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Hematopoietic stem cell transplantation rescues the hematological, immunological and vascular phenotype in DADA2

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On Behalf of deficiency of adenosine deaminase type 2 (DADA2) Foundation, European Society for Blood and Bone Marrow Transplantation (EBMT) and European Society for Immunodeficiencies (ESID), Inborn Errors Working Party

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Key Points:

- Hematopoietic stem cell transplantation (HSCT) represents an effective and definitive treatment for DADA2.
- HSCT can cure the immunological, hematological, and vascular phenotype of DADA2 with 100% survival at median follow-up of 18 months.

Abstract

Deficiency of adenosine deaminase 2 (DADA2) is caused by biallelic deleterious mutations in *CECR1*. DADA2 results in variable autoinflammation and vasculopathy (recurrent fevers, livedo reticularis, polyarteritis nodosa, lacunar ischemic strokes and intracranial hemorrhages), immunodeficiency and bone marrow failure. TNF-α blockade is the treatment of choice for the autoinflammation and vascular manifestations. Hematopoietic stem cell transplantation (HSCT) represents a potential definitive treatment. We present a cohort of 14 patients from 6 countries who received HSCT for DADA2. Indication for HSCT was bone marrow dysfunction or immunodeficiency. Six of 14 patients had vasculitis pre-HSCT. The median age at HSCT was 7.5 years. Conditioning regimens were myeloablative (9), and reduced intensity (5). Donors were HLA-matched sibling (n=1), HLA-matched unrelated (n=9), HLA-mismatched unrelated (n=3), and HLA haploidentical sibling (n=1). All patients are alive and well with no new vascular events and resolution of hematological and immunological phenotype at a median follow-up of 18mo (range 5mo to 13yr). Plasma ADA2 enzyme activity normalized in those tested post-HSCT (7/7), as early as D+14 (myeloid engraftment). Post-HSCT hematological autoimmunity (cytopenias) was reported in 4 patients, acute graft versus host disease (GvHD) grade 1 in 2, grade 2 in 3 and grade 3-4 in 1, and moderate chronic GvHD in 1 patient. In conclusion, in 14 patients, HSCT was an effective and definitive treatment for DADA2.

Introduction

In 2014, two independent studies reported biallelic deleterious mutations in the cat eye chromosome region 1 gene (*CECR1*), encoding adenosine deaminase 2 (ADA2), as the cause of a new and rare auto-inflammatory condition, named human deficiency of ADA2 syndrome (DADA2) (OMIM # 615688).[1,2] In the initial reports, DADA2 was hallmarked by recurrent episodes of fever with elevation of acute phase reactants and vasculopathy ranging from early-onset cutaneous livedo reticularis, Raynaud's phenomenon and polyarteritis to life-threatening intracranial vasculopathy with lacunar strokes and hemorrhages.[3,4] Bone marrow dysfunction, which may include pure red cell aplasia (PRCA), Diamond-Blackfan like anemia, thrombocytopenia and neutropenia, is now recognized as an equally important manifestation.[5,6] In addition, immunodeficiency can be found with hypogammaglobulinemia (esp. IgM) as well as disturbances in the T cell compartment.[7] Inflammatory bowel disease, reminiscent of the bowel involvement found in patients with common variable immunodeficiency (devoid of plasma cells) can also be found. Eight of the nearly 100 DADA2 patients (8%) reported to date in the literature are deceased.[1,2,8,9] The most common reason of death was stroke (in 4/8 patients). The diagnosis of DADA2 is based on the measurement of low/absent plasma ADA2 enzymatic activity and on the identification of biallelic loss-of-function mutations in *CECR1,* which is located within chromosome 22q11. Patients with homozygous deletions encompassing the *CECR1* locus on 22q11 show the same phenotype. Also, absent plasma ADA2 enzymatic activity has been described in singular patients with monoallelic mutations in *CECR1*. It is unclear at this point whether in these cases a second (cryptic) mutation is present on the other allele, in an intron, in the promoter region, or rather in a different gene affecting ADA2 expression. Alternatively copy number variations affecting the *CECR1* locus may have been missed.

In humans, two ADA proteins have been described: ADA1 and ADA2. Deficiency of ADA1, encoded by the *ADA* gene on chromosome 20q, causes severe combined immunodeficiency

owing to profound depletion of T, B, and NK lymphocytes, which normally show high expression of ADA1. In these patients, plasma levels of adenosine and deoxyadenosine are elevated, and their red cells show a marked accumulation of dATP (derived from deoxyadenosine). Lymphopenia has been attributed primarily to dATP pool expansion, which blocks DNA synthesis and induces apoptosis in immature lymphoid cells. Aberrant signalling via receptors for adenosine may contribute to non-immunologic features that occur in some ADA1-deficient patients. In contrast to ADA1, ADA2 is primarily expressed by myeloid cells and is mainly secreted in plasma by monocytes.[10] The function of ADA2 in cellular pathways is still enigmatic and its absence in mice has hampered investigation of pathogenic mechanisms. However, where studied, the levels of adenosine and deoxyadenosine in plasma, and of dATP in red cells, have not been elevated in DADA2.[1,2] It has been postulated that ADA2 is an adenosine deaminase related growth factor.[11] In vitro studies suggest that ADA2 secreted from monocytes, acts as an autocrine factor to stimulate their differentiation into macrophages and dendritic cells.[12] ADA2 may play a role in maintaining the balance between M1 and M2 macrophages with ADA2 deficiency leading to an M1 proinflammatory phenotype as reviewed by Martinon and Aksentijevich.[13] A mixed neutrophil and type I interferon signature was observed in two ADA2 deficient patients.[14] This correlated with increased levels of proinflammatory cytokines such as IL-6 in the plasma of ADA2 deficient patients in two studies and with the vasculitis seen in patients with DADA2. Despite *CECR1* not being expressed in the endothelium, deficiency of ADA2 leads to endothelial instability as also shown in the zebrafish model and in co-culture systems of endothelial cells.[1,2]

Medical management of patients with DADA2 is challenging. None of the commonly used immunosuppressive drugs have been particularly effective in controlling disease manifestations. Etanercept in particular or anti-TNF agents have shown promise in the management of the inflammatory syndrome and vasculitis.[7,15] However, at least etanercept does not appear to reverse the hematological phenotype (Polina Stepensky, unpublished observation, n=5). Soon after the first description of DADA2, hematopoietic stem cell

transplantation (HSCT) was reported to result in a rapid and sustained immune reconstitution and resolution of the systemic inflammation.[16,17] Indeed, given that monocytes are the main cellular source for ADA2, there is a strong rationale for HSCT as a potential cure. Additional patient case reports have since confirmed these findings.[6,18–20] The aim of the present study is to better define the role for HSCT as a potential cure for DADA2. We report the results of a multinational cohort study of 14 patients with proven DADA2 who underwent HSCT, including the previously reported cases. All are alive, well and cured.

Patients and Methods

We conducted an international cohort study on patients with DADA2 via the European Group for Blood and Marrow Transplantation (EBMT), European Society for Immunodeficiencies (ESID), Primary Immunodeficiency Transplant Consortium (PIDTC), and Center for International Blood and Marrow Transplant Research (CIBMTR). Data collection started after the Inaugural International Conference on Deficiency of ADA2 hosted by the DADA2 Foundation and held in Bethesda, Maryland on November 11th, 2016. Criteria for patient inclusion into the study were: 1) diagnostic findings consistent with DADA2 and confirmed by genetic testing and/or 2) diagnostic findings consistent with DADA2 and absent plasma ADA2 activity, and 3) HSCT performed with a follow-up time more than 5 months after HSCT. A questionnaire was completed by participating physicians. Data were obtained in accordance with the Declaration of Helsinki. All patients or their guardians had given written informed consent for data collection. The study was approved by the Ethical Committee of the University Hospitals Leuven. Neutrophil engraftment was defined as the first of 3 consecutive days when the absolute neutrophil count was ≥ 0.5 x 10^9/L, platelet engraftment defined as the first of 7 days without a platelet transfusion that the platelet count was ≥ 20 x 10^9/L, and full donor chimerism was defined as ≥ 95% of leukocytes being of donor origin in peripheral blood or bone marrow samples. The diagnosis and grading of acute and chronic GvHD were defined according to international standard criteria.

Results

We received complete data sets for 14 DADA2 patients transplanted between 2000-2016. Patients received treatment in 11 different centers in 6 countries in Europe and North America. Five patients were reported previously.[16–21] The male/female ratio was 8/6. The median age at initial presentation was 1.5 years (range birth to 16 years). The median age at genetic diagnosis was 10 years (range 2-25 years) (**Table 1**). The diagnosis of DADA2 was confirmed at the molecular level by demonstration of biallelic deleterious *CECR1* mutations in all patients. Plasma ADA2 enzyme activity prior to HSCT was low in 6/6 patients tested (**Table 2**). Six of 14 patients had vasculitis prior to HSCT, 3 of whom had intracranial hemorrhage. All patients except one had received at least one line of immunosuppressive treatment prior to HSCT. Hematological manifestations were prominent in this cohort with PRCA documented in 7/14 patients, neutropenia in 6/14, immune mediated thrombocytopenia (ITP) in 2/14 and pancytopenia in 3/14 at presentation. Low IgG was present in 7/10, low IgA was present in 7/10 and low IgM in 7/10 measured. Seven of 14 patients had recurrent infections, of whom 3/7 especially viral infections. Ten of 14 patients were on immunoglobulin substitution prior to HSCT and only 2 continue to require replacement post HSCT (both less than a year from HSCT). Splenomegaly was present in 11/14 patients.

The median age at HSCT was 7.5 years (range 2-23 years). Overall survival of patients undergoing HSCT was 100% (14/14 patients alive). Six of 14 patients had received HSCT prior to description of the condition in 2014. Indications for HSCT were bone marrow dysfunction (neutropenia, pure red cell aplasia (PRCA), thrombocytopenia), and/or immunodeficiency (hypogammaglobulinemia with recurrent infections) (**Table 3**). A total of 20 HSCT procedures were performed for 14 patients: two patients required a second transplant for engraftment failure due to use of an apparently healthy sibling donors, who later were discovered to carry the deleterious *CECR1* mutations and be ADA2 deficient. They received salvage HSCT from matched unrelated donors. One patient required a second HSCT for graft

failure. Another patient required a boost due to insufficient stem cell dose at first transplant. Yet another patient originally received myeloablative conditioning but required two unconditioned boosts following a drop in whole blood chimerism to 30%. This coincided with a decline of plasma ADA2 enzyme activity to pretransplant levels and a new presentation of auto-immune PRCA followed by agranulocytosis. Regimens were different across transplant centers. Nine patients received myeloablative conditioning and 5 received a reduced intensity regimen. The most commonly used regimen (in 5 patients) was treosulfan/fludarabine +/- thiotepa with ATG or alemtuzumab. Serotherapy was used in 12/14 patients: ATG in 4 and alemtuzumab in 8 patients. Bone marrow was the source of stem cells in 10 grafts, peripheral blood stem cells in 4. Donors were matched sibling (n=1), haplo-identical sibling (n=1), 10/10 matched unrelated (n=9), and mismatched unrelated (n=3) for the final curative transplant procedures. Methotrexate associated with a calcineurin inhibitor was the prophylactic regimen for graft-versus-host-disease in 9/14 patients (**Table 3**). All patients received antimicrobial and graft versus host disease (GvHD) prophylaxis as per discretion of the transplant physician/center.

All patients engrafted at a median of day +20 for neutrophils and at day +23 for platelets. All patients are alive and well with a median follow-up of 18 months (range 5m-13y). All patients had resolution of the hematological and immunological phenotype and no further vascular events were reported in 13/14 patients (**Figure 1**). One patient suffered from a pineal gland hemorrhage immediately post-HSCT at the time of prolonged thrombocytopenia as described earlier.[18] He has had no further vascular events since with a follow-up of 76 months. Chimerism analysis showed full donor white blood cell chimerism in all patients. ADA2 plasma enzyme activity normalized in all patients in whom ADA2 activity was monitored post-HSCT (n=7) (**Table 2**). This occurred as early as 14 days post-HSCT, concurring with monocyte reappearance in the peripheral blood as verified by prospective monitoring of plasma ADA2 enzyme activity in 1 patient (Isabelle Meyts, unpublished observation). As for HSCT related morbidity: two patients developed veno-occlusive disease, which responded to

fluid restriction. Acute GvHD developed in 6/14 and resolved after standard treatment. Moderate chronic GvHD developed in only one patient. Viral reactivation was a frequent complication post-HSCT as it occurred in 10/14 patients. Adenoviremia was the most common in 6/14 patients (42%). The patients who developed more than 2 viral reactivations all received peripheral blood stem cells. Post-HSCT hematological autoimmune phenomena were reported in 4 of 14 patients: ITP in 2, auto-immune hemolytic anemia (AIHA) in 1 and neutropenia with immune mediated PRCA in 1, responding to various treatment regimens (high dose IVIG / steroids / sirolimus / rituximab / bortezomib / Romiplostim). Alopecia as a feature of DADA2 was present in 1 patient and completely resolved post-HSCT.

Discussion

We report the results of the first international survey on the outcome of HSCT for DADA2. The results support the use of HSCT as a definitive cure for DADA2 with 14 out of 14 patients alive and well and 12/14 patients off all treatment at a median time post-HSCT of 18 months. Two patients are still on immunosuppression as both are less than a year out of HSCT. HSCT proved curative for the hematological, immunological as well as vascular phenotype associated with the condition. Although the number of patients reported here is too limited to issue a general recommendation for HSCT in DADA2, the data clearly support the feasibility of HSCT as a definitive treatment option.

Importantly for all the DADA2 patients described here the indication for HSCT was the hematological and/or immunological disorder, whereas the disease emphasis in the initial reports was on the vasculopathy with livedo, polyarteritis and lacunar infarction. Indeed, PRCA, severe neutropenia, and refractory thrombocytopenia as well as combined immunodeficiency (including recurrent infection with herpesviridae) prevailed in all transplanted patients. Prior to HSCT, treatment with TNF inhibitors (etanercept, infliximab) was attempted, but proved unsuccessful in 4 patients, and interleukin-1 receptor antagonist (anakinra) failed in 2 patients as far as restoration of the hematological and immunological

phenotype was concerned. HSCT not only resolved the hematological and immunological phenotype, it also resulted in resolution of the vascular phenotype in all 6 patients who had vasculitis prior to HSCT. Only one patient experienced a single vascular event early post-HSCT at the time of severe thrombocytopenia. The same patient had an episode of late VOD, which responded to fluid restriction. No further vascular events were reported in any of the transplanted patients, in line with the current notion that the basic pathophysiology lies in macrophage skewing towards an M1 proinflammatory phenotype and that the vasculopathy phenotype can also be cured by HSCT. In this limited number of patients, no genotype-phenotype correlation could be found. Therefore, the mere presence of a given mutation alone cannot support the decision to proceed to HSCT for this rare condition; rather the phenotype should be weighed in the decision to proceed to HSCT or to provide conventional treatment.

As becomes clear from our data, donor selection is important. Prior to the unravelling of the genetics of the disease, two patients received a graft from apparently healthy sibling donors who later, were shown to carry disease causing *CECR1* mutations and to be ADA2 deficient, resulting in engraftment failure. The question of whether a sibling donor who carries a single deleterious mutation in *CECR1* is a good option remains unclear at present. However, it can be considered safer to opt for a matched unrelated donor in these cases given that a drop in chimerism in 1 patient resulted in PRCA. Moreover at present, since absent ADA2 activity has been reported in patients with monoallelic *CECR1* mutations, haplo-insufficiency of *CECR1* cannot be excluded as a cause for minor disease manifestations. Because recipient cells may have impaired ability to reject graft hematopoietic cells, and because in cases of immunodeficiency, donor lymphocytes could have survival advantage over ADA2-deficient recipient cells, non-myeloablative conditioning can be a reasonable option as shown in some of the reported patients. In addition, a reduced intensity-conditioning (RIC) regimen may be a reasonable option until there is a better understanding of what degree of donor is necessary to cure the disease.

Infectious viral complications were frequent in our cohort, although they did not result in mortality. Adenovirus reactivation was the most common viral complication present in 6/14 patients (42%). This goes with previously published reports [22,23]. Thus, frequent prospective monitoring of patients, and pre-emptive treatment is essential.

Importantly, post-HSCT hematologic autoimmune complications were common in our cohort (ITP, PRCA, AIHA, neutropenia), seen in 4/14 patients (28%). Previous reports suggested the association of post-HSCT autoimmune hematologic disorders with non-malignant disorders, unrelated donors, serotherapy, and younger age at HSCT.[24–26] In our cohort, the association with serotherapy, in particular alemtuzumab, and younger age at HSCT might represent a potential explanation. However, at present we have no solid explanation for the auto-immune phenomena post-HSCT.

In conclusion, we report the successful treatment by HSCT in 14 DADA2 patients. All patients are alive and well and cured of immunological, hematological, and vascular manifestations. Thus, HSCT is an effective treatment option for definitive treatment of DADA2. Given the important morbidity and potential mortality associated with this disease, HSCT can be envisaged in any patient with DADA2 and severe immunological or hematological manifestations or even vasculopathy unresponsive to anti-inflammatory treatment. A matched unrelated donor can be considered in cases where there is no unaffected matched sibling donor.

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Author contributions HH and I. Meyts collected, analysed, and interpreted data, and wrote the manuscript; HH, ARK, I. Müller, FB, RGB, JD, APH, SMH, DDH, SJ, RK, GS, MT, MK, JS, JVM, KW, MH, BB, LM, and I. Meyts provided clinical information from patients and edited the manuscript. MH provided the ADA2 enzyme assay activity for most of the patients and edited the manuscript. All authors approved the final manuscript.

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Table 1: Demographic and clinical features of the 14 DADA2 patients before HSCT

Patient ID	Gender/Ethnicity	Age at disease onset (y)	Age at genetic diagnosis (y)	DADA2 clinical manifestations	CD4, CD8, CD19, CD56 (n x 10*6/L)	IgG	IgA (mg/dl)	IgM	Previous treatment	Reference
P001	M, Caucasian	3	4	PRCA, HSM, alopecia, strabismus, recurrent fevers, aphthous ulcers	**814, 459, 104, 60**	605	40	**< 6**	Prednisone, MMF, Sirolimus, CsA	6,21
P002	F, Hispanic	7	21	Recurrent infections, ID	**361, 262, 0, 32**	NA	NA	NA	Prednisone	20
P003	F, Caucasian	7	11	Splenomegaly, ICH, livedo, arthritis	**528, 211, 26, 30**	NA	NA	NA	Infliximab	
P004	F, Caucasian	1	9	Pancytopenia, stroke, ICH, vasculitis, arthritis, HSM, HTN, CMP, ID	**273, 222, 23, 29**	**354**	**34**	**9**	Prednisone, Etanercept, Anakinra, Azathioprine	19
P005	M, Caucasian	0	2	PRCA, LNP, HSM, recurrent infections	1763, 1037, **415, 104**	**426**	**<8**	**< 6**	Prednisone	
P006	F, Caucasian	14	15	PRCA, neutropenia	**441, 276, 43, 35**	**499**	**11**	**15**	Prednisone	
P007	M, Caucasian	0.5	13	PRCA, neutropenia, SAA, HSM, livedo, IDDM, GHD	NA	**360**	60	30	None	17
P008*	M, Caucasian	0.5	5	PRCA, pancytopenia, splenomegaly, recurrent infections, LNP	**653, 93, 11, 0.5**	997	**2.4**	**7**	Prednisone, Sirolimus, Tacrolimus	16, 18
P009*	M, Caucasian	0.4	4	Anemia, neutropenia, HSM, LNP, IBD, SAH, TIA, recurrent infections	**599, 278, 228, 147**	**436**	**17**	54	Prednisone, Azathioprine, Sirolimus, Etanercept	
P010	M, Caucasian	2	22	Anemia, lymphopenia, HSM, Livedo, ICH, HTN, optic nerve atrophy, PAN	**50, 50, 1, 10**	**465**	**47**	**17**	Prednisone, Azathioprine, Infliximab	
P011	F, Caucasian	2.5	4	PRCA, Vasculitis, pancytopenia, epilepsy, HSM, aphthous ulcers	**386, 257, 237, 41**	1000	**2.4**	74	Prednisone	
P012	F, Caucasian	16	25	ITP, HSM, lymphoproliferation, recurrent infections, lung granuloma	1670, 1721, **106**, 101	NA	NA	NA	Prednisone, ATG	
P013	M, Caucasian	0.3	9	PRCA, neutropenia, splenomegaly, IBD, recurrent fevers, aphthous ulcers	**607**, 781, 685, **90**	**290**	119	**9**	Prednisone, Anakinra	
P014	M, Hispanic	0	18	PRCA, hepatopathy, aphthous ulcers	NA	NA	NA	NA	Prednisone	

*: Siblings; Bold font indicates low values for age. Y: year; M: male; F: female; HSM: hepatosplenomegaly; URI: upper respiratory infection; ID: immunodeficiency; ICH: intracranial hemorrhage; HTN: hypertension; CMP: cardiomyopathy; PRCA: pure red cell aplasia; LNP: lymphadenopathy; GHD: growth hormone deficiency; IDDM: insulin dependent diabetes mellitus; IBD: inflammatory bowel disease; SAH: subarachnoid hemorrhage; TIA: transient ischemic attack; PAN: polyarteritis nodosa; ATG: antithymocyte globulin.

Table 2: Genetic and Biochemical Basis for Diagnosis of the 14 DADA2 patients

Patient ID	*CECR1* allele 1	*CECR1* allele 2	ADA2 activity pre HSCT	ADA2 activity post HSCT
P001	c.1110C>A (p.N370K)	c.1072G>A (p.G358R)	0.6[a]	19.7[a] at 1y
P002	c.794C>G (p.S265X)	c.794C>G (p.S265X)	0.0[a]	10.8[a] at 1y
P003	c.660C>A (p.Y220X)	c.660C>A (p.Y220X)	2.5[b]	NA
P004	c.506G>A (p.R169Q)	c.506G>A (p.R169Q)	0.8[a]	7.0[a] at 1y
P005	c.144delG (p.R49fs)	c.506G>A (p.R169Q)	NA	NA
P006	c.144dupG (p.R49fs)	c.506G>A (p.R169Q)	NA	NA
P007	c.506G>A (p.R169Q)	c.506G>A (p.R169Q)	NA	15.3[a] at 3m
P008*	c.506G>A (p.R169Q)	c.506G>A (p.R169Q)	NA	22.07[a]
P009*	c.506G>A (p.R169Q)	c.506G>A (p.R169Q)	0.11[a]	NA
P010	c.506C>T (p.R169Q)	c.2T>C (p.M1T)	NA	NA
P011	c.506G>A (p.R169Q)	c.506G>A (p.R169Q)	**NA**	NA
P012	c.140G>T (p.G47V)	c.336C>G (p.H112Q)	NA	NA
P013	c.144del (p.R49fs)	c.47+2T>C (splice site)	0.2[a]	11.7[a]
P014	c.506G>A (p.R169Q)	c.1072G>A (p.G358R)	NA	22.3[a]

*: Siblings

a) Plasma ADA2 (mU per mL): Healthy controls (n=20 + pooled normal plasma), 14.2 ± 5.2 (4.8 - 27.2). DADA2 patients (n=25), 0.4 ± 0.5 (0.02 - 1.7).

b) Dried Plasma Spots ADA2 (mU/g protein): Healthy controls (n=39), 130.5 ± 49.8 (58 - 271). DADA2 patients (n=40), 4.4 ± 4.3 (0.04 -17.2)

Table 3: Transplant data and post-HSCT complications for the 14 DADA2 patients

Patient ID	Year of HSCT	Age at HCT (y)/Gender	Indication of HSCT	HLA match/graft source	Conditioning	GvHD prophylaxis	aGvHD/ grade	cGvHD	Viral comp	Autoimmune comp	Last chimerism	Last follow up (m)
P001	2016	4/M	PRCA, neutropenia	10/10 MUD BM	Flu/Mel/Alem (RIC)	MTX/Tacrolimus	No	No	EBV/HHV6	ITP	1y 98%	14
P002	2013	20/F	Neutropenia	5/10 Haplo BM (Boost for low CD34 dose- $0.5x10^6$/kg)	Flu/Bu/Cy/TBI200	Tacro/MMF/PTCy	No	No	None	None	3y 100%	41
P003	2016	11/F	Pancytopenia Autoimmunity	10/10 MUD PB	Flu/Treo/TT/ATG	MTX/CsA	Skin grade 1	No	Adeno/HHV6, BK	None	6m 100%	12
P004	2012	8/F	Pancytopenia	9/10 MMUD BM	Flu/Treo/Alem	MTX/CsA	No	No	Adeno/VZV	None	5y 100%	60
P005	2016	2/M	PRCA, recurrent CMV	10/10 MUD BM	Flu/Treo/TT/ATG	MTX/CsA	Skin grade 2	No	Adeno	None	6m 100%	12
P006	2016	16/F	PRCA, neutropenia	9/10 MMUD PB (Second HSCT due to GF)	Flu/TT/ATG (RIC)	MTX/CsA	No	No	BK/CMV	None	1m 100%	4
P007	2003	4/M	Refractory SAA	10/10 MUD BM (First HSCT from affected MSD)	Flu/TBI/Alem (RIC)	MTX/CsA	No	No	Adeno	None	3y 100%	161
P008*	2009	3/M	PRCA, neutropenia	MSD BM	Bu/Cy	MMF/CsA	Colon grade 3-4	No	VZV	ITP	3y >95%	76
P009*	2016	5/M	Recurrent TIA, immunodeficiency	10/10 MUD PB (2 boosts for declining chimerism)	Flu/Treo/Alem	MMF/CsA	Skin grade 1	No	Adeno/HSV1/ BK	PRCA, neutropenia	1y >95%	15
P010	2016	23/M	Severe lymphopenia, hypogamm	10/10 MUD BM	Flu/Bu/Alem	MTX/CsA	No	No	None	None	6m >95%	11
P011	2016	5/F	PRCA, neutropenia	10/10 MUD BM	Flu/Treo/TT/Alem	MMF/CsA	Skin grade 1	No	CMV, EBV	None	6m 100%	10
P012	2014	23/F	Severe neutropenia	10/10 MUD BM	Flu/Mel/Alem (RIC)	Prednisone/CsA	No	No	None	None	2y 98%	39
P013	2015	7/M	PRCA	10/10 MUD BM	Bu/Cy/ATG	MTX/CsA	No	No	None	None	1y 100%	21
P014	2007	9/M	PRCA	MMUD PB (First HSCT from affected MSD)	Flu/TBI450/Alem (RIC)	MTX/CsA	Skin grade 2	Skin +liver moderate	CMV/adeno/ BK	AIHA	3y 95%	117

*: Siblings; GvHD: graft versus host disease; comp: complications; m: month; y: year; HSCT: hematopoietic stem cell transplant; MSD: matched sibling donor; MUD: matched unrelated donor; MMUD: mismatched unrelated donor; BM: bone marrow; PB: peripheral blood; Flu: fludarabine; Mel: melphalan; Alem: alemtuzumab; ATG: antithymocyte globulin; MTX: methotrexate; MMF: mycophenolate mofetil; CsA: cyclosporine A; Treo: treosulfan; TT: thiotepa; Bu: busulfan; Cy: cyclophosphamide; AIHA: autoimmune hemolytic anemia; Adeno: adenovirus.

Figure Legends:

Figure 1: Effect of HSCT in clinical features resolution. Dark grey squares represent the presence of a clinical feature/phenotype. Light grey squares represent major improvement in clinical features. White squares represent complete resolution of clinical feature. Each patient is presented by two attached columns (before and after transplant) for comparison. Follow-up time post-HSCT for each patient is shown in months in first row. Severe infections represent any viral, bacterial, or fungal infection that required antiviral, or antifungal treatment, or led to sepsis. IBD: inflammatory bowel disease; FTT: failure to thrive.

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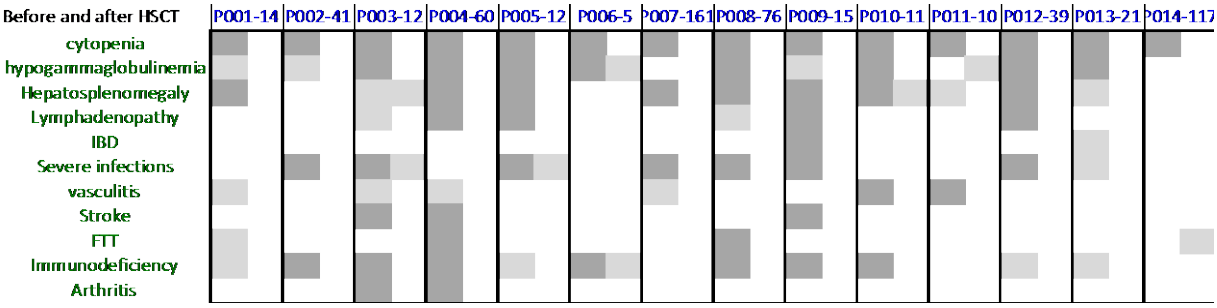

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