low-dose TBI or Flu + ATG were categorised as those receiving reduced-intensity conditioning (RIC). L-PAM dose was ≤140 mg/m^2 in patients receiving RIC.

GVHD was graded according to the standard criteria (Przepiorka, *et al* 1995).

Statistical analyses

The probability of survival was estimated by the product-limit method, and the log-rank test was used for group comparisons. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of neutrophil, platelet and reticulocyte recovery and that of acute and chronic GVHD. Death before recovery was the competing event for haematological recovery, and death without GVHD was the competing event for GVHD. Gray's test was used for group comparisons of cumulative incidence (Gooley, *et al* 1999, Gray 1988). The Cox regression model was used for analysing data to be used for identification of prognostic factors. Factors found to be significant ($p < 0.05$) or marginally significant ($p < 0.1$) in univariate analysis were included in multivariate analysis. The variables considered were patient's age at the time of transplantation, diagnosis, duration from diagnosis to transplantation, second or third transplantation, HLA disparity, presence of infection at the time of transplantation, conditioning regimen and cell dose infused. Variables with >2 categories were included in the final model using dichotomized dummy variables when at least one of the categories showed significant effect on survival. Continuous variables were dichotomised for the prognostic factor analyses. Variables were dichotomized as follows; patient age greater or less than twelve months at transplantation, dichotomized at median for nucleic cell dose of < 8.2 x 10^7/kg versus ≥8.2 x 10^7/kg and CD34 cell dose of < 2.1 x 10^5/kg and ≥2.1 x 10^5/kg, shorter than or equal to or longer than 180 days for time to transplant. All p values were two-sided.

Results

Engraftment

Sixty-seven patients (76%) achieved stable engraftment. The cumulative incidence of neutrophil, platelet and reticulocyte recovery at day 100 after transplantation was 77% (95% confidence interval [CI], 66%–85%), 56% (95% CI, 45%–65%) and 64% (95% CI, 53%–73%), respectively (**Fig 1A, B**; data not shown). The median time for neutrophil, platelet and reticulocyte recovery was 19 days (range, 9–104 days), 40 days (range, 10–122 days) and 27 days (range, 12–98 days), respectively. The cumulative incidences of neutrophil recovery were not statistically different among the disease groups (SCID, 74%; WAS, 91% and others, 68% at day 100 after transplantation) (**Fig 1C**), although incidence was low in CGD patients ($N = 7$, 43%).

The time required for neutrophil recovery was similar in all disease groups, while that required for platelet recovery varied to some extent among the different disease groups. Platelet engraftment was slightly delayed in WAS patients, but the time required for engraftment in these patients was not significantly different from that required in other patients (**Fig 1D**).

Forty-three, 31 and 14 patients received MAT, RIC and no conditioning, respectively. No difference was observed in the incidence of neutrophil recovery between the MAT and RIC groups (84% vs 87% at day 100). Similarly, no difference was observed in platelet recovery between these 2 groups (data not shown).

The cell dose infused ranged from 1.89 to 31.1 $\times 10^7$/kg, with a median of 8.60 $\times 10^7$/kg. No correlation was observed between the cell dose infused and engraftment.

Five of 21 patients with engraftment failure received a second transplantation. Two WAS

patients achieved successful engraftment in the second transplantation, while 1 SCID and 2 CGD patients did not survive the second transplantation. Only 2 of the remaining 16 patients who rejected the UCB graft remained alive at the latest data analysis.

Survival and causes of death

Of the 88 PID patients who underwent UCBT, 62 remained alive at the latest follow-up. 5Y-OS for all patients was 69% (95% CI, 57%–78%) (**Fig 2**), while that for SCID and WAS patients was 71% and 82%, respectively. All 5 SCN patients remained alive, although 1 patient had rejected the graft on day 79 after UCBT. Three of 7 CGD patients survived UCBT; this low survival rate may be due to the fact that UCBT was selected in 5 patients after the first or second failed bone marrow transplantation (BMT). Seven of 14 patients categorised as 'other diseases' remained alive at the latest follow-up.

Table II summarises the survival and causes of death after UCBT. Of the 26 patients who died, 19 had died within day 100 (17 from infection) and 7 (SCID, 6 and congenital CD4 lymphopaenia, 1) had died within day 28 after UCBT.

Causes of early death (≤28 days) were cytomegalovirus (CMV) disease (3 patients), *Pneumocystis* pneumonia (1 patient), interstitial pneumonia (1 patient), bacterial infection (1 patient) and veno-occlusive disease (VOD) (1 patient). All those who died of CMV disease had CMV pneumonia before transplantation.

The cause of death between day 28 and 100 in the remaining 12 patients was bacterial infection (7 had concomitant fungal infection, 1 also had VOD and 1 had CMV disease), CMV disease (2 patients), fungal infection (1 patient), multiple organ failure (1 patient) and VOD (1 patient). Four of 7 CGD patients died of bacterial or fungal infection without engraftment. Although detailed data on bacterial/fungal infections at the time of transplantation were not

collected, all the CGD patients were administered both antimicrobial and antifungal agents at the time of transplantation.

The causes of death after day 100 were GVHD (5 patients), Epstein–Barr virus (EBV)-associated post-transplant lymphoproliferative disorder (EBV-PTLD, 1 patient) and adrenal insufficiency (1 patient). None of the other patients died of infection after day 100.

GVHD

All but 3 patients in the present study received either CyA- or tacrolimus-based immunosuppressant prophylaxis for GVHD. The cumulative incidence of grade 2–4 acute GVHD at day 100 was 28% (95% CI, 19%–38%), and that of grade 3–4 GVHD was 8% (95% CI, 4%–15%) (**Fig 3A, D**).

The incidence of grade 2–4 GVHD was higher in patients who underwent 2- or 3-antigen-mismatched UCBT compared with those who underwent HLA-matched or HLA-1-antigen–mismatched UCBT, but it was not statistically significant ($p = 0.071$) (**Fig 3B**). On the other hand, no difference was observed in the incidence of grade 3–4 GVHD between <2-antigen-mismatched and >2-antigen-mismatched transplants (**Fig 3E**), although grade 3–4 GVHD was not observed by high-resolution DNA typing in patients who underwent genotypically HLA-matched transplantation.

When focusing on differences among the disease groups (**Fig 3C**), a significantly higher incidence of grade 2–4 GVHD was observed in WAS patients than in non-WAS patients, $p = 0.021$. In addition, 3 of 5 WAS patients who developed grade 3–4 GVHD died of either GVHD (2 patients) or VOD (1 patient).

Chronic GVHD was observed in 9 patients, and its cumulative incidence at day 180 was 13%

(95% CI, 7%–23%) (**Fig 3F**).

Infections

Twenty-eight patients (SCID, 11; WAS, 8; CGD, 3 and other diseases, 6) developed bacterial infection after UCBT. Sixteen of the 28 patients remained alive at the time of data collection.

Fungal infection mainly caused by *Aspergillus* species was observed in 8 patients (CGD, 3; SCID, 2; WAS, 2 and X-linked hyperIgM syndrome, 1). Three of the 8 patients died of bacterial infection, bacterial/fungal infection or GVHD.

Twenty patients (SCID, 8; WAS, 4; CGD, 2; SCN, 2 and others, 4) developed CMV disease after UCBT. CMV was detected before conditioning in all 8 SCID patients of which 4 patients died of CMV disease after transplantation. Twelve of the 20 patients remained alive at the time of analysis.

Other notable virus-related complications were respiratory syncytial virus bronchiolitis accompanied by chronic GVHD in 1 SCID patient and EBV-PTLD in 1 patient with Blau's syndrome; both infections led to a fatal outcome. One WAS patient had severe haemorrhagic colitis caused by Coxsackie virus B infection, which was treated successfully by infusion of expanded CD4 T cells prepared from the infusion residua of donor cord blood {Tomizawa, 2005 #14}. Another WAS patient had persistent norovirus infection. Interstitial pneumonia not due to CMV or *Pneumocystis* was noted in 3 patients of which 1 patient had parainfluenza/rhinovirus infection, while the causative agent for infection in the remaining 2 patients was not identifiable.

Risk factors for overall mortality

Lastly, we analysed the factors contributing to overall survival. Using univariate analyses,

the following were found to be significant contributory factors to the poor prognosis: HLA mismatch of ≥2 antigens, time to transplant >180 days, second or third transplantation, ongoing infection at the time of transplantation, no conditioning for UCBT and diagnosis other than SCID, SCN or WAS (**Table III**). The dose of transfused nucleated cells or CD34-positive cells did not effect 5Y-OS.

Using multivariate regression analyses, the following were found to be significant contributory factors to patient death: infection at the time of transplantation, no conditioning, HLA mismatch of >2 antigens and diagnosis other than SCID, SCN or WAS (**Table IV**). RIC was detected as the favourable factor for patient survival ($p = 0.01$) (**Fig 4 and Table IV**).

Discussion

In this manuscript, we report the outcome of UCBT for 88 PID patients, which is a report on the largest cohort of PIDs receiving UCBT up to the present. The overall survival rate for PID patients undergoing UCBT was comparable to that previously reported for 46 Japanese PID patients undergoing BMT from either HLA-identical siblings or unrelated donors (Sakata, *et al* 2004), and also to that reported by the European Society of Immunodeficiency and other stem cell transplantation centres for PID patients receiving BMT from HLA-matched related donors, HLA-mismatched related donors or unrelated donors (Antoine, *et al* 2003, Dvorak and Cowan 2008, Rao, *et al* 2005). The time for haematopoietic recovery was comparable to or better than the median recovery time observed in a large cohort of UCBT in children with haematopoietic disorders (Michel, *et al* 2003, Thomson, *et al* 2000) and in adults with leukaemia (Atsuta, *et al* 2009, Laughlin, *et al* 2004). The incidence of grade 2–4 GVHD (28%) in UCBT was lower compared with that reported in URBMT in PID patients in Japan (47%) (Sakata, *et al* 2004), with that reported in BMT in 90 SCID patients (34%) (Neven, *et al* 2009) and with that observed in the studies of UCBT for childhood haematological malignancies (Michel, *et al* 2003, Sawczyn, *et al* 2005, Thomson, *et al* 2000). The incidence of chronic GVHD (13%) after UCBT was slightly lower than that after URBMT in PID patients in Japan (20%) (Sakata, *et al* 2004), and was lower compared to that in UCBT studies for childhood leukaemia (Michel, *et al* 2003, Sawczyn, *et al* 2005). Thus, UCBT in PID patients in the present study was associated with a good survival rate, good engraftment rate, rapid haematological recovery and a lower incidence of acute and chronic GVHD.

Since 5Y-OS for SCID patients (71%) was better than that for SCID patients receiving bone marrow from HLA-mismatched related donors in both Japan (5Y-OS, 36%, manuscript in

preparation) and Europe (5Y-OS, 52%) (Antoine, *et al* 2003), UCBT would be particularly beneficial for patients requiring rapid access to donor units yet lacking a matched related donor.

Here several key risk factors were associated with overall mortality.

First, infection was the major cause of mortality during the first 100 days after UCBT in PID patients, and the frequency was much higher than that observed in other disorders following UCBT (Kurtzberg, *et al* 2008, Rocha, *et al* 2006, Szabolcs, *et al* 2008). As predicted and reported in previous studies (Antoine, *et al* 2003, Cuvelier, *et al* 2009), infection at the time of transplantation was associated with poor survival ($p < 0.0001$), suggesting that the control of pre-existing infection at the time of UCBT is critically important.

Eight of 11 SCID patients who had active infection, mainly CMV pneumonia, died before day 50, while 26 of 28 patients without infection at the time of UCBT remained alive at the time of data collection. UCBT without conditioning was selected for 12 patients of which 7 had CMV infection and 1 had *Pneumocystis* pneumonia at the time of transplantation. Six out of the 7 patients died of CMV infection; and one patient with *Pneumocystis* pneumonia did not survive UCBT.

UCBT in WAS patients achieved good 5Y-OS, as reported in a previous study of 15 cases (Kobayashi, *et al* 2006). One of the key factors would have been the time from diagnosis to transplantation. In our WAS patients, UCBT was performed at a median age of 14 months (range, 4–84 months) at which most patients showed thrombocytopaenia, but did not yet have uncontrolled infection or autoimmunity.

Four CGD patients died of bacterial or fungal infection without engraftment. Although these patients were not categorised as those with active infection at the time of transplantation, they required intravenous administration of antimicrobial and antifungal agents before and after transplantation.

Second, HLA disparity was a risk factor associated with overall mortality. Lower survival was observed in UCB recipients transplanted with a >2 antigen-mismatched graft compared with those transplanted with a <2 antigen-mismatched graft (HR = 3.87, $p = 0.002$). Although no difference was observed in 5Y-OS between recipients of HLA-matched and those of HLA 1-antigen-mismatched UCBT in the present study, we would need data from a larger number of patients with information on more extensive and sensitive HLA typing to discuss the impact of fully matched HLA on transplant outcome.

Finally, non-SCID/SCN/WAS patients showed a significantly lower survival rate (HR = 5.40, $p < 0.0001$ by multivariate analyses). Although previous large-scale study showed that results of HSCT according to disease did not show obvious disease-specific findings (Antoine, *et al* 2003), it is not yet known UCBT is suitable for all types of PIDs. This may indicate donor source other than USB is preferable for certain types of PID. Although the success of UCBT noted for X-linked hyperIgM syndrome, bare lymphocyte syndrome and X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency (Tono, *et al* 2007) is encouraging, optimisation of transplantation procedures and determination of suitable timing for UCBT may be necessary for this group of patients. Alternatively, this may simply indicate an expansion of transplantation to less favorable clinical conditions or to less favorable transplantation condition. Apparently, studies on the larger cohort are necessary for drawing any conclusion on whether diagnosis is significant overall.

Recent studies suggest improved survival after BMT for PID with the RIC regimen; however, to date, comparison of CBT using RIC versus MAT has not been made. In our study, 87% of patients on the RIC regimen and 66% on the MAT regimen remained alive at the latest follow-up. The multivariate analyses revealed that the RIC regimen is associated with higher 5Y-OS than the MAC regimen (HR = 0.20, $p = 0.011$). Although it is premature to conclude

that RIC provides an equal or superior outcome to MAT for all PID patients, non-myeloablative treatment may be beneficial at least for certain types of PID. RIC was selected preferentially in SCID and CGD patients with good survival rates: 17 of 18 SCID patients and 3 of 4 CGD patients remain alive. With this result, we are in the process of initiating a clinical trial of UCBT with RIC in SCID patients. On the other hand, only 2 of 23 WAS patients received RIC. Our previous data showed that a conditioning regimen other than BU/CY or BU/CY/ATG was the only independent factor associated with failure in HSCT for WAS patients (Kobayashi, *et al* 2006). However, whether this holds true for UCBT in younger WAS patients should be determined.

Notably, although the outcome of UCBT for WAS in this cohort was excellent compared with that from previously reported HSCT results using different donor sources {Friedrich, 2009 #262; Kobayashi, 2006 #35} , UCBT in WAS patients was associated with a high rate of grade 2–4 acute GVHD (11 of 23 patients) and a post-transplant infectious episode (13 of 23 patients). Eight patients experienced bacteraemia/sepsis and 6 experienced viral infection (CMV pneumonia, 4; Coxsackie virus enterocolitis, 1 and persistent norovirus infection, 1). The high rate of serious infections and GVHD in WAS patients after transplantation warrants further study in search of preventive measures that might include RIC for severe, transplantation-related toxicities.

Long-term follow-up of the clinical and immunological status is necessary when considering the lifespan of PID patients. Recent studies on the long-term outcome after HSCT for SCID revealed the presence of relatively late complications such as chronic GVHD, autoimmune events, severe or recurrent infections, chronic human papilloma virus infection, nutritional problems and late rejection in 50% of patients (Mazzolari, *et al* 2007, Neven, *et al* 2009). Similarly, long-term follow-up of HSCT in WAS patients revealed that 20% of patients

developed cGVHD-independent autoimmunity (Ozsahin, *et al* 2008). One possible measure that might be taken to avoid the chronic problems associated with CBT would be to select a HLA-matched UCB unit, since HLA disparity was a risk factor for both overall survival and the development of GVHD in our study. The advantage of RIC over MAT in preventing late complications needs careful assessment, together with data on mortality, engraftment and early post-transplant complications.

Finally, the issue of SCID patients who died before or without receiving SCT most likely due to uncontrolled infection still remains unresolved. This suggests that the early diagnosis of SCID and prevention of opportunistic infection within a protected environment and with appropriate prophylactic drugs is critically important for the improvement of survival in SCID patients in general. To that end, neonatal screening with the employment of T-cell receptor excision circles should be beneficial for an improved outcome in SCID patients (McGhee, *et al* 2005, Morinishi, *et al* 2009).

We report the results of UCBT for 88 PID patients in Japan. Despite the limitations of a retrospective, non-randomised study, our study suggests that unrelated umbilical cord blood can be considered as a promising stem cell source for children with congenital immunodeficiency when a HLA-matched related donor is not available.

Acknowledgements

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Kawa, K. Koike, T. H., and M. K. contributed to the acquisition and interpretation of data; Y. A. and S.K. edited the manuscript; T. M. designed research and wrote the manuscript; an all authors reviewed and approved the manuscript.

Figure legends

Figure 1

Cumulative incidence of neutrophil and platelet recovery after UCBT

The cumulative incidence of neutrophil recovery 77% (95% CI, 66%–85%) (A). . The cumulative incidence of platelet recovery 56% (95% CI, 45%–65%) (B). The cumulative incidence of neutrophil (C) and platelet (D) recovery according to disease category is shown.

Figure 2

Kaplan–Meier estimates of overall survival after umbilical cord transplantation

Figure 3

Cumulative probability of acute and chronic GVHD after UCBT

The cumulative incidence of grade 2–4 acute GVHD at day 100 was 28% (95% CI, 19%–38%) (A). The incidence was higher in transplantation mismatched for ≤2 antigens (B) and in that for WAS patients (C). The cumulative incidence of grade 3–4 acute GVHD at day 100 was 8% (95% CI, 4%–15%) (D) and the incidence was not different between patients undergoing transplantation for ≥2-antigen-mismatched transplant and those undergoing <2-antigen-mismatched transplant (E). The cumulative incidence of chronic GVHD at day 180 was 13% (95% CI, 7%–23%) (F).

Figure 4

Kaplan–Meier estimates of overall survival after umbilical cord transplantation

Comparison of overall survival among RIC, MAT, and no conditioning is shown. In 5Y-OS,

MAT vs RIC, p=0.111, MAT vs no conditioning, p =0.017 in univariate analysis

Figure 1

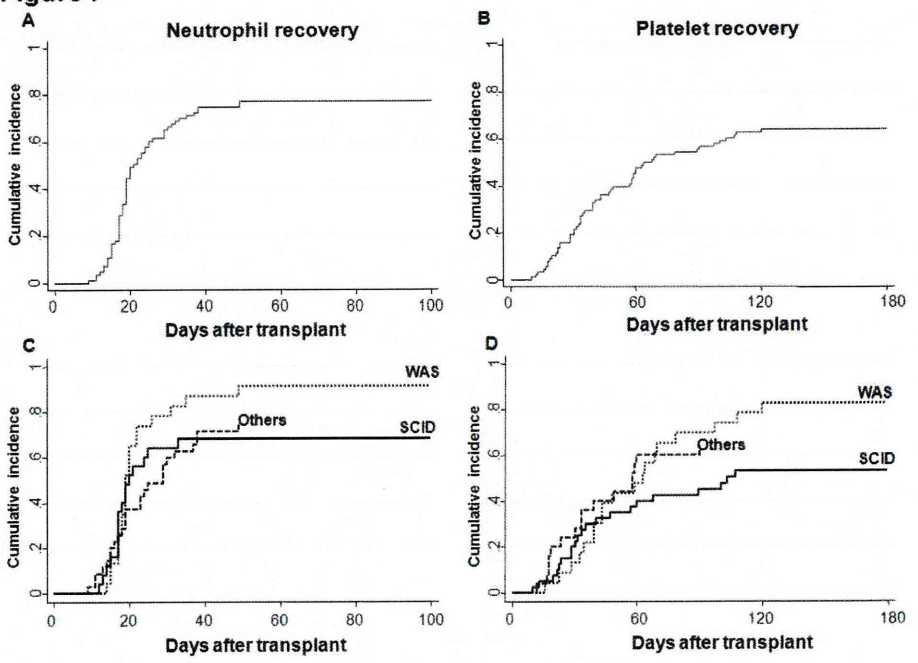

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Figure 2

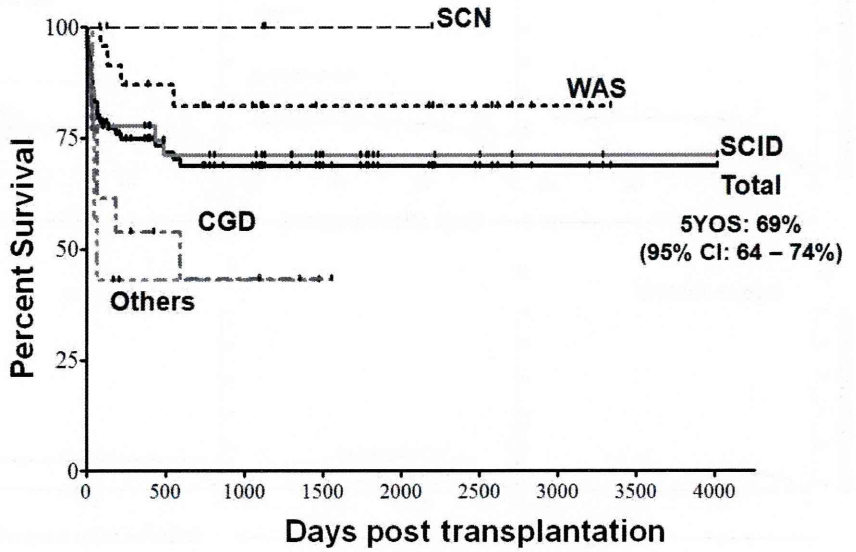

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Figure 3

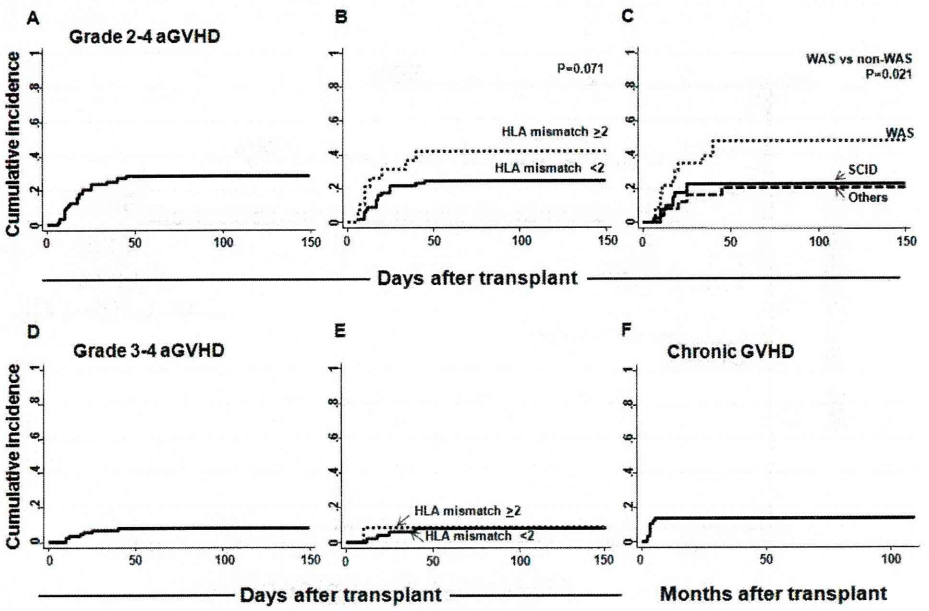

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Figure 4

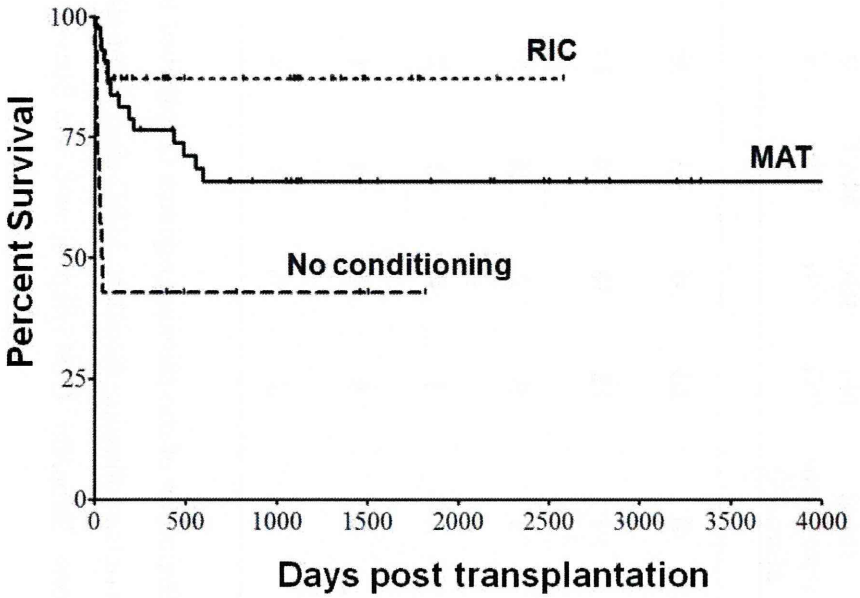

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Table I Age at the time of transplantation, type of conditioning and HLA disparity

					Conditioning			HLA disparity			
	Patients (N)	Median age of transpl (Months). (range)	Median cell dose ($\times 10^7$/kg) (range)	Second or third transplantation (N)	No (N)	RIC (N)	MAT (N)	0 (N)	1 (N)	2 (N)	3 (N)
TOTAL	88	9 (0–248)	8.60 (1.89–31.1)	8	14	31	43	29	40	15	4
SCID	40	6.5 (0–27)	11.4 (4.55–31.1)	1	12	18	10	17	15	5	3
WAS	23	14 (4–84)	6.49 (2.89–13.6)	1	0	2	21	7	10	6	0
CGD	7	63 (31–248)	6.00 (1.89–12.3)	5	1	4	2	2	4	1	0
SCN	5	10 (4–124)	5.99 (4.16–9.19)	0	0	1	4	1	4	0	0
Others	13	37 (6–194)	8.11 (3.01–19.8)	1	1	6	6	2	7	3	1

RIC, reduced-intensity conditioning; MAT, myeloablative therapy. Definition of conditioning regimen is described in 'Materials and Methods' section. Others include 4 CD40L deficiency, 2 CVID and one of each of the following disorders: MHC class II deficiency, DiGeorge syndrome, X-linked lymphoproliferative disorder, NEMO deficiency, IPEX syndrome, Idiopathic CD4 lymphopaenia and Blau syndrome.

Table II Survival and causes of death

	Cases (N)	Alive (N)	Death (day)			Infection at CBT (N)	Cause of death (<day 100)			Cause of death (≥day 100)	
			<28 (N)	<100 (N)	≥100 (N)		Bac/Fung infection (N)	Viral infection (N)	Others (N)	GVHD (N)	Others (N)
TOTAL	88	62	7	19	7	18	10	7	VOD 3 MOF1	5	PTLD 1 AI 1
SCID	40	29	6	9	2	11	2	6	1 (VOD)	1	1 (AI)
WAS	23	19	0	1	3	1	1	0	0	3	0
CGD	7	3	0	4	0	5	4	0	1 (VOD)	0	0
SCN	5	5	0	0	0	0	0	0	0	0	0
Others	13	6	1	5	2	1	3	1	1 (VOD) 1 (MOF)	1	1 (PTLD)

Bac/Fung infection, Bacterial and/or fungal infection. VOD, Veno-occlusive disease; MOF, Multiple organ failure; AI, Adrenal insufficiency and PTLD, Post-transplant lymphoproliferative disorder. Cause of death does not add to number of deceased patients since 1 patient had died of VOD and bacterial infection.