplantation lymphoproliferative disorders were observed. Epstein-Barr virus (EBV) monitoring was initiated if a patient showed symptoms suspected of post-transplantation lymphoproliferative disease, such as unknown fever and lymphadenopathy. In 3 recent cases, EBV load was measured at 3 to 4 weeks post-transplantation, and weekly EBV monitoring was continued if EBV DNA was detected. Case 1 showed a slight increase in EBV load up to 1380 copies/μg DNA in whole blood at day +179, followed by a decrease in EBV load without treatment. An EBV load of >1000 copies/μg DNA was not observed in other patients.

The median number of lymphocytes at day +60 was 1775/μL (range, 1050 to 2240/μL). Lymphocyte subsets at 2 to 3 months post-UCBT were analyzed in 4 patients (cases 1, 3, 4, and 5), with median $CD3^+$, $CD4^+$, and $CD8^+$ T cell and $CD19^+$ B cell counts of 774/μL (range, 627 to 2431/μL), 306/μL (range, 289 to 390/μL), 375/μL (range, 299 to 2133/μL), and 532/μL (range, 27 to 1256/μL), respectively. In addition, all cases were assessed for lymphocyte subsets at 12 to 13 months, resulting in median $CD3^+$, $CD4^+$, and $CD8^+$ T cell and $CD19^+$ B cell counts of 908/μL (range, 818 to 1927/μL), 500/μL (range, 294 to 579/μL), 401/μL (range, 348 to 972/μL), and 1057/μL (range, 430 to 1941/μL), respectively. The outcomes of UCBT are summarized in Table 3.

DISCUSSION

In this study, all patients successfully achieved neutrophil and platelet engraftment after UCBT using a conditioning regimen of Flu 150 mg/m^2 plus CY 120 mg/kg with TBI 4 Gy. Complete donor chimerism was confirmed early after transplantation, at a median of 14 days. All 6 patients were alive and experienced complete hematologic recovery. Despite the lack of ATG in the conditioning regimen, neither grade III-IV aGVHD nor extensive GVHD necessitating systemic steroid therapy occurred.

A recent study using registry data from pediatric patients with AA suggested a negative impact of ATG in conditioning for UCBT, because primary GF was observed in 8 of 15 patients receiving the ATG regimen [19]. The use of ATG in conditioning might reduce donor T cells, which would adversely affect achievement of sustained engraftment with complete donor chimerism. Moreover, data from the European Society for Blood and Marrow Transplantation (EBMT) group showed higher non-relapse mortality in patients with hematological malignancies and who received the ATG regimen for UCBT as compared with the non-ATG group [20]. Additionally, rates of infection and post-transplant lymphoproliferative disorders were significantly higher in the ATG-group. Because AA patients usually receive ATG during the preceding IST before

Table 3
Outcomes of Transplantation

Case	Engraftment, d		Lymphocyte Count at Day 60, /μL	Complete Donor Chimerism, d	Onset of aGVHD, d, Maximum Grade (Involved Organ)	cGVHD (Involved Organ)	Tac Daily Dose at 1 Year Post-UCBT	Survival, mo
	Neutrophils	Platelets						
1	37	86	1050	16	37, grade II (skin, gut)	Limited (skin)	1.4 mg	131
2	18	38	2010	32	26, grade II (skin)	limited (skin)	0.5 mg	79
3	16	31	2240	14	28, grade II (skin, gut)	limited (skin)	0.9 mg	18
4	14	78	2100	14	None	None	0.8 mg	14
5	17	36	1540	14	None	Extensive (skin)	0.8 mg	13
6	24	39	1060	14	None	None	1.6 mg	12

UCBT, repeated use of ATG during preconditioning might induce more severe immunosuppressive conditions.

The AA working party of the European Society for Blood and Marrow Transplantation reported the critical role of TNC dose for engraftment and survival in AA patients undergoing UCBT. The probability of neutrophil engraftment was greater in cases using cord blood units with a prefrozen TNC dose $>3.9 \times 10^7$/kg (58% versus 33%) [9]. Recently, Peffault et al [21] reported excellent outcomes in 26 patients who underwent UCBT with an RIC regimen containing ATG. Neutrophil engraftment was achieved in 23 of these 26 patients, and their 1-year overall survival was 88.5%. In that study, cord blood units with a median prefrozen TNC dose of 5.8×10^7/kg were used. These results suggest that when cord blood units at a higher TNC dose are available, ATG-containing regimens might not negatively impact patients with AA.

CY is a key drug in the conditioning regimen for patients with AA, with 200 mg/kg CY the standard dose for young patients undergoing BMT from an HLA-matched sibling [22,23]. However, a greater risk of organ toxicities has been associated with a CY dose of 200 mg/kg in older patients. Recently, Flu plus lower-dose CY with ATG or alemtuzumab was recommended for patients age >30 years [1,24,25]. The appropriate CY dose for use in combination with Flu remains an open question. In a UBMT setting, a study of CY dose deescalation suggested an association between 150 mg/kg CY in combination with Flu, TBI 2 Gy, and ATG and excess organ toxicity [26]. Currently, from 50 to 100 mg/kg CY is recommended in the Flu-based regimen for UBMT [27]. Regarding the appropriate dose of TBI, Deeg et al [28] reported that 2 Gy was optimal when combined with 200 mg/kg CY and ATG for UBMT, and 2 Gy TBI is frequently used, even in Flu-based conditioning for UBMT.

However, in the setting of UCBT for patients with AA, the optimal doses of CY and TBI have not yet been identified. Because the risk of GF is greater with UCBT compared with UBMT, more intensive conditioning might be needed to achieve engraftment of cord blood in patients with AA. Our findings suggest that a combination of 120 mg/kg CY and 4 Gy TBI with Flu is tolerable and sufficient to prevent GF of cord blood; however, for older patients, a dose reduction of CY and/or TBI might be necessary to prevent cardiac and pulmonary toxicities. Yamamoto et al [15] reported a promising result of UCBT using a RIC including 80 mg/m^2 Mel with Flu and low-dose TBI for much older patients. Further consideration is needed to determine whether CY or Mel is more effective based on regimen-related toxicities and engraftment rates for each age group.

Eapen et al [29] reported that 3 or more mismatches out of 8 HLA alleles was associated with worse survival and engraftment rates in pediatric patients undergoing UCBT for nonmalignant disorders. In their cohort, most of the patients received cord blood units with a TNC dose $>5 \times 10^7$/kg. This suggests that it is important to select a unit with fewer HLA allele mismatches and a high TNC dose for nonmalignant diseases.

However, and especially in adult patients, it is often difficult to obtain a cord blood unit meeting the requirements of both sufficient TNC dose and fewer than 3 mismatches out of 8 HLA alleles. In the present study, all 6 patients achieved sustained engraftment, despite the lower TNC number (2.9×10^7/kg) at a median dose and 2 or more HLA allele mismatches (2 mismatches in 2 patients, 3 mismatches in 3 patients, and 5 mismatches in 1 patient). Early donor lymphocyte recovery by avoiding the use of ATG might explain the favorable engraftment in our cohort. In fact, the lymphocyte counts recovered to >1000/μL by day +60 after UCBT in all patients.

The optimal GVHD prophylaxis in UCBT is a matter of continuing debate. A retrospective study comparing Tac/MTX and Tac/MMF in patients with acute leukemia who underwent UCBT using RIC showed a higher incidence of engraftment with Tac/MMF than with Tac/MTX [30]. In contrast, the risk of grade II-IV aGVHD was significantly higher in the Tac/MMF group. In our cohort, grade II aGVHD occurred in 3 patients and skin cGVHD occurred in 4 patients, but these patients improved with topical or low-dose steroid treatment. A low incidence of severe steroid-refractory GVHD might be an advantage of UCBT for patients with nonmalignant diseases. Our 4 patients given Tac/MMF showed early achievement of complete donor chimerism and did not experience stomatitis. In a patient who developed zygomycosis, early neutrophil engraftment worked favorably and contributed to curing pneumonia. Although Tac/MMF might be more advantageous than Tac/MTX in terms of cord blood engraftment, it was difficult to compare the 2 types of GVHD prophylaxis in our cohort owing to the small number of patients. Further investigation in a prospective setting is needed to address this issue.

The anti-HLA antibody is an important factor associated with engraftment rates after UCBT [31]. A previous study reported that donor-specific antibodies were associated with graft failure and decreased survival in UCBT using RIC [32]. All patients in this study were screened for anti-HLA antibodies, and 2 patients positive for anti-HLA antibodies were able to find a unit of cord blood lacking antibody-reacting HLA antigens, resulting in sustained engraftment. The use of numerous transfusions can be associated with the production of anti-HLA antibodies, and an early decision concerning transplantation might contribute to decreasing the transfusion burden. Moreover, a shorter time to transplantation from diagnosis is associated with superior survival after UBMT in patients with AA [33]. Therefore, early UCBT could potentially improve survival rates for patients with refractory AA if cord blood with a sufficiently high TNC dose is available. A prospective comparison study is needed to clarify whether early UCBT or UBMT requiring a longer coordination time is better after IST failure.

Limitations of this study include its small cohort, retrospective nature, and short duration of follow-up. However, the other specific regimens described in previous studies of UCBT in patients with AA were also based on a small number of cases [12,13,15,19]. Although this study investigated only 6 patients, findings of their successful outcomes might be helpful when considering the optimal regimen for UCBT, especially in adult patients with AA. However, a longer follow-up period is needed to appropriately evaluate late GF, late-onset organ deficiency, and secondary malignancies that could be associated with the TBI regimen.

In conclusion, the conditioning regimen of Flu 150 mg/m^2 plus CY 120 mg/kg with TBI 4 Gy was effective in achieving engraftment and early complete donor chimerism in UCBT in our adult patients with AA. When an HLA-matched donor is unavailable, UCBT using this non-ATG conditioning might represent a promising treatment approach for refractory AA. Based on the results of our successful cases, a prospective clinical trial is needed to evaluate this non-ATG regimen (Flu/CY/TBI4Gy) in UCBT for patients with AA.

ACKNOWLEDGMENTS

Conflict of interest statement: There are no conflicts of interest to report.

REFERENCES

1. Killick SB, Bown N, Cavenagh J, et al. Guidelines for the diagnosis and management of adult aplastic anaemia. *Br J Haematol*. 2016;172:187–207.
2. Scheinberg P, Young NS. How I treat acquired aplastic anemia. *Blood*. 2012;120:1185–1196.
3. Bacigalupo A. How I treat acquired aplastic anemia. *Blood*. 2017;129:1428–1436.
4. Jeong DC, Chung NG, Cho B, et al. Long-term outcome after immunosuppressive therapy with horse or rabbit antithymocyte globulin and cyclosporine for severe aplastic anemia in children. *Haematologica*. 2014;99:664–671.
5. Vallejo C, Montesinos P, Polo M, et al. Rabbit antithymocyte globulin versus horse antithymocyte globulin for treatment of acquired aplastic anemia: a retrospective analysis. *Ann Hematol*. 2015;94:947–954.
6. Kosaka Y, Yagasaki H, Sano K, et al. Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. *Blood*. 2008;111:1054–1059.
7. Eapen M, Rocha V, Sanz G, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. *Lancet Oncol*. 2010;11:653–660.
8. Terakura S, Atsuta Y, Tsukada N, et al. Comparison of outcomes of 8/8 and 7/8 allele-matched unrelated bone marrow transplantation and single-unit cord blood transplantation in adults with acute leukemia. *Biol Blood Marrow Transplant*. 2016;22:330–338.
9. Peffault de Latour R, Purtill D, Ruggeri A, et al. Influence of nucleated cell dose on overall survival of unrelated cord blood transplantation for patients with severe acquired aplastic anemia: a study by Eurocord and the Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2011;17:78–85.
10. Mao P, Zhu Z, Wang H, et al. Sustained and stable hematopoietic donor-recipient mixed chimerism after unrelated cord blood transplantation for adult patients with severe aplastic anemia. *Eur J Haematol*. 2005;75:430–435.
11. Ohga S, Ichino K, Goto K, et al. Unrelated donor cord blood transplantation for childhood severe aplastic anemia after a modified conditioning. *Pediatr Transplant*. 2006;10:497–500.
12. Chan KW, McDonald L, Lim D, Grimley MS, Grayson G, Wall DA. Unrelated cord blood transplantation in children with idiopathic severe aplastic anemia. *Bone Marrow Transplant*. 2008;42:589–595.
13. Yoshimi A, Kojima S, Taniguchi S, et al. Unrelated cord blood transplantation for severe aplastic anemia. *Biol Blood Marrow Transplant*. 2008;14:1057–1063.
14. Liu HL, Sun ZM, Geng LQ, et al. Unrelated cord blood transplantation for newly diagnosed patients with severe acquired aplastic anemia using a reduced-intensity conditioning: high graft rejection, but good survival. *Bone Marrow Transplant*. 2012;47:1186–1190.
15. Yamamoto H, Kato D, Uchida N, et al. Successful sustained engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with severe aplastic anemia. *Blood*. 2011;117:3240–3242.
16. Kuwatsuka Y, Kanda J, Yamazaki H, et al. A comparison of outcomes for cord blood transplantation and unrelated bone marrow transplantation in adult aplastic anemia. *Biol Blood Marrow Transplant*. 2016;22:1836–1843.
17. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825–828.
18. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204–217.
19. Kudo K, Muramatsu H, Narita A, et al. Unrelated cord blood transplantation in aplastic anemia: is anti-thymocyte globulin indispensable for conditioning? *Bone Marrow Transplant*. 2017;52:1659–1661.
20. Pascal L, Tucunduva L, Ruggeri A, et al. Impact of ATG-containing reduced-intensity conditioning after single- or double-unit allogeneic cord blood transplantation. *Blood*. 2015;126:1027–1032.
21. Peffault de Latour R, Chevret S, Jubert C, et al. Unrelated cord blood transplantation in patients with idiopathic refractory severe aplastic anemia: a nationwide phase 2 study [e-pub ahead of print]; *Blood*. 2018;132:750–754. doi.org/10.1182/blood-2018-01-829630. accessed XXX.
22. Storb R, Etzioni R, Anasetti C, et al. Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia. *Blood*. 1994;84:941–949.
23. Champlin RE, Perez WS, Passweg JR, et al. Bone marrow transplantation for severe aplastic anemia: a randomized controlled study of conditioning regimens. *Blood*. 2007;109:4582–4585.
24. Maury S, Bacigalupo A, Anderlini P, et al. Improved outcome of patients older than 30 years receiving HLA-identical sibling hematopoietic stem cell transplantation for severe acquired aplastic anemia using fludarabine-based conditioning: a comparison with conventional conditioning regimen. *Haematologica*. 2009;94:1312–1315.
25. Aljurf M, Al-Zahrani H, Van Lint MT, Passweg JR. Standard treatment of acquired SAA in adult patients 18-40 years old with an HLA-identical sibling donor. *Bone Marrow Transplant*. 2013;48:178–179.
26. Tolar J, Deeg HJ, Arai S, et al. Fludarabine-based conditioning for marrow transplantation from unrelated donors in severe aplastic anemia: early results of a cyclophosphamide dose deescalation study show life-threatening adverse events at predefined cyclophosphamide dose levels. *Biol Blood Marrow Transplant*. 2012;18:1007–1011.
27. Anderlini P, Wu J, Gersten I, et al. Cyclophosphamide conditioning in patients with severe aplastic anaemia given unrelated marrow transplantation: a phase 1-2 dose de-escalation study. *Lancet Haematol*. 2015;2:e367–e375.
28. Deeg HJ, O'Donnell M, Tolar J, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. *Blood*. 2006;108:1485–1491.
29. Eapen M, Wang T, Veys PA, et al. Allele-level HLA matching for umbilical cord blood transplantation for non-malignant diseases in children: a retrospective analysis. *Lancet Haematol*. 2017;4:e325–e333.
30. Terakura S, Kuwatsuka Y, Yamasaki S, et al. GvHD prophylaxis after single-unit reduced intensity conditioning cord blood transplantation in adults with acute leukemia. *Bone Marrow Transplant*. 2017;52:1261–1267.
31. Takanashi M, Atsuta Y, Fujiwara K, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. *Blood*. 2010;116:2839–2846.
32. Ruggeri A, Rocha V, Masson E, et al. Impact of donor-specific anti-HLA antibodies on graft failure and survival after reduced intensity conditioning-unrelated cord blood transplantation: a Eurocord, Société Francophone d'Histocompatibilité et d'Immunogénétique (SFHI), and Société Française de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC) analysis. *Haematologica*. 2013;98:1154–1160.
33. Bacigalupo A, Socié G, Hamladji RM, et al. Current outcome of HLA identical sibling versus unrelated donor transplants in severe aplastic anemia: an EBMT analysis. *Haematologica*. 2015;100:696–702.