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Haploidentical haematopoietic stem cell transplantation using CD3 or CD3/CD19 depletion and conditioning with fludarabine, cyclophosphamide and antithymocyte globulin for acquired severe aplastic anaemia

The treatment of patients with severe aplastic anaemia (SAA) who lack a suitable donor remains a significant challenge. Although haematopoietic stem cell transplantation (HSCT) using a haploidentical related donors is a possible alternative for patients without a suitable donor, limited reports are available on the use of haploidentical HSCT for patients with acquired SAA (Tzeng *et al*, 1996; Kremens *et al*, 2001; Tsutsumi *et al*, 2004; Woodard *et al*, 2004; Lacerda *et al*, 2005; Wang *et al*, 2010). The majority of reports to date have involved a T-cell depleted strategy using purified $CD34^+$ cells to prevent fatal graft-versus-host disease (GVHD). However, haploidentical HSCT using $CD34^+$-selected stem cells was complicated by a high rate of graft rejection and opportunistic infection due to delayed immune reconstitution.

In this prospective trial, CD3- or CD3/CD19-depleted grafts instead of purified $CD34^+$ cells were employed to reduce the risk of severe GVHD and facilitate engraftment by selectively removing T cells and repleting non-$CD34^+$ cells. For conditioning, a fludarabine-based regimen with dose-reduced cyclophosphamide and rabbit-antithymocyte globulin (rATG) without total body irradiation (TBI) was used to enhance engraftment and reduce regimen-related toxicity. Here, we report the successful preliminary results of haploidentical HSCT using CD3- or CD3/CD19-depleted grafts and a non-TBI, dose-reduced regimen for children and adolescents with acquired SAA.

Between 2009 and 2010, four patients with acquired SAA (including two with very severe aplastic anaemia) who lacked a suitable sibling or an unrelated donor received HSCT from a haploidentical related donor at the Asan Medical Centre, Seoul, Korea. Clinical characteristics of the patients are shown in Table I. This study was registered at http://www.clinicaltrials.gov (NCT01105273).

The conditioning regimen consisted of fludarabine (30 mg/m^2 once daily on days −6 to −2), cyclophosphamide (60 mg/kg once daily on days −3 to −2) and rATG (Thymoglobulin; Genzyme, Cambridge, MA, USA) (2·5 mg/kg once daily on days −3 to −1). For prophylaxis of GVHD, a combination of mycophenolate mofetil and ciclosporin was used in cases where the $CD3^+$ cell count in the graft exceeded 5×10^4/kg. Peripheral blood stem cells (PBSCs) were processed for CD3 or CD3/CD19 depletion by negative selection using the Milteny CliniMACS® system (Miltenyi-BioTec, Bergisch-Gladbach, Germany). The depletion procedure was described previously in detail (Bethge *et al*, 2008). If infused $CD34^+$ cells were less than $3{\cdot}0 \times 10^6$/kg of recipient body weight, a second PBSC collection was performed and the pooled product was processed for CD34 enrichment by positive selection of $CD34^+$ cells.

Total infused $CD34^+$ cells ranged from $3{\cdot}0–5{\cdot}5 \times 10^6$/kg of recipient weight. T-cell depletion was variably effective, with 1·3–3·1 log reduction. In terms of $CD3^+$ cells, two grafts contained $15{\cdot}6$ and $19{\cdot}0 \times 10^4$/kg, while the remaining 3 grafts

Table I. Patient characteristics and outcomes of haematopoietic stem cell transplantation from an HLA-haploidentical familial donor.

Patient	1	2	3		4
Sex	M	M	M		M
Age at diagnosis (years)	13·5	13·0	14·8		11·8
Age at HSCT (years)	16·7	13·6	21·7		19·5
Time to HSCT (months)	39	7	83		92
Disease status	Severe	Very severe	Very severe		Severe
Blood transfusion (units, n)					
RBC	14	12	246		16
Platelets	32	22	159		51
Previous IST	Yes	No	Yes		Yes
Donor	Mother	Mother	Mother* (1st HSCT)	Brother† (2nd HSCT)	Mother
Graft manipulation	$CD3^-$	$CD3/CD19^-$	$CD3/CD19^-$ with $CD34^+$	$CD3/CD19^-$ with $CD34^+$	$CD3/CD19^-$ with $CD34^+$
Graft composition					
$CD34^+$ cells ($\times 10^6$/kg)	5·5	3·0	3·5	5·4	3·7
$CD3^+$ cells ($\times 10^4$/kg)	15·6	795	125	155	19·0
Engraftment (d)					
Neutrophils	10	12	12	11	10
Platelets	14	17	22	22	19
Graft failure	None	None	Secondary	None	None
GVHD					
Acute GVHD	None	Grade 1	None	Grade 1	None
Chronic GVHD	None	None	NA	None	None
Infection					
Bacterial	None	None	*C. tertium*	None	None
Fungal	None	None	None	None	None
CMV reactivation	Yes	None	None	Yes	Yes
CMV disease	Retinitis	None	None	None	Retinitis
BKV cystitis	None	None	Yes	None	None
PTLD	Yes	None	None	None	None
Transfusion independence (months)	21+	20+	9+		4+
Survival (months after HSCT)	Alive (23+)	Alive (21+)	Alive (18+)		Alive (16+)

HSCT, haematopoietic stem cell transplantation; RBC, red blood cells; IST, immunosuppressive therapy; GVHD, graft-versus-host disease; NA, not applicable; CMV, cytomegalovirus; BKV, BK virus; PTLD, posttransplant lymphoproliferative disorder.
*Donor of the first HSCT for Patient 3.
†Donor of the second HSCT for Patient 3 after graft rejection.

contained unexpectedly higher doses of 125, 155, and 795×10^4/kg, respectively (Table I).

All patients achieved rapid neutrophil and platelet engraftment (Table I). Patients 1 and 2 achieved sustained engraftment with full donor chimerism and remained transfusion-independent. After initial engraftment, Patient 3 developed secondary graft failure at day 31. Following reconditioning, the patient received a second CD3/CD19-depleted graft from an alternative haploidentical donor at 78 days after the first transplant. The patient remained transfusion-independent for 9 months with complete donor chimerism after the second transplant. Patient 4 showed 90–95% donor chimerism, but had poor graft function requiring infrequent transfusion. The patient received a boost of $CD34^+$-selected cells without further conditioning from the same donor as the first transplant at 11 months after transplantation. The patient was alive and transfusion-independent for 4 months.

T-cell and Natural Killer (NK) cell reconstitution data are depicted in Fig 1. Apart from patient 4 who showed a prolonged delay in immune reconstitution, the other patients achieved $CD3^+$ cell counts of $>0{\cdot}15 \times 10^9$/l on day 90, $>0{\cdot}4 \times 10^9$/l on day 180, and $>0{\cdot}7 \times 10^9$/l on day 365, and $CD4^+$ cell counts of $> 0{\cdot}15 \times 10^9$/l on day 180 and $>0{\cdot}4 \times 10^9$/l on day 365 (Fig 1).

Three patients experienced cytomegalovirus (CMV) reactivation, which was managed with preemptive ganciclovir or foscarnet. Two displayed complications of CMV retinitis, which were resolved with intravenous and intraocular ganciclovir, leaving no visual impairment. Patient 1 developed Epstein-Barr virus-related post-transplant lymphoproliferative disorder, which was treated successfully with rituximab, cyclophosphamide, and corticosteroids (Table I).

No patients developed clinically significant acute or chronic GVHD. The regimen was well tolerated without significant

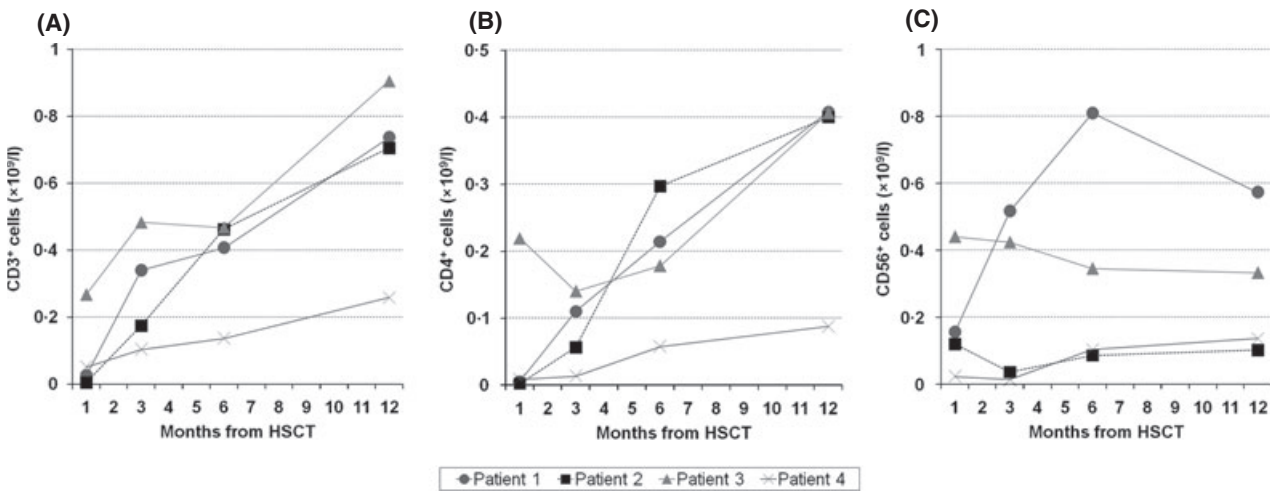

Fig 1. Reconstitution of T and NK cells after haploidentical HSCT. (A) $CD3^+$ cells (B) $CD4^+$ cells, and (C) $CD56^+$ cells at 1, 3, 6, 12 months after HSCT. Data from Patient 3 were acquired after the second HSCT. HSCT, haematopoietic stem cell transplantation.

toxicity or transplant-related mortality. All four patients survived for a median period of 19 (range, 16–23) months. All patients were transfusion-independent with sustained complete donor chimerism for a median period of 14 (range, 4–21) months (Table I).

While this preliminary study only involved four patients with refractory SAA, all subjects were long-term survivors without transfusion requirement. Our findings suggest that rapid engraftment without significant GVHD can be achieved even in heavily transfused patients using CD3- or CD3/CD19-depleted grafts and fludarabine-based, dose-reduced conditioning, and haploidentical HSCT using negative depletion of T cells can be a curative treatment option for SAA patients with no suitable donors.

T-cell reconstitution in our study was comparable with previous reports of haploidentical HSCT using CD3 or CD3/CD19 depletion and reduced-intensity conditioning (Chen *et al*, 2006; Bethge *et al*, 2008; Federmann *et al*, 2011), and appeared more rapid than that of haploidentical HSCT using CD34 selection and myeloablative conditioning (Eyrich *et al*, 2001). Notably, Patient 4 experienced prolonged CMV reactivation and CMV retinitis due to delayed immune reconstitution. Accordingly, we will adopt early CMV prophylaxis with foscarnet or ganciclovir instead of preemptive strategy or transfer of antigen-specific donor T cells in the next trial.

Despite successful initial engraftment, graft rejection remained a problem for one patient who had a long history of disease and had been heavily transfused. In our next trial, strategies to facilitate successful engraftment will include increasing the dose of $CD34^+$ cells to more than $5{\cdot}0 \times 10^6$/kg and introduction of low-dose TBI to enhance recipient immunosuppression for heavily transfused patients before HSCT. In addition, the decision to transplant in cases of highly transfusion-dependent patients without a suitable donor needs to be made without prolonged delay.

Authorship contributions

KN Koh analysed the data and wrote the article. HJ Im conceived and designed the prospective study, including the conditioning regimen. BE Kim collected clinical data. ES Choi was responsible for collection of mononuclear cells and acquisition of the related data. SW Kwon took part in graft manipulation. S Jang and CJ Park performed flow cytometric analysis of the products. HJ Im and JJ Seo revised the article critically for improvement of its content.

Disclosure of conflicts of interest

The authors have no relevant financial relationships to disclose or conflicts of interest to report.

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Correct interpretation of T-ALL oncogene expression relies on normal human thymocyte subsets as reference material

Ectopic oncogene expression is common to T-cell acute lymphoblastic leukaemia (T-ALL). The presence of oncogene transcripts, such as *LMO2, LYL1, TLX1*, *TLX3*, *TAL1* and *NKX2-1,* is regarded as a defining element of a T-ALL signature (Ferrando *et al*, 2002; Homminga *et al*, 2011). This signature enables the molecular subtype classification of T-ALL and identification of the stage of leukaemic arrest. Subgroup classifications based on oncogene expression often rely on comparisons made with incorrect biological reference material (Ballerini *et al*, 2002; Ferrando *et al*, 2002, 2004). This can result in incorrect interpretation and misclassification of T-ALL subgroups. Usage of the right reference material is essential for correct T-ALL subgroup classification, particularly when such T-ALL classification aims to identify prognostic subgroups with different clinical outcome. Although many have speculated and made assumptions regarding the usage of relevant reference material for T-ALL, no real attempt has been made to show that normal human thymocyte subsets are the most optimal biologically relevant reference material for comparison to T-ALL.

To redefine the concept of ectopic oncogene expression in T-ALL based on human thymocyte subsets as a reference, we evaluated the transcriptional programme of T-ALL oncogenes during normal human thymocyte development by means of real-time quantitative polymerase chain reaction (RQ-PCR) (Table SI) (Beillard *et al*, 2003; Dik *et al*, 2005). *LMO2* and *LYL1* were expressed in the most immature thymic stages and expression gradually declined up to the pre-T-cell receptor (TCR)αβ stages (Fig 1). Murine *Lyl1* transcription is restricted to the double negative (DN)1/DN2 stage (Ferrando *et al*, 2004), while in humans, *LYL1* transcripts can be detected up to the immature single positive (ISP) stage. Traces of *LMO2* and *LYL1* transcripts were also detected in TCRγδ$^+$ thymocytes, at levels comparable to those seen in DN3 subsets (Fig 1). This concurs with the fact that TCRγδ$^+$ thymocytes descend from the early DN stages (Carpenter & Bosselut, 2010).

In contrast to findings that *Tal1* is expressed in murine thymocytes (Ferrando *et al*, 2004), no *TAL1* transcripts were detected in any human thymic subset. Likewise, no transcripts of *TLX1, TLX3* and *NKX2-1* were detected in