

Letter to the Editor

Haploidentical CD3 TCR $\alpha\beta$ and CD19-depleted second stem cell transplant for steroid-resistant acute skin graft versus host disease

To the Editor:

Steroid-resistant acute graft versus host disease (aGvHD) is a significant cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT).¹⁻³ Although many treatments are available, there is significant morbidity and mortality from the biological effects of aGvHD and the toxic adverse effects of therapy. Patients who fail to respond to steroids have a worse outcome. Second-line treatment options include extracorporeal photopheresis, mycophenolate mofetil (MMF), anti-TNF- α antibodies, or mammalian target of rapamycin inhibitors. In concept, GvHD is a form of autoimmunity (or at least alloimmunity), and so replacing damaging T lymphocytes may give the opportunity to “re-set” the immune system, enabling thymic education and development of central tolerance.⁴ Patients who undergo transplantation for primary immunodeficiency often have preexisting comorbidities, particularly viral infections and tissue damage secondary to inflammation, exacerbated by alloreactive T lymphocytes that cause aGvHD and the intense immunosuppression used to treat it. Performing a second transplant with further tissue-damaging chemotherapy to eliminate alloreactive lymphocytes, and subsequent replacement with new alloreactive lymphocytes, capable of being primed by cytokines released by tissue damage and viral infection may therefore seem counterintuitive. Ikegame et al⁵ reported 15 adult patients with malignant disorders with severe, steroid-refractory aGvHD who were treated by allogeneic HSCT using grafts from a second allogeneic donor. The response rate was 80.0%, with an overall survival of

44.6% in this high-risk population at 6 months.⁵ Although T-lymphocyte-depleted allografts reduce the risk of aGvHD, the patient remains extremely vulnerable to viral infection until thymopoiesis is achieved, around 120 days post-HSCT (or longer in the face of aGvHD-induced thymic damage). A recent modification in T-lymphocyte-depleted HSCT, CD3 TCR $\alpha\beta$ /CD19-depletion, has shown promising results in speed of immune reconstitution, lack of aGvHD, and clearance of viral infection in various malignant and nonmalignant diseases.⁶⁻⁸

We report a 3-year-old girl with Artemis-deficient severe combined immunodeficiency (homozygous c.103C>G exon 1 *DCLRE1C*), the first child of nonconsanguineous parents. She presented at age 4 months with inflammation at her BCG vaccination site, associated axillary lymphadenopathy, and oral candidiasis. A matched unrelated donor cord blood transplant was performed (see Table I). She had 100% donor chimerism from day +28. The posttransplant course was complicated by disseminated adenovirus infection and prolonged norovirus excretion, requiring total parenteral nutrition, and she developed BCG immune reconstitution syndrome. Despite the use of low-dose alemtuzumab pre-HSCT,⁹ a 10 out of 10 HLA-matched cord donor, and ciclosporin plus MMF as GvHD prophylaxis, she developed skin aGvHD grade 3. Biopsy showed parakeratosis, necrotic keratinocytes in all layers of the epidermis, an interstitial inflammatory infiltrate in the superficial dermis with lymphocyte exocytosis, spongiosis, and focal basal cell vacuolation. This did not resolve with topical therapy plus systemic methylprednisolone 2 mg/kg, MMF, weekly infliximab, and extracorporeal photopheresis (16 cycles) over the next 10 months. Her rash resolved briefly after rabbit ATG 25 mg/kg (Thymoglobulin), but flared up 10 days later, again confirmed histologically as skin aGvHD grade 3. There was no evidence

TABLE I. Transplant characteristics

Age at HSCT	Donor	Conditioning	GvHD prophylaxis	Nucleated/kg ($\times 10^8$)	CD34+/Kg ($\times 10^6$)	CD19+/kg ($\times 10^5$)	TCR $\alpha\beta$ /kg ($\times 10^4$)	TCR $\gamma\delta$ /kg ($\times 10^7$)	NK/kg ($\times 10^7$)	Neutrophil engraftment	Lineage-specific donor chimerism (%)
First transplant	Alemtuzumab	CsA/MMF		0.97	0.46	—	—	—	—	Day +33	T 100
7 mo	0.3 mg/kg										B 100
10/10 HLA-matched unrelated donor	Treosulfan										CD15 100
	36 g/m ²										
cord	Fludarabine										
Patient and donor	150 mg/m ²										
A 0101, A 0201											
B 0801, B 4102											
C 0701, C 1701											
DR 0301, DR 1303											
DQ 0201, DQ 0301											
Second transplant	Alemtuzumab	MMF/		8.8	10.2	<3.5	3.9	0.98	2.7	Day +12	T 100
18 mo	1 mg/kg	Tacrolimus									B 100
Mother	Treosulfan										CD15 100
A 0101, A 0301	36 g/m ²										
B 0702, B 0801	Fludarabine										
C 0701, C 0702	150 mg/m ²										
DR 0101, DR 0301	Thiotepa										
DQ 0201, DQ 0501	15 mg/kg										

CsA, Ciclosporin; NK, natural killer.

Boldface indicates standard tissue typing values.

TABLE II. Latest lymphocyte subsets and immunoglobulins

Subset markers/ immunoglobulins	Results	Age-related normal
CD3+	967 cells/ μ L	900-4500 cells/ μ L
CD4+	652 cells/ μ L	500-2400 cells/ μ L
CD8+	263 cells/ μ L	300-1600 cells/ μ L
CD19+	465 cells/ μ L	200-2100 cells/ μ L
NK	272 cells/ μ L	100-1000 cells/ μ L
CD4+ naive	251 cells/ μ L	NA
CD4- naive	193 cells/ μ L	NA
IgG	6.9 g/L	4.9-16.1 g/L
IgA	0.21 g/L	0.4-2.0 g/L
IgM	1.66 g/L	0.5-2.0 g/L

NA, Not applicable/available; NK, natural killer.

of gut or liver GvHD at any time (she had upper and lower gut biopsies 3 and 10 months after the first transplant) and no evidence of chronic GvHD. Despite first-, second-, and third-line treatments, aGvHD was ongoing, she had had disseminated adenovirus, had persistent norovirus enteritis necessitating total parenteral nutrition, and required antihypertensive medication because of prolonged administration of steroids and calcineurin inhibitors. We felt that we were unlikely to resolve the ongoing alloimmune response without drastic measures, and that the patient would succumb to viral infection or other complications of intense and ongoing immunosuppression.

A second transplant was performed after 11 months to treat refractory GvHD using her mother as a haploidentical donor with G-CSF-mobilized peripheral blood stem cells and CD3 TCR $\alpha\beta$ /CD19 depletion using the CliniMACs (Miltenyi Biotech, Bergisch Gladbach, Germany) system due to the reported low risk of aGvHD using this technique.⁶ She developed capillary leak and mild pneumonitis with engraftment and adenovirus detected by PCR in blood. She was treated with methylprednisolone 2 mg/kg, infliximab, and cidofovir. From day +25 posttransplant she developed increasing platelet and red blood cell transfusion requirements, increasing creatinine, uncontrolled hypertension, and proteinuria. Based on low haptoglobin, increase in lactate dehydrogenase, and widespread fragmented red cells in blood film together with her clinical picture, a diagnosis of thrombotic microangiopathy was made. Tacrolimus was discontinued, steroids weaned, and defibrotide given for 6 weeks. Defibrotide is a recognized treatment for endothelial dysfunction and in this case led to a clinical improvement.¹⁰ Her adenoviremia cleared on day +127. Ten months after second HSCT she developed autoimmune thrombocytopenia with antiplatelet antibodies detected, which responded to high-dose immunoglobulin and methylprednisolone, which was subsequently weaned and stopped (see Table E1 in this article's Online Repository at www.jacionline.org).

She is now more than 24 months post second transplant. She has 100% donor chimerism consistently since day +28. She has normal lymphocyte subsets including naive T cells and a normal IgM level (see Table II). All immunosuppression has been discontinued. Her skin has hypopigmented and hyperpigmented patches but no inflammatory changes. Norovirus cleared from feces 8 months after the second transplant. She is beginning to eat but currently has a nasogastric tube in place to facilitate enteral feeding. She has normal renal function with a creatinine level

of 53 μ mol/L (normal, 7-62 μ mol/L for age 2-12 years) and is off antihypertensive medication.

We report a patient who has been cured of long-standing acute GvHD following a rescue CD3 TCR $\alpha\beta$ /CD19-depleted haploidentical HSCT. Her posttransplant course was complicated by engraftment pneumonitis, viral reactivation and thrombotic microangiopathy, and autoimmune thrombocytopenia. Further follow-up is required to ascertain long-term immunoreconstitution. However, for recalcitrant or resistant aGvHD, which fails to respond to conventional treatments, CD3 TCR $\alpha\beta$ /CD19-depleted haploidentical HSCT may be considered as a curative procedure.

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TABLE E1. Steroid doses post second transplant

Day/date	Dose	Reason
23/10/13 Day 0	5 mg	
Weight 9 kg		
Day +3	2.5 mg	
Day +26	2 mg/kg	Pneumonitis
Day +29	1.5 mg/kg	
Day +31	2 mg/kg	Pneumonitis
Day +40	1.5 mg/kg	
Day +52	1 mg/kg	
Day +59	0.5 mg/kg	
Day +75	2 mg/kg	Pneumonitis but also adenovirus
Day +77	1 mg/kg	
Day +85	2 mg/kg	Worsening respiratory status thrombotic microangiopathy diagnosis made so steroids weaned
Day +94	1 mg/kg	
Day +101	0.75 mg/kg	
Day +107	0.5 mg/kg	
Day +111	0.25 mg/kg	
Day +121	0.125 mg/kg	
Day +125	0.125 mg/kg alternate days	
Day +164	Stopped	
10 mo post 04/09/14	2 mg/kg	Autoimmune thrombocytopenia
18/9/14	1.5 mg/kg	
October 2014	0.5 mg/kg	
31/10/14	1.5 mg/kg then weaned	Further drop in platelets
January 2015	7.5/5 mg alternate days	
Weight 10 kg		
February 2015	5/2.5 mg alternate days	
April 2015	2.5 mg daily	
June 2015	2.5 mg alternate days	
September 2015	Prednisolone stopped. Switched to hydrocortisone maintenance	
End of October 2015	Off all steroids	