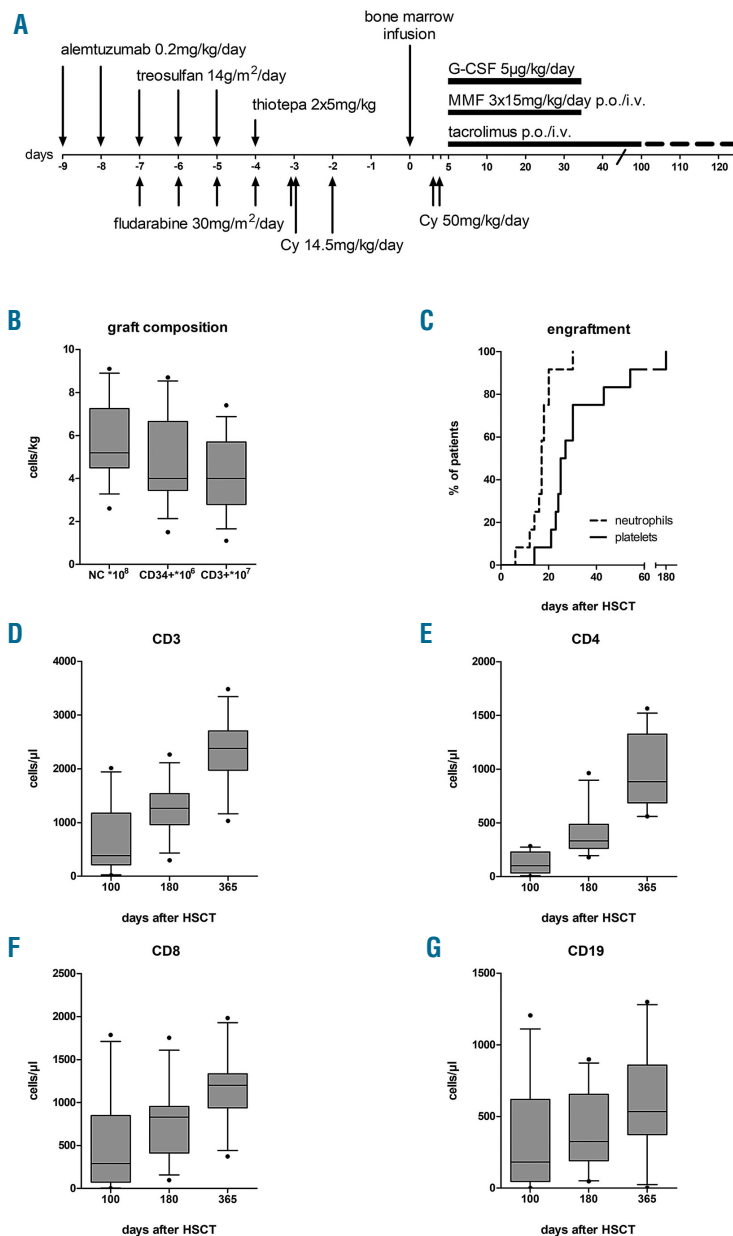


## T-cell replete haploidentical bone marrow transplantation and post-transplant cyclophosphamide for patients with inborn errors

Matched donor hematopoietic stem cell transplantation (HSCT) cures most patients with inborn hematopoietic disorders, but haploidentical HSCT was historically associated with graft rejection, graft-versus-host disease (GvHD) and infections. *In vivo* depletion of haploidentical alloreactive T cells with post-transplant cyclophosphamide (haplo pTCy) has been shown to result in outcomes comparable to those with matched related or unrelated donors in adults with malignant diseases.<sup>1</sup> Published results of this approach in patients with non-malignant diseases are still relatively scarce.<sup>2</sup> We therefore analyzed the results of haplo pTCy in combination with upfront serotherapy and a myeloablative conditioning regimen in patients with non-malignant diseases at

our center, showing good overall survival, little GvHD, and few infectious complications.

Between 2013 and 2018, all patients with inborn errors of hematopoiesis who lacked a suitable matched related or 10/10 HLA matched unrelated donor, or who had failed a previous HSCT, were treated according to this institutional protocol. The graft was unmanipulated bone marrow from familial HLA-haploidentical donors. Conditioning consisted of alemtuzumab 2x0.2 mg/kg/day (days -9 to -8), fludarabine 5x30 mg/m<sup>2</sup>/d (days -7 to -3), treosulfan 3x14 g/m<sup>2</sup>/d (days -7 to -5), thiotepa 2x5 mg/kg (day -4), and cyclophosphamide 2x14.5 mg/kg/d (days -3 to -2) in all patients except patient n. 5 who received submyeloablative busulfan [total Area Under the Curve (AUC) 55,000 ng x hour/mL] instead of treosulfan/thiotepa with the intention to enable central nervous system engraftment of donor-derived microglial cells.<sup>3,4</sup> GvHD prophylaxis consisted of cyclophosphamide 50 mg/kg/d on days +3 and +4, fol-



**Figure 1. Conditioning regimen, graft composition, engraftment and immune reconstitution.** (A) Schematic diagram of the conditioning regimen and immunosuppression based on the original Baltimore protocol.<sup>8</sup> (B) Composition of the bone marrow grafts. (C) Cumulative incidence of neutrophil and platelet engraftment. (D-G) Immune reconstitution of CD3, CD4, CD8 and CD19 positive cells. Solid line: median; gray shaded boxes: interquartile range; whiskers: 10-90 percentile; dots: outliers. Cy: cyclophosphamide; G-CSF: granulocyte colony stimulating factor; MMF: mycophenolate mofetil; NC: nucleated cells; p.o.: oral administration; i.v.: intravenous.

lowed by mycophenolate mofetil (days +5 to +35), and tacrolimus (days +5 to +100 with consecutive tapering) (Figure 1A and *Online Supplementary Appendix*).

We included thirteen patients with a median age of 9.3 years at HSCT (range: 0.2-21.6). All patients received T-cell replete HLA-haploidentical bone marrow grafts. Five patients suffered from immunodeficiencies, seven from sickle cell disease (SCD) or thalassemia, and one from congenital erythropoietic porphyria (Table 1). Three patients (ns. 3, 6, 7) have been published previously.<sup>5</sup> Patients n. 9 [matched sibling donor (MSD)] and n. 12 [matched unrelated donor (MUD)] had undergone a previous unsuccessful allogeneic HSCT after a myeloablative regimen. The grafts contained a median of  $5.2 \times 10^8$ /kg (range: 2.6-9.1) total nucleated,  $4.0 \times 10^6$ /kg (range: 1.5-8.7) CD3<sup>+</sup> cells and  $4.0 \times 10^7$ /kg (range: 1.1-7.4) CD3<sup>+</sup> cells (Table 1 and Figure 1B).

After a median follow up of 34.8 months (range: 0.2-72.0) overall survival is 92%, and event-free survival (event defined as disease recurrence or death) is 77% (*Online Supplementary Figure S1*). Neutrophil and platelet engraftment occurred in all evaluable patients on day +17 (median: range 6-30) and +26 (range: 14-180), respective-

ly (Figure 1C). There was no impact of ABO matching on the degree of erythroid engraftment. Patients were discharged from the ward at median day+34 (range: 24-47).

Two patients are stable mixed chimeras but remain disease free: Patient n. 3 has stable donor chimerism  $\geq 90\%$  and has no signs of his original disease; Patient n. 7 (SCD) has 75% donor chimerism in whole blood and 50% in the erythroid lineage. With a sickle hemoglobin (HbS) level of 45% at last follow up, she has no symptoms of SCD, and is off hydroxycarbamide. The other SCD patients with complete donor chimerism all have HbS levels comparable to their heterozygous donors (approx. 35%). Patient n. 5 experienced partial autologous reconstitution and disease recurrence 13 months after HSCT. She was successfully retransplanted from the same donor and has full donor chimerism. Patient n. 8 experienced secondary graft loss three months after HSCT and is alive with 0% donor chimerism. Thus, 77% of patients have  $\geq 90\%$  donor chimerism at last follow up and 85% are disease free (Table 2).

Anti-donor HLA antibodies have been recognized as a risk factor for graft failure in haploidentical HSCT.<sup>6</sup> In this cohort, four patients (ns. 7, 9, 10, 11) had such anti-

**Table 1. Patients' and donor characteristics.**

Patient (years)	Diagnosis	Sex	Age at HSCT	Donor	HLA match	ABO mismatch	CMV serostatus recipient/donor	Pre HSCT rituximab	Pre HSCT complications
1	IL-10R	m	10.3	father	5/10	none	+/+	yes	IBD, B-NHL
2	XIAP	m	9.4	father	5/10	minor	-/-	-	IBD, perianal abscess
3	SCD	m	8.5	father	5/10	none	+/+	-	ACS, silent stroke, recurrent VOC, splenic sequestration crisis, osteomyelitis
4	WAS	m	1.5	father	6/10	major	+/+	yes	colitis, skin vasculitis, CMV viremia
5	PNP	f	0.2	father	7/10	minor	+/+	-	-
6	SCD	f	5.4	father	6/10	none	+/+	-	recurrent VOC
7	SCD	f	20.3	mother	5/10	none	+/+	yes	RBC alloimmunization with hemolytic crisis, recurrent VOC, PRES, cerebral seizures
8	SCD	f	6.9	mother	7/10	none	+/-	-	stroke
9	THAL	f	14.9	brother	5/10	major	+/+	yes	grand mal seizure, hemosiderosis
10	SCD	f	21.6	mother	5/10	none	+/+	yes	RBC alloimmunization with hemolytic crisis, recurrent VOC, ACS
11	THAL	m	15.7	father	5/10	none	+/+	yes	hemosiderosis
12	CEP	m	9.3	father	7/10	none	-/-	yes	photosensitivity, osteopenia, pathologic fracture, hemolysis, thrombocytopenia
13	AD-EDA-ID	f	0.4	father	5/10	none	-/-	-	PCP, invasive aspergillosis, septicemia with ARDS and CPR

ACS: acute chest syndrome; AD-EDA-ID: autosomal-dominant anhidrotic ectodermal dysplasia with immunodeficiency; ARDS: acute respiratory distress syndrome; B-NHL: B-cell non-Hodgkin lymphoma; CEP: congenital erythropoietic porphyria; CMV: cytomegalovirus; CPR: cardiopulmonary resuscitation; HSCT: hematopoietic stem cell transplantation; M: male; F: female; IBD: inflammatory bowel disease; IL10R: interleukin-10 receptor deficiency; MSD: matched sibling donor; MUD: matched unrelated donor; PCP: pneumocystis jirovecii pneumonia; PNP: purine nucleoside phosphorylase deficiency; PRES: posterior reversible encephalopathy syndrome; RBC: red blood cell; SCD: sickle cell disease; THAL: thalassemia major; VOC: vaso-occlusive crises; WAS: Wiskott-Aldrich syndrome; XIAP: X-linked inhibitor of apoptosis deficiency.

bodies, in three of whom a reduction in antibody titers with plasmapheresis and rituximab was attempted (*Online Supplementary Table S1*). All patients primarily engrafted, including patient n. 11 who declined plasmapheresis.

Cellular immune reconstitution was timely with a median of 384/ $\mu$ L CD3<sup>+</sup> cells (range: 17-2013), 103/ $\mu$ L CD4<sup>+</sup> (range: 4-284), 291/ $\mu$ L CD8<sup>+</sup> (range: 4-1786) and 182/ $\mu$ L CD19<sup>+</sup> (range: 0-1206) on day +100, after HSCT (Figure 1D-G). Patient n. 7 remained severely B-lymphocytopenic at one year after HSCT (3/ $\mu$ L) and is still on intravenous immunoglobulin (IVIg) substitution at 35 months of follow up. She had received six doses of rituximab preceding HSCT due to severe hemolytic crisis. All other patients were off immunosuppression and off IVIg at last follow up.

One patient died on day +7 after HSCT from unexplained neurotoxicity; no infectious agent was identified, leading to the interpretation of fludarabine-associated toxicity in this infant patient. Acute GvHD was limited to transient disease of the skin (overall grade I) in two patients. Higher grade acute or chronic GvHD was not observed. Patient n. 4 had cytomegalovirus (CMV) viremia before and after HSCT. Reactivation of CMV

occurred in six other patients, but no overt CMV disease was observed. Patient n. 7 developed varicella zoster virus (VZV) reactivation (shingles) after cessation of acyclovir prophylaxis. No adenovirus or Epstein-Barr virus reactivations were observed. Patient n. 5 experienced veno-occlusive disease of moderate severity on day+9 after HSCT despite an AUC of 55,000 ng x hour/mL which was successfully treated with defibrotide for two weeks.<sup>7</sup> No renal or hepatic toxicity greater than Common Terminology Criteria for Adverse Events grade 2 was observed. No patient developed hemorrhagic cystitis.

Bolanos-Meade *et al.* provided proof of concept that haplo pTCy was a feasible therapeutic strategy for patients with SCD lacking a matched donor, but the submyeloablative, irradiation-containing preparative regimen resulted in high rates of autologous reconstitution.<sup>8</sup> Treosulfan/fludarabine conditioning had been reported to result in limited acute and possibly little long-term toxicity in matched donor HSCT.<sup>9</sup> We added thiotepea to increase myeloablation and a low dose of cyclophosphamide and alemtuzumab for additional recipient immunosuppression in the face of a greater HLA barrier in the haploidentical setting. The addition of either agent

**Table 2.** Hematopoietic stem cell transplantation outcome and complications.

Patient	Follow up (months)	Donor chimerism at last f/u	IVIg at last f/u	End of IS (day)	Acute GvHD (I-IV)	Chronic GvHD (1-3)	Viral reactivation	Other severe complications	Status at last f/u
1	72	100%	–	+97	–	–	–	–	alive + free of disease
2	58	100%	–	+50	–	–	–	–	alive + free of disease
3	54	100%	–	+111	–	–	CMV (d+12)	–	alive + free of disease
4	41	whole blood: 90%, CD3: 100%, CD14: 70%, CD19: 90%	–	+75	–	–	CMV (present before HSCT)	–	alive + free of disease
5	38	100% (before second HSCT: whole blood: 50%, CD3: 60%)	–	+100	I (skin)	–	–	moderate VOD (d+9)	alive + free of disease after second HSCT
6	38	100%	–	+120	I (skin)	–	CMV (d+25)	–	alive + free of disease
7	35	whole blood 75%, RBC 50%	yes	+124	–	–	VZV (d+230)	bacterial bloodstream infection ( <i>Citrobacter koseri</i> , d+8)	alive + free of disease
8	26	0%	–	+61	–	–	CMV (d+21)	bacterial bloodstream infection ( <i>Staph. aureus</i> , d+9; <i>Pseudomonas aeruginosa</i> , d+12) parotitis (d+13)	alive with disease
9	23	100%	–	+126	–	–	CMV (d+62)	bacterial bloodstream infection ( <i>Staph. aureus</i> , d+128)	alive + free of disease
10	20	100%	–	+125	–	–	CMV (d+19)	–	alive + free of disease
11	17	100%	–	+145	–	–	CMV (d+12)	–	alive + free of disease
12	12	100%	–	+145	–	–	–	–	alive + free of disease
13	0	n/a	n/a	n/a	n/a	n/a	–	cerebral edema (d+1)	dead (d+7)

CMV: cytomegalovirus; f/u: follow up; GvHD: graft-versus-host disease; HSCT: hematopoietic stem cell transplantation; IS: immunosuppression; IVIg: intravenous immunoglobulin; RBC: red blood cells; VOD: veno-occlusive disease; VZV: varicella zoster virus; n/a: not available; d: day.

had previously been found not to add significant acute toxicities to treosulfan/fludarabine.<sup>10</sup> In the future, pharmacokinetic monitoring of treosulfan may mean that targeting the degree of myeloablation can be improved even more, and possibly thiotepa could be omitted. The myeloablative conditioning with the addition of serotherapy may have enabled us to spare ionizing radiation, which we believe should be avoided in pediatric patients with non-malignant diseases due to its inherent risk for long-term sequelae. Overt immune-mediated rejection was not observed in our cohort, but two patients experienced graft loss through autologous reconstitution probably due to incomplete myeloablation. Durable complete donor engraftment after the first haploidentical HSCT was achieved in 75% of evaluable patients, indicating that even more myeloablation (e.g. with pharmacokinetically monitored busulfan or treosulfan) may be necessary to achieve full engraftment in patients in whom a complete chimerism is desired, and that the required degree of myeloablation may be disease specific. In theory, the tolerizing effect of pTCy obviates the need for serotherapy. We still chose to add alemtuzumab as an immunoablative agent directed at recipient cells to the conditioning regimen. We used a lower dose than is commonly used in T-cell replete matched transplants<sup>11</sup> and applied it on days -9 and -8 in order not to compromise donor T-cell immunity. We observed viral reactivations in 67% of patients, but no overt viral disease. T-cell immune reconstitution did not seem to be negatively affected by the serotherapy. In fact, median CD3<sup>+</sup> T-cell and CD19<sup>+</sup> B-cell numbers on day +180 were higher than in two studies using HLA-haploidentical grafts and TCR $\alpha\beta$  depletion.<sup>12,13</sup> Future studies should address the pharmacokinetics of alemtuzumab in order to use this potent drug in the most safe and effective way in this setting.

Our results compare well with other published experience in similar patient populations. Shah *et al.* describe their experience with haplo pTCy in seven patients with dedicator of cytokinesis 8 (DOCK8) deficiency after conditioning with reduced-dose busulfan, fludarabine, cyclophosphamide and low-dose total body irradiation (200 cGy), resulting in full engraftment in all patients, 86% survival, and no chronic GvHD.<sup>14</sup> Balashov *et al.* reported their experience with haploidentical TCR $\alpha\beta$ /CD19-depleted peripheral blood stem cell transplantation in ten primary immunodeficiency disease (PID) patients with an incidence of grade III-IV acute GvHD of 10%, extensive chronic GvHD of 10%, and graft failure of 20%.<sup>12</sup> Bertaina *et al.* used a similar strategy in 23 patients with non-malignant diseases, and saw no grade III-IV acute GvHD or extensive chronic GvHD, 17% graft failure, and 9% transplant-related mortality.<sup>15</sup> A recent paper from the UK reporting on 24 PID patients who underwent a haploidentical TCR $\alpha\beta$ /CD19-depleted HSCT described 84% overall survival, 4% graft failure, 4% grade III-IV acute GvHD, no chronic GvHD, and an infection-related mortality of 16%.<sup>15</sup> TCR $\alpha\beta$ /CD19 depletion is demanding from a technical, logistic and economic perspective, and may thus not be available to all centers.

While the number of treated patients in this single-center retrospective analysis is relatively low, the diseases are heterogeneous and the conditioning not entirely uniform, it still represents a larger cohort of patients treated with a uniform conditioning regimen and haplo pTCy in a variety of non-malignant diseases than in any other published report that is known to us. It remains unclear whether our approach is applicable to all inborn error dis-

ease categories and in all age groups. Even though a direct comparison to other studies is difficult, due to the heterogeneity of patients and conditioning regimens, none of the previously reported approaches with either *in vivo* or *ex vivo* depletion of alloreactive T cells seem to be superior to our experience. Myeloablative conditioning and haplo pTCy were safe and resulted in good disease-free survival in this cohort of patients with inborn errors in the absence of relevant GvHD. In general, haplo pTCy is an inexpensive T-cell depletion strategy that could be easily transferrable to centers with limited access to graft manipulation resources. However, larger prospective, and preferably comparative studies, are warranted to evaluate this approach in comparison to other donors, such as, for example, mismatched unrelated donors.

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