

# Stem Cell Transplantation for Primary Immunodeficiency Disease: Experience of a Singapore Hospital

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**Abstract:** We retrospectively analyzed the outcomes of hematopoietic stem cell transplantation in 7 patients with primary immunodeficiency diseases treated at the National University Hospital, Singapore, over the period from December 1996 to January 2010. The primary immunodeficiency diseases managed were X-linked hyperimmunoglobulin M syndrome (n = 3), severe combined immunodeficiency (n = 1), leukocyte adhesion deficiency type 1 (n = 1), chronic granulomatous disease (n = 1), and Wiskott–Aldrich syndrome (n = 1). The age of the patients ranged from 5 months to 17 years. Conditioning regimen depended on the type of immunodeficiency, whereas supportive treatment was tailored for differing pretransplant conditions. Eight stem cell transplantations were performed for 7 patients. Donors were HLA-matched sibling donors for 2 patients and unrelated donors for the rest. At the median follow-up of 8.6 years (range 2.2–15.0 years) as of December 2011, 6 patients were alive and cured of their primary diseases.

**Key Words:** primary immunodeficiency, hematopoietic stem cell transplantation

(*WAO Journal* 2012; 5:41–44)

Stem cell transplantation (SCT) is the curative treatment for severe primary immunodeficiency diseases (PIDs). If left untreated, severe PIDs are associated with high mortality risk and poor quality of life from serious infections in childhood. Overall survival rates for matched-related donor transplants ranged from 69 to 100% depending on the underlying disease type and promptness of the transplant.<sup>1–3</sup> For matched-unrelated donor transplants, the increase in the donor pool together with improvements in supportive care and novel conditioning regimens to reduce risks of graft-versus-host disease (GVHD) has resulted in overall survival to up to 81%.<sup>1,4</sup> In

addition, early transplantation before the development of serious infections has proven beneficial.<sup>1–6</sup> In our center, we have performed SCT for the following PIDs: hyperimmunoglobulin M (HlgM) syndrome, severe combined immunodeficiency (SCID), leukocyte adhesion deficiency type 1 (LAD1), chronic granulomatous disease (CGD), and Wiskott–Aldrich syndrome. This article aims to describe the experience over the period from 1996 to 2008 in treating 7 children with PIDs at the National University Hospital, Singapore.

## METHODS

This was a retrospective study of 7 patients with PID who underwent SCT at Singapore's University Children's Medical Institute, National University Health System, from December 1996 to January 2010 (Table 1). There were 8 SCTs, which took place for these 7 patients, as 1 patient required a second transplant. All the SCTs for PIDs that had been performed in our center were included in this study description. We traced the patient files to determine the events pre-SCT and to assess survival rate and event-free survival after SCT. Ethics approval was obtained from the Institutional Review Board of the National University Hospital.

## Stem Cell Transplants and Conditioning

Two SCTs were HLA-matched, sibling donor transplants (1 bone marrow and 1 peripheral blood), 3 patients underwent unrelated transplants, which were fully HLA-matched at the antigen level (1 peripheral blood, 1 bone marrow, and 1 cord blood), and 3 patients had cord blood unrelated transplants, which were HLA-matched at 4 or 5 (out of 6) antigens (Table 2).

Except for patient 3, busulfan was used in all regimens in combination with cyclophosphamide, fludarabine, and antithymocyte globulin (rabbit). Only the patient with SCID (patient 3) underwent a reduced intensity preconditioning regimen comprising cyclophosphamide and fludarabine. All patients received GVHD prophylaxis with cyclosporine A and/or a combination of short-course methotrexate, methylprednisolone, and mycophenolate mofetil. The median total nucleated cell dose was  $22.0 \times 10^7$  per kilogram (range,  $4.78$ – $46.9 \times 10^7$ /kg), whereas the median CD34 cell dose was  $2.62 \times 10^6$  per kilogram (range,  $0.15$ – $5.54 \times 10^6$ /kg) (Table 2).

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The authors have no funding or conflicts of interest to disclose.

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**TABLE 1.** Clinical Features and Source of Graft of Patients With Primary Immunodeficiency

Patient	Sex	Diagnosis	Age at Presentation	Age at Diagnosis (mo)	Complications Before SCT
1	M	WAS	3 d	52	CMV retinitis and colitis, infectious chronic diarrhea with rectal prolapse, hypoalbuminemia, iron deficiency anemia, failure to thrive
2	M	CGD	4 mo	12	Intra-abdominal sepsis, right elbow subcutaneous abscess ( <i>Pseudomonas fluorescens</i> ), oral candidiasis, failure to thrive
3	M	X-linked SCID	13 d	4	Systemic CMV disease (hepatitis, colitis, retinitis), chronic diarrhea, tinea, oral thrush
4	M	X-linked HIGM/CD40 ligand deficiency	12 mo	68	Bronchiectasis and recurrent lung infections, <i>Pneumocystis jiroveci</i> pneumonitis, chronic diarrhea, pin worm infestations, chicken pox
5	M	LAD1	4 mo	26	Tracheostomy (laryngotracheoesophageal cleft type 2), bilateral aspiration pneumonia, left lower lobe abscess, skin ulcers, hiatus hernia with severe gastro-oesophageal reflux disease, gastrostomy site wound breakdown, hypertension, iron deficiency anemia, hypoalbuminemia, failure to thrive
6	M	X-linked HIGM/CD40 ligand deficiency	3 mo	22	Pneumonia, recurrent upper respiratory tract infection, <i>Salmonella</i> septicemia and enteritis, gastroenteritis, failure to thrive
7	M	X-linked HIGM/CD40 ligand deficiency	27 mo	44	Recurrent bronchitis, gastroenteritis, allergic rhinitis

WAS, Wiskott–Aldrich syndrome.

### Supportive Treatment

Bacterial prophylaxis comprised oral bactrim until day -2 of SCT, which was restarted when the absolute neutrophil count was  $>1 \times 10^9/L$ . Antifungal prophylaxis comprised oral itraconazole solution for all patients. Weekly intravenous immunoglobulin (from day -5 to day +84 of SCT) was given for antiviral prophylaxis. Acyclovir was given to patients who were seropositive for herpes viruses. Other supportive therapy included granulocyte colony-stimulating factor.

### Engraftment

Myeloid engraftment was taken as the first of 3 consecutive days with absolute neutrophil count  $>0.5 \times 10^9/L$ , red cell engraftment as the first of 7 days without transfusions,

and platelet engraftment as the day when platelet count was  $>50 \times 10^9/L$  without transfusions for 7 days prior.

### RESULTS

The PIDs managed were X-linked HIGM syndrome (n = 3), SCID (n = 1), LAD1 (n = 1), CGD (n = 1), and Wiskott–Aldrich syndrome (n = 1) (Table 1). Genetic studies were done for all patients except patient 1 for whom it was not available. For patient 2 (CGD), chorionic villus sampling was done for the brother—mutation in the gp91-phox gene located on chromosome X (Xp21.1). Tissue sample of patient 3 (X-linked SCID) could not be genetically typed; however, the mother was heterozygous for a deleterious splice mutation at complementary DNA (cDNA) 868 in the IL2RG gene [cDNA mutation, 868(+5) splice del ga]. Patient 4 (HIGM syndrome) had an X-linked insertion deletion (indel) mutation

**TABLE 2.** Characteristics of Graft Source and Conditioning Regimes Used

Patient	Age at SCT (mo)	Time From Diagnosis to SCT (mo)	Graft Source	Graft Type	HLA-Identity	Conditioning Regimen	GVHD Prophylaxis	NC $\times 10^7/kg$	CD34 $\times 10^6/kg$
								Infused	Infused
1	56	4	BM	MRD	6/6	BU 16.0 mg/kg; CY 200 mg/kg	CsA + MTX	23.7	4.8
2	20	8	PB	MRD	6/6	BU 16.0 mg/kg; CY 200 mg/kg	CsA + MTX	22.1	5.54
3	5	1	CB	MUD	6/6	CY 100 mg/kg; FLU 150 mg/m <sup>2</sup>	CsA + MTX	10.1	2.43
4	204	136	CB	MUD	5/6	BU 12.8 mg/kg; FLU 150 mg/m <sup>2</sup> ; ATG 8 mg/kg	CsA + Pred + MMF	4.78	0.15
5	32	6	CB	MUD	4/6	BU 8.0 mg/kg; ATG 6.0 mg/kg	CsA + Pred	21.8	0.44
6	71	49	CB	MUD	5/6	BU 16 mg/kg; CY 200 mg/kg; ATG 4.5 mg/kg	CsA + MTX + Pred	6.36	0.24
7 (first SCT)	97	53	PB	MUD	6/6	BU 16 mg/kg; CY 200 mg/kg; ATG 5 mg/kg	CsA + MTX + Pred	42.7	3.22
7 (second SCT)	100	55	BM	MUD	6/6	CY 50 mg/kg; ATG <sub>equine</sub> 90 mg/kg; rituximab 375 mg/m <sup>2</sup>	CSA + Pred + MMF	46.9	2.81

ATG, antithymocyte globulin (rabbit; thymoglobuline); BM, bone marrow; BU, busulfan; CsA, cyclosporine A; CY, cyclophosphamide; FLU, fludarabine; CB, cord blood; MRD, matched related donor; MUD, matched unrelated donor; MTX, methotrexate; PB, peripheral blood; Pred, Methylprednisolone.

**TABLE 3.** Time to Engraftment for Each Cell Lineage

Patient	Duration of Admission (d)	Myeloid Engraftment (d)	Red Cell Engraftment (d)	Platelet Engraftment (d)	Time (Yr) From SCT to Last Date of Follow-up
1	208	17	18	35	15.0
2	30	11	10	28	10.3
3	105	14	14	12	9.8
4	76	41	31	75	7.5
5	*	17	*	*	*
6	36	22	31	48	2.2
7	45	19	27	†	†
7 (second SCT)	113	18	25	145	3.4
Median (range)	76 (30–75)	17.5 (11–41)	25 (10–31)	41.5 (12–145)	8.6 (2.2–15.0)

\*Patient demised.

†Patient rejection and re-transplantation.

in exon 3 on the CD40 ligand gene (cDNA mutation, 347-355delTATAATGTTinsG). Patient 5 (LAD1) had a 2490 base pair deletion on intron 8, exon 9, and intron 9 of the CD18 gene located on chromosome 21q22.3. Patient 6 and 7 (HlgM syndrome) are brothers and have the same X-linked deletion mutation on exon 5 in the CD40 ligand gene (“TA” del at nucleotide positions 775-776, 775-776delTA).

PIDs were diagnosed 4 to 56 months after presentation (median, 18 months), with the SCT performed at a median of 28.5 months after diagnosis (range, 0.7–135 months). The median age at SCT was 63.5 months (range, 5–204 months). Median time to myeloid engraftment was 17.5 days (range, 30–75 days). Median time to red cell engraftment was 25 days (range, 10–31 days). Median time to platelet engraftment was 41.5 days (range, 12–145 days) (Table 3).

Of the 7 patients, there was 1 death (patient 5) after SCT. Pre-SCT morbidity for patient 5 was considerable. He was in poor nutritional state and suffered multiple infections and required a tracheostomy for upper airway obstruction. Post-SCT, he primarily experienced acute respiratory distress syndrome secondary to parainfluenza type 3 and *Pseudomonas pneumonia* as well as *Escherichia coli* sepsis, renal impairment requiring hemodialysis, and gastrointestinal bleed secondary to refractory thrombocytopenia. He underwent a cord blood unrelated transplant matched at 4 of 6 loci at the antigen level but failed to engraft.

For the surviving patients, all except one had sustained engraftment. Patient 7 had his first graft rejected before platelet engraftment was achieved. The second transplant using the same donor for this patient was successful, with platelet engraftment occurring after 145 days. GVHD prophylaxis was modified by replacing methotrexate with mycophenolate mofetil. The conditioning regimen for the second transplant, performed 57 days after the first transplant, was with cyclophosphamide 50 mg/kg, antithymocyte globulin 90 mg/kg, and rituximab 375 mg/m<sup>2</sup> (Table 2).

Only 1 patient was reported to have GVHD (grade I) after transplant. Two patients had reactivated cytomegaloviral (CMV) infections. Patient 1, who entered transplant with CMV retinitis and colitis, received foscarnet and CMV hyperimmune globulin, although this was limited by secondary renal impairment. Patient 3, who also had CMV retinitis and hepatitis before transplant, received daily intravenous immunoglobulin and gancyclovir before transplant and foscarnet after transplant. Other posttransplant infections included lung infections from a variety of causes including *Pseudomonas*, *Aspergillus*, parainfluenza, and fungi.

### Follow-up and Outcome

Patients were followed up at a median time of 8.6 years (range, 2.2–15.0 years) based on the last follow-up (Table 3).

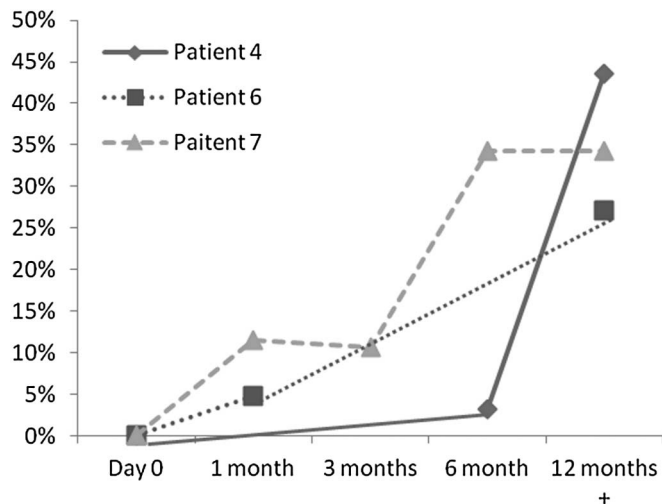
**TABLE 4.** Summary of Chimerism Studies Demonstrating Donor Engraftment

No. Days After SCT	Donor Mononuclear Cells (%)				Donor Granulocytes (%)				IgG Levels (g/dL)
	Day 21	6 mo	1 yr	2 yr	Day 21	6 mo	1 yr	2 yr	
Patient 1	*	*	*	*	*	*	*	*	†
Patient 2	*	*	*	*	*	*	*	*	†
Patient 3	12.5%	75%	75%	75%	12.5%	25%	5%	12.50%	4.95
Patient 4	70%	90%	95%	95%	70%	100%	100%	100%	2.27
Patient 5	‡	‡	‡	‡	‡	‡	‡	‡	‡
Patient 6	89%	91%	93%	96%	—	96%	95%	96%	5.88
Patient 7 (second transplant)	100%	100%	100%	100%	99%	100%	100%	100%	11.28

\*Studies not available at these time points (transplanted more than 10 years ago).

†IgG levels not relevant to the patient's immunodeficiency disease type.

‡Patient demised.



**FIGURE 1.** CD40 ligand expression (percentage positive on stimulated CD3 cells) in patients with HIgM syndrome (CD40 ligand deficiency).

All surviving patients were well, infection free, and leading a good quality of life. One patient had been discharged from follow-up 15 years post-SCT. Chimerism studies demonstrated donor engraftment at 21 days, 6 months, 1 year, and 2 years after SCT (Table 4). None of the surviving patients required immunological replacement 1 year after SCT, and all had been given hepatitis B and measles, mumps, and rubella vaccinations with good response in specific IgG levels 2 years after SCT. IgG levels measured at 1 year from SCT for those with HIgM syndrome and SCID improved from pretransplant (Table 4). For patient 2 with CGD, the nitroblue tetrazolium test also improved from 6% (pretransplant) to 50% (22 days after transplant). Patient 4, 6, and 7 with HIgM syndrome secondary to CD40 ligand deficiency, and there were increased CD40 ligand levels after SCT (Fig. 1).

## DISCUSSION

Although SCTs have been considered the definitive treatment for PIDs, studies continue to be performed to demonstrate efficacy and determine what factors may improve survivability. Advances in conditioning regimens have allowed the survivability rates for matched related donor and matched unrelated donor to be almost on par, 69 to 100% compared with 42 to 81%, respectively. The survival improved with earlier timing of the transplant and varied with the type of PID being treated (SCID having a higher survivability compared with other PIDs).<sup>1,7</sup>

Although it has been demonstrated by Buckley<sup>2</sup> that overall survival for SCTs improves greatly if performed

before the patient is 3.5 months old for patients with SCID, most studies have been unable to perform a SCT within that time frame.<sup>6,8,9</sup> Our center was no exception with 1 patient diagnosed with SCID at 4 months and entering transplant at 5 months of age. Patients with other PIDs were transplanted at a median age of 63.5 months (range, 5–204 months). Our median age for diagnosis was also quite late, at a median of 35 months (range, 4–68 months). Consequently, we had a high rate (4 of 7 patients) for pulmonary infections pretransplant among our patients, a factor associated with reduced survival. Although our patient numbers were small, we had 1 fatality out of 7 subjects who had significant infections before transplant. This further emphasizes the need for early diagnosis of PIDs and early transplantation for improved outcomes.

## CONCLUSIONS

Our small series of patients adds to the experience of others and demonstrates the importance of SCT in severe PIDs as a curative strategy. Despite significant pre-SCT morbidity from the underlying PID in our patients (eg, systemic CMV infection, pneumonias, and bronchiectasis), the outcome of our patients, with the exception of 1 death, was satisfactory.

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