Journal of Clinical Immunology (2019) 39:37–44
https://doi.org/10.1007/s10875-018-0575-y

ORIGINAL ARTICLE

Hematopoietic Stem Cell Transplantation in Patients with Heterozygous *STAT1* Gain-of-Function Mutation

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Received: 20 December 2017 / Accepted: 21 November 2018 / Published online: 13 December 2018
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Abstract

Purpose Human signal transducer and activator of transcription 1 (STAT1) gain-of-function (GOF) mutations present with a broad range of manifestations ranging from chronic mucocutaneous candidiasis and autoimmunity to combined immunodeficiency (CID). So far, there is very limited experience with hematopoietic stem cell transplantation (HSCT) as a therapeutic modality in this disorder. Here, we describe two patients with heterozygous *STAT1* GOF mutations mimicking CID who were treated with HSCT.

Methods Data on the HSC sources, conditioning regimen, graft-versus-host disease (GvHD) and antimicrobial prophylaxis, and the post-transplant course including engraftment, GvHD, transplant-related complications, infections, chimerism, and survival were evaluated. Pre- and post-transplant immunological studies included enumeration of circulating interferon gamma (IFN-γ)- and interleukin 17 (IL-17)-expressing $CD4^+$ T cells and analysis of IFN-β-induced STAT1 phosphorylation in patient 1 (P1)'s T cells.

Results P1 was transplanted with cord blood from an HLA-identical sibling, and P2 with bone marrow from a fully matched unrelated donor using a reduced toxicity conditioning regimen. While P1 completely recovered from her disease, P2 suffered from systemic CMV disease and secondary graft failure and died due to severe pulmonary involvement and hemorrhage. The dysregulated IFN-γ production, suppressed IL-17 response, and enhanced STAT1 phosphorylation previously found in the $CD4^+$ T cells of P1 were normalized following transplantation.

Conclusion HSCT could be an alternative and curative therapeutic option for selected *STAT1* GOF mutant patients with progressive life-threatening disease unresponsive to conventional therapy. Morbidity and mortality-causing complications included secondary graft failure, infections, and bleeding.

Keywords STAT1 · gain-of function mutation · mucocutaneous candidiasis · autoimmunity · hematopoietic stem cell transplantation

Introduction

Signal transducer and activator of transcription 1 (STAT1) mediates the actions of many cytokines involved in innate and adaptive immune responses to viruses and intracellular bacteria [1]. Autosomal dominant (AD) gain-of-function (GOF) *STAT1* mutations are frequently associated with chronic mucocutaneous candidiasis (CMC), immunodeficiency, and autoimmune phenomena, accompanying with exaggerated T helper cell type 1 (T_H1) and defective T helper cell type 17 (T_H17) responses [2–7].

There is ambiguity concerning the optimal management approaches for patients with *STAT1* GOF mutations. The standard of care usually consists of supportive therapy options including antimicrobial prophylaxis, alone or together with

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immunoglobulin replacement therapy, especially for patients who have recurrent respiratory tract infections, with or without antibody deficiency [7, 8]. In addition, a case report of a patient with a *STAT1* GOF mutation has shown that treatment with granulocyte-colony-stimulating factor (G-CSF) improved the generation of T_H17 cells and recovery from fungal infections [9]. Oral therapy with ruxolitinib, a Janus kinase 1/2 (JAK1/2) inhibitor that attenuates STAT1 activation by cytokine receptors, was also shown to be a viable alternative in controlling disease complications, including mucocutaneous candidiasis and autoimmunity [10, 11]. On the other hand, a small number of patients have been treated with hematopoietic stem cell transplantation (HSCT) with mixed outcomes, rendering a recommendation for this therapy inconclusive [7, 12–15]. Therefore, the accrual of additional HSCT results on patients with *STAT1* GOF mutations would offer valuable information relevant to the management of this disease.

Here, we report the results of HSCT carried out on two previously reported patients with *STAT1* GOF mutations who presented with a severe disease course mimicking combined immune deficiency (CID) [16].

Methods

Patient Demographics and HSCT Procedure Clinical data on the patients were collected retrospectively. HSCT was applied according to the EBMT/ESID guidelines for HSCT for primary immunodeficiencies. Data collected included HSC sources, details of the conditioning regimen, graft-versus-host disease (GvHD) and antimicrobial prophylaxis, and post-transplant (engraftment, GvHD, transplant-related complications, infections, chimerism, survival) period. Chimerism analysis was assessed starting at the fourth week of transplantation and onward by using polymerase chain reaction (PCR)-based amplification of sort tandem repeat sequences. Neutrophil and platelet recovery were defined according to the EBMT guidelines as the first of the three consecutive days with a neutrophil count > 500/mm^3 and an unsupported platelet count > 20.000/mm^3, respectively. Viral infections were treated preemptively, considering previous patients' infections. Viral pathogens, including cytomegalovirus (CMV), Epstein Barr virus (EBV), herpes simplex virus (HSV) types 1 and 2, adenovirus, parvovirus, and human herpesvirus 6 (HHV-6), were assessed weekly in the blood by quantitative PCR. CMV disease was defined as CMV infection involving organ tissues. The presence of a CMV infection was ascertained by a positive blood CMV PCR test result. A written informed consent for transplantation was obtained from the parents.

Antibodies and Flow Cytometry Monoclonal antibodies (mAbs) to the following human proteins were used for staining: CD3 (UCHT1), CD4 (RPA-T4), IFN-γ (4S.B3), IL-17 (BL168) (Biolegend), phospho (p)-STAT1 (KIKSI0803) (all from eBioscience), and STAT1 (246523; R&D Systems). Appropriate isotype controls were used in parallel. Peripheral blood mononuclear cells (PBMCs) were incubated with mAbs against surface markers for 30 min on ice. Intracellular staining with STAT1 mAb was performed using an eBioscience fixation/permeabilization kit according to the manufacturer's instructions. For p-STAT1 staining, PBMCs were stimulated for 20 min with appropriate cytokines in complete medium, fixed with 2% paraformaldehyde for 20 min on ice, permeabilized with 90% methanol for 30 min on ice, and stained using CD3, CD4, and p-STAT1 mAbs in PBS for 30 min. For cytokine detection, cell suspensions were incubated with phorbol myristate acetate (PMA) (Sigma-Aldrich; 50 ng/mL), Ionomycin (Sigma-Aldrich; 500 ng/mL), and GolgiPlug™ (BD Biosciences; according to the manufacturer's instructions) for 4 h in complete medium before surface staining. Permeabilization and intracellular interferon (IFN)-γ and IL-17 staining were carried out using an eBioscience Fixation/Permeabilization kit as described above. Data were collected with an LSRFortessa™ cytometer (BD Biosciences) and analyzed with the FlowJo software (Tree Star, Inc.).

Statistical Analysis Comparisons between the results on patient and healthy controls were analyzed using the two-way ANOVA with post-test analysis, with p values less than 0.05 considered statistically significant.

Results

Patient 1 (P1) Patient 1 is a 3-year-old girl who presented soon after birth with recurrent urosepsis and refractory oral candidiasis despite oral fluconazole therapy. The details of the patient's clinical features were previously described, and are summarized in Table 1 [16]. She also suffered from CMV pneumonitis, mycobacterial lung disease, and hepatitis of possible autoimmune etiology. A mutation was found in the DNA binding domain (DBD) of *STAT1*; c.1154C > T, p.T385M [16]. The patient's T cells exhibited STAT1 hyperphosphorylation in response to treatment with IFN-β as compared to T cells of control subjects. Analysis of her circulating CD4$^+$ T cells revealed increased frequencies of IFN-γ$^+$CD4$^+$ (T_H1 type) T cells, and decreased frequencies of IL-17$^+$CD4$^+$ (T_H17 type) T cells, consistent with the effects of the *STAT1* GOF mutation of T_H cell skewing [16].

At age 2 years, and despite medical therapy, she continued to suffer from unrelenting lung infections, giving the impetus for HSCT. She received unmanipulated, CMV seronegative, umbilical cord HSC from a genetically HLA-identical sister who had normal *STAT1* gene sequencing. A reduced toxicity conditioning regimen with treosulfan/fludarabine/thymoglobulin (ATG) was applied. She received valganciclovir (CMV blood

Table 1 The clinical features of the patients before HSCT

Patients	Sex	Age of onset	Mutation and domain	Infections before HSCT	Autoimmunity	Therapy received before HSCT
P1	Female	2 months	c.1154C > T, p.T385M (DBD)	CMC CMV *M. tuberculosis* Pneumonia Urosepsis	Hepatitis Hypopigmented skin lesions (like vitiligo)	Ganciclovir Valganciclovir Anti-TB drugs[a] TMP-SMX Fluconazole Voriconazole CS IVIG
P2	Male	4 months	c.971G > T; p.C324F (DBD)	CMC CMV Pneumonia	–	Ganciclovir Valganciclovir TMP-SMX Fluconazole Voriconazole IVIG

CMC chronic mucocutaneous candidiasis, *CMV* cytomegalovirus, *CS* corticosteroid, *TMP-SMX* trimethoprim-sulfamethoxazole, *IVIG* intravenous immunoglobulin, *TB* tuberculosis

[a] Anti-TB drugs used until HSCT: isoniazid, rifampicin, ethambutol, pyrazinamide, cycloserine, and levofloxacin

PCR was negative at transplantation) and voriconazole prophylaxis in addition to the anti-bacterial (ciprofloxacin) and anti-mycobacterial therapies (ethambutol, cycloserine, levofloxacin), which was started at 9 months of age. The details of the HSCT procedure are presented in Table 2. The total infused stem cells was 3.4×10^5 $CD34^+$cells/kg. Prophylaxis therapy was initiated, consisting of cyclosporine (CsA) and corticosteroid (CS) for GvHD and defibrotide for sinusoidal obstruction syndrome (SOS). Hematological recovery was achieved for granulocytes on day + 28 and for platelets on day + 41. Despite oral valganciclovir prophylaxis, she had CMV infection at day + 4, which was controlled with ganciclovir therapy. Screening for other viral agents including EBV, HSV types 1 and 2, adenovirus, parvovirus, and HHV-6 were negative during the follow up.

There were no signs for acute or chronic GvHD, and due to mixed lymphocyte chimerism (23% donor) on day + 32, CsA was stopped on day + 40. At day + 83, she suffered from acute toxic hepatitis that was ascribed to prolonged anti-TB prophylaxis, which resolved after drug discontinuation. Sequential chimeric analyses at days + 90, + 153, + 190, + 270, and + 420 post-transplant showed 80, 90, 85, 82, and 100% donor chimerism, respectively. The chimerism increased spontaneously after stopping immunosuppressive drugs without other interventions. At day + 720, the whole blood and lymphocyte (including both T and B cells) chimerism levels were 100% donor cells. Lymphocyte subset analysis at that time revealed the peripheral blood $CD3^+$ T cells at 3190/mm^3 (58%), $CD3^+CD4^+$ T cells at 1815/mm^3 (33%), $CD3^+CD8^+$ T cell at 1265/mm^3 (23%), $CD19^+$ B cells at 1870/mm^3 (34%), and $CD16^+56^+$ NK cells at 330/mm^3 (6%).

To confirm the resolution of her underlying STAT1 GOF signaling phenotype post -transplantation, we compared the induction of STAT1 phosphorylation in her $CD4^+$ T cells obtained pre- and post-transplant in response to treatment with IFN-β. Analysis revealed that the enhanced STAT1 phosphorylation previously noted upon treatment of her $CD4^+$ T cells with IFN-β significantly decreased down to normal control levels following transplantation ($p < 0.0001$, Fig. 1a, b). Similarly, her dysregulated IFN-γ/IL-17 $CD4^+$/$CD8^+$ T cell responses were also normalized (Fig. 2). She is now at 24 months after transplantation with continued 100% donor chimerism without medication.

Patient 2 (P2) Patient 2 is a 3-year-old male who was originally reported as P2 in our earlier publication [16]. Details of his pre-transplant clinical features are presented in Table 1 [16]. Sanger sequencing revealed heterozygous de novo *STAT1* missense mutation (c.971G > T; p.C324F) at DBD. At age 2.5 years, and in view of uncontrolled recurrent CMV disease with severe lung involvement, oral candidiasis resistant to azole treatment, and overall poor quality of life, the patient was transplanted using 10/10 matched unrelated bone marrow donor, who was CMV seronegative. At that time, the patient's blood CMV PCR was negative under valganciclovir therapy. The patient received a reduced toxicity conditioning regimen with treosulfan/fludarabine/ATG (Table 2). The total transfused stem cells were 6.95×10^6 $CD34^+$cells/kg. CsA and mycophenolate mofetil (MMF) were used for GvHD prophylaxis and defibrotide for SOS. G-CSF was used from day + 8 until + 19. Hematological recoveries were achieved for granulocytes on day + 13 and on day + 17 for platelets. The granulocyte numbers remained higher than 1000/mm^3 between days + 15 and + 22, but they declined to 500/mm^3 on day at day + 23. G-CSF therapy was therefore initiated at a frequency of once every 4 days, which helped sustain the granulocyte numbers above 1000/mm^3.

Table 2 The details and outcome of the HSCT procedure

	P1 (3 years/girl) T385M	P2 (3 years/boy) C324F
Age at HSCT	2 years	2.5 years
Source of donor	HLA-identical sister	Match unrelated donor
Source of HSC (matching)	UCB 6/6	Bone marrow (10/10)
Conditioning	- Treo 14 g/m^2/day, from − 5 to − 3 - Flu 40 mg/m^2/day, from − 5 to − 2 - ATG 2.5 mg/kg/day, from − 9 to − 6	- Treo 14 g/m^2/day, from − 5 to − 3 - Flu 30 mg/m^2/day, from − 5 to − 2 - ATG 2.5 mg/kg/day, from − 5 to − 2
Prophylaxis		
Anti-viral	Valganciclovir	Valganciclovir
Anti-fungal	Voriconazole	Voriconazole
Anti-mycobacterial	Ethambutol, cycloserine, levofloxacin	–
Anti-bacterial	Ciprofloxacin	Azithromycin, ciprofloxacin
Donor CD34$^+$cells/kg	3.4×10^5	6.95×10^6
GvHD prophylaxis	- CsA, days − 1 to + 40 - CS, days 0 to + 14; tapered in 2 weeks (a total of 28 days)	- CsA, days − 1 to + 40 - MMF, days + 1 to + 40 (2 × 400 mg/m^2)
Myeloid engraftment	Day + 28	Day + 13
Platelet engraftment	Day + 41	Day + 17
GvHD	No	Grade II skin
Transplant-related infections	CMV infection *Klebsiella pneumonia* bacteremia	CMV pneumonia *S. epidermidis* bacteremia *Klebsiella pneumonia* bacteremia *Acinetobacter pittii* (catheter-related infection)
Outcome	100% donor chimerism, alive	Secondary graft failure, disseminated CMV disease, deceased

UCB umbilical cord blood, *BM* bone marrow, *Flu* fludarabine, *Treo* treosulfan, *ATG* thymoglobulin, *GvHD* graft-versus-host disease, *CsA* cyclosporine, *MMF* mycophenolate mofetil, *CS* corticosteroid, *CMV* cytomegalovirus

Despite CMV prophylaxis with valganciclovir, CMV infection was detected on day + 26 (blood CMV PCR 1294 copies/ml) and systemic ganciclovir was initiated in addition to intravenous immunoglobulin therapy. On day + 35, systemic and local methyl prednisolone therapy was initiated in addition to the ongoing CsA and MMF due to the clinically diagnosed grade II acute skin GvHD. The skin lesions were alleviated by day + 39 in response to therapy, and a skin biopsy was not undertaken to confirm the GvHD. Meanwhile, the result of the bone marrow chimerism analysis performed on day + 40 showed absence of donor cells. The absent chimerism was thought to reflect a secondary graft failure; consequently, all immune suppressive agents were stopped. His lung infiltrates progressed, and on day + 45, *Klebsiella pneumonia* was detected in blood culture, while *Acinetobacter pittii* was cultured from his central catheter, leading to the initiation of broad-spectrum antibiotic therapy. During this period, his absolute lymphocyte and granulocyte numbers were persistently less than 100 and 500/mm^3, respectively. Later on, owing to the control of the CMV infection, ganciclovir therapy was switched to valganciclovir on day + 53. However, CMV infection was observed to recur on day + 64, with the virus detected in the blood at 5884 copies/ml. Accordingly, ganciclovir therapy was started again in an attempt to control the CMV infection. Nevertheless, the patient's clinical condition progressively deteriorated with septicemia, pancytopenia, and severe diffuse interstitial lung involvement associated with an elevated CMV load. Foscarnet and CMV hyper-immune globulin were added to the ganciclovir therapy at day + 86. However, the patient was admitted to the intensive care unit on day + 90 and supported by mechanical ventilation. High levels of CMV were detected in the blood, endotracheal aspirates, and urine as 583,580, 3,450,000, and 2170 copies/ml, respectively. Despite the broad range of antimicrobial coverage, including cidofovir therapy, he developed acute respiratory distress syndrome and pulmonary hemorrhage, which led to his death at day + 106.

Discussion

In this report, we evaluated the outcome of HSCT in two patients with heterozygous *STAT1* mutations in the DBD [16]. HSCT resulted in successful and sustained disease control in one patient (P1), highlighting the decisive role of the hematopoietic cell lineages in disease pathogenesis and manifestations. The fatal outcome due to secondary graft failure in the other

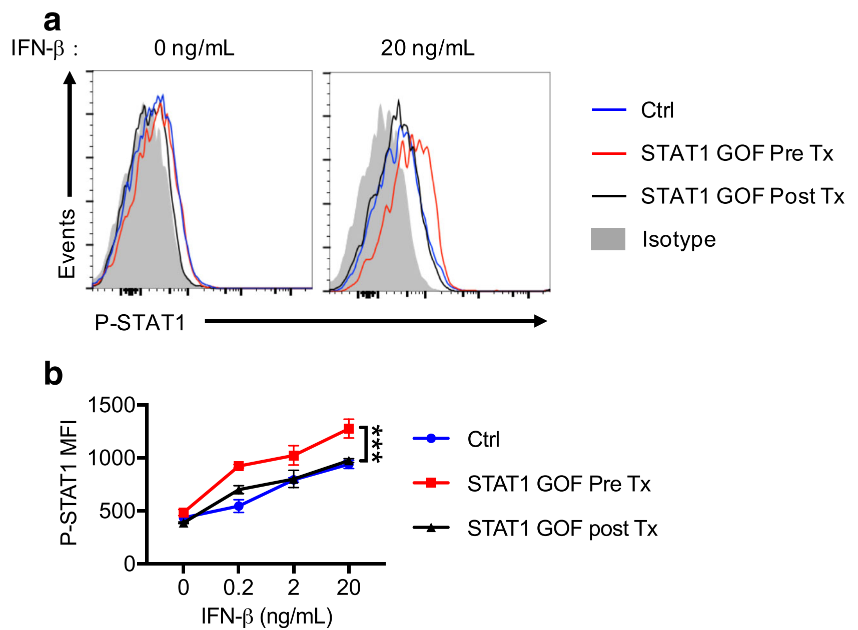

Fig. 1 Normalization of STAT1 hyperphosphorylation in *STAT1* GOF mutated patient after transplantation. **a** Pre- and post-transplantation total p-STAT1 expression in CD4$^+$ T cells stimulated with IFN-β (20 ng/mL) by flow cytometry in patient (P1) compared to healthy control. **b** The dose response curve of STAT1 phosphorylation induced with IFN-β in patient and control CD4$^+$ T cells. ***$p < 0.0001$ by two-way ANOVA. *Tx* transplantation

(P2) emphasizes the remaining challenges in optimizing this therapy for high-risk patients with severe disease.

HSCT has been performed in a few *STAT1* GOF mutated patients with inconsistent results and high mortality [7, 12–15 and this report]. A comparative analysis of the details of the HSCT procedure is presented in Table 3. In a recent cohort including 15 *STAT1* GOF transplanted patients, HSCT was conducted due to severe persistent CMC and bacterial or systemic viral infections, Immune dysregulation-Polyendocrinopathy-Enteropathy-X-linked (IPEX)-like symptoms refractory to medical therapy, CID, or hemophagocytic lymphohistiocytosis (HLH) [7, 12, 13, 15]. Interestingly, the majority of transplanted patients had DBD mutations (M390T, N397D, T385M, N397D, S466R, C324F), while the rest had coiled coil domain mutations (D165G, R274W, R274Q, D292E, I294T, H328R), reflecting perhaps a more severe disease course in patients with DBD mutations, for whom HSCT might be considered as an earlier therapeutic option.

A major concern with HSCT in *STAT1* mutated patients is the high rates of secondary graft failure, the cause of which is

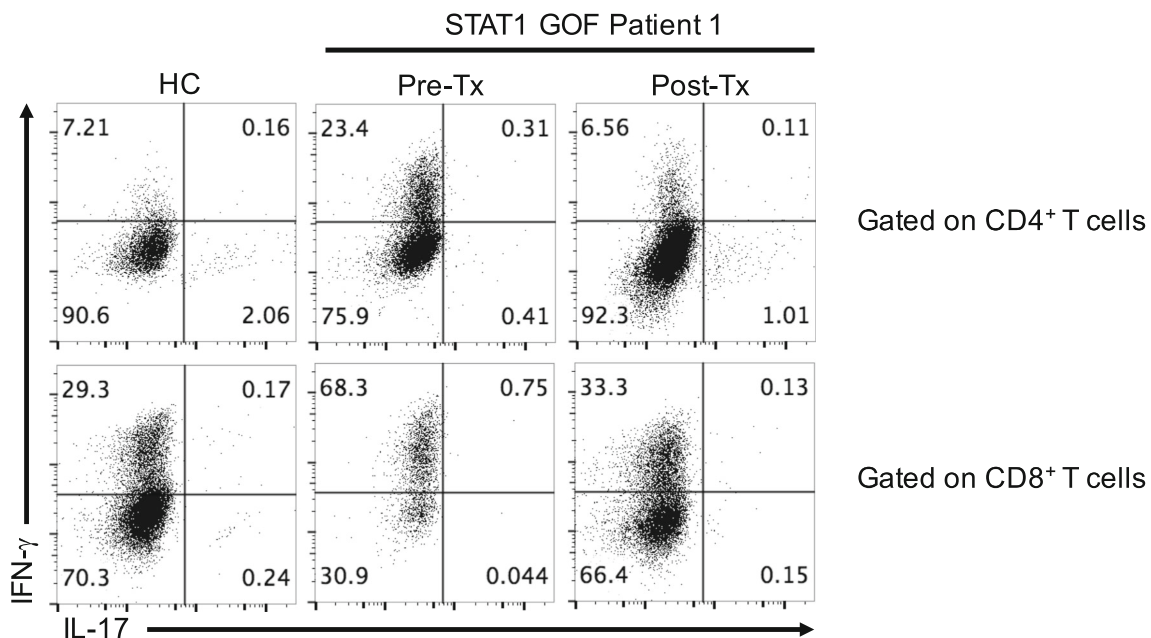

Fig. 2 Normalization of T_H1 cell skewing and improved T_H17 cell responses in *STAT1* GOF mutated patient following transplantation. Flow cytometric analyses of IL-17 and IFN-γ expression in peripheral blood CD4$^+$ and CD8$^+$ T cells of P1 before and after transplantation as compared to a control subject

Table 3 The details and outcome of HSCT in this study and other studies for *STAT1* GOF mutated patients

Parameters	Present study P1 (3 years/girl)	Present study P2 (3 years/boy)	Aldave et al [13] (13 months/girl)	Faitelson et al [12] (10 years/girl)
Age at HSCT	2 years	2.5 years	13 months	10 years
Mutation	T385M	C324F	N397D	N397D
Source of donor	MSD, UCB (6/6)	MUD, BM (10/10)	MRD, BM (6/6)	MUD (10/10)
Conditioning regimen	Myeloablative reduced toxicity (Flu/Treo/ATG)	Myeloablative reduced toxicity (Flu/Treo/ATG)	RIC (Flu/Mel/ATG)	MAC (Bus/Cy/VP)
GvHD prophylaxis	CsA, CS	CsA, MMF	MTX, CsA	CsA, CS, MTX
Acute GvHD	No	Yes (grade II)	No	Yes (grade II)
Chronic GvHD	No	No	No	No
Graft failure	No	Yes (secondary)	Yes (secondary)	Yes (secondary)
Interventions to prevent graft failure	–	Discontinuation of immunosuppressive drugs (methyl prednisolone, CsA, MMF)	Not available	Not available
Transplant-related complications	CMV infection *Klebsiella pneumonia* Toxic hepatitis	CMV infection and disease (pneumonia) *S. epidermidis* bacteremia *Klebsiella pneumonia* bacteremia *Acinetobacter pittii* Severe lung involvement Pulmonary hemorrhage	Severe thrombocytopenia High transaminase levels Interstitial pneumonia	Refractory HLH Pulmonary hemorrhage GI bleeding Renal failure TEN
Outcome	Alive 100% donor chimerism 24 months post-HSCT	Died (106 + days) Secondary graft failure Disseminated CMV disease	Died 10 months post-HSCT Graft failure Fulminant interstitial lung infection	Died Day + 42 from multiorgan failure

Parameters	Grunebaum et al [14] (7 years/boy)	Toubiana et al [7] (cohort study—5/274 transplanted)	Leiding et al [15] (cohort study—15 transplanted)
Age at HSCT	7 years	Unknown	16.5 years (range, 4–12 years)
Mutation	T385M	N397D, D292E, M390T, I294T, S466R	T385M, N397D, H328R, D165G, R274W, R274Q, D292E, I294T, N397D, S466R, M390T
Source of donor	MRD, BM	First patient—a) MSD, UCB, b) Haploidentical donor Second patient—full matched Third patient—MSD, BM Fourth patient—MMUD, UCB Fifth patient—matched donor	MRDx4, MUDx5, MMUD, haploidentical, partially matched UCBx3, full matched UCB 4xPBSC, 4xCB, 3xBM
Conditioning regimen	MAC (Bus/Cy)	Unknown	RIC 10/19, MAC 7/19 with various regimen
GvHD prophylaxis	CsA, CS	Unknown	Applied in 14/15 patients in different combinations (CsA, MMF, MTX, tacrolimus, sirolimus, CS)
Acute GvHD	Yes (skin and GI tract)	Unknown	Yes—8/15 patients
Chronic GvHD	No	Unknown	No
Graft failure	No	Unknown	Yes Primary—40% Secondary—50%
Interventions to prevent graft failure	–	Not available	Retransplatation
Transplant-related complications	GI ulcers with bleeding Hemorrhagic cystitis, pulmonary aspergillosis	Refractory HLH Disseminated CMV Interstitial lung disease	Total 80 post-HSCT events: Viral reactivation and sepsis most common Vascular (thrombus, microangiopathy, aneurysms) Various (pancreatitis, hepatitis, pulmonary edema, GI bleeding)
Outcome	Alive 95% donor chimerism	2/5 alive 3/5 died	6/15 alive, 1/6 with mixed chimera, 5/6 with full donor chimerism (95–100%)

ATG thymoglobulin, *BM* bone marrow, *Bus* busulfan, *CMV* cytomegalovirus, *CsA* cyclosporine, *CS* corticosteroid, *VP* etoposide, *Flu* fludarabine, *GvHD* graft-versus-host disease, *MAC* myeloablative conditioning, *Mel* melphalan, *MTX* methotrexate, *MMF* mycophenolate mofetil, *MSD* matched sibling donor, *MRD* matched related donor, *MUD* matched unrelated donor, *MMUD* mismatched unrelated donor, *PBSC* peripheral blood stem cells, *RIC* reduced intensity conditioning, *Treo* treosulfan, *TEN* toxic epidermal necrosis, *UCB* umbilical cord blood

not fully understood [15]. It was not found to be related to genotype, phenotype, conditioning regimen, patient's age, donor type, and HSC source. On the other hand, enhanced interferon signaling in *STAT1* GOF might be mechanistically related to the secondary graft failure, but such a link remains to be firmly established [15].

An international STAT1 GOF study group published five transplanted patients with AD *STAT1* GOF mutations [7]. Only two of them are currently alive without serious complications. In the Leiding et al. transplantation cohort, 6 patients (40%) survived [15]. One of the patients who survived had complete secondary graft loss and underwent a second HSCT and then had full immune reconstitution. Another patient had split donor chimerism, while the rest of the patients were reported to have 95–100% immune reconstitution [15]. Our patient P1 continues to live with complete disease remission and evidence by in vitro functional analyses of normalized IFN-γ/IL-17 responses and STAT1 phosphorylation in T cells post-transplantation.

Despite using HLA-identical donors, the majority of *STAT1* GOF mutated patients who received HSCT died due to transplant-related complications [7, 15 and this report]. In the Toubiana et al. STAT1 cohort, the three patients who died post-transplant suffered complications that included CMV infection, diffuse interstitial lung involvement, and uncontrolled HLH [7]. In the Leiding et al. cohort, those patients who manifested a CID phenotype prior to their HSCT experienced increased infections post-transplant [15]. Younger age at the time of transplantation and the use of a reduced intensity regimen were found to be associated with increased overall survival [15]. Our patient P2 and the other reported patients who did not survive after HSCT therapy had serious disease-related morbidities, including severe infections and in some cases HLH prior to transplantation, which could amplify transplant-related complications and foster poor prognosis [13, 15]. Similar to patient P2, bleeding complications were also reported in transplanted *STAT1* GOF mutated patients [14, 15]. Hyperactivation of the IFN-γ pathway may also be associated with poor engraftment and outcome after transplantation in *STAT1* GOF mutated patients. In this regard, the pre-transplant use of ruxolitinib to suppress hyperactive IFN responses was found to be associated with full immune reconstitution and less post-transplant complications [15].

In our two *STAT1* GOF mutated patients, valganciclovir was chosen for CMV prophylaxis due to the frequent virus infection and the need to maintain sustained CMV suppression before transplantation. In recent years, valganciclovir has been used as an effective and safe drug for preemptive therapy in the adult and pediatric populations after allogeneic HSCT [17, 18]. Previously, we demonstrated that treatment with oral valganciclovir or intravenous ganciclovir alone were equally effective in reducing CMV DNA load in pediatric HSCT patients [17]. Nevertheless, and despite prophylaxis, breakthrough CMV infection was observed in both of our patients, emphasizing the need to aggressively suppress CMV disease in the context of HSCT for this disorder.

Our study has some limitations, which need to be mentioned. Patient P2 likely had engrafted donor cells after transplantation and developed GvHD (albeit not confirmed by a skin biopsy), which was controlled by immunosuppressive therapy. However; we could not ascertain the status of donor chimerism in the early post-transplantation period until day + 40. With the lack of this information, the treatment of the patient's presumptive GvHD with immunosuppressive drugs might have increased the risk of graft rejection. On the other hand, it should be kept in mind that GvHD could itself be a cause of graft rejection. These issues emphasize the need for performing early chimerism studies in patients at high risk of graft rejection, which in the case of low or mix chimerism would allow early interventions such as tempering the use of immunosuppressive drugs and/or infusion of donor lymphocytes or stem cells to stabilize the graft. Finally, using a CMV seronegative donor for patients with active CMV infection could be one of the reasons for the progression of CMV disease, as in the case of patient P2. Therefore, selection of CMV seropositive donors in patients with CMV may result in a better outcome.

In conclusion, HSCT provides an alternative therapeutic option for subjects with *STAT1* GOF mutations who have an unrelenting disease course despite conventional therapy. Close disease monitoring, aggressive treatment of complications, and early donor screening for HSCT should be provided for patients with severe phenotype, especially before development of end organ damage. Furthermore, stabilizing patients before transplantation and anticipating common complications post-transplantation for early intervention (e.g., bleeding and graft failure) may enhance the survival rates.

Acknowledgments This manuscript is dedicated to the memory of Professor Dr. Isil Berat Barlan (1958-2015). This work was supported by the National Institutes of Health (5R01AI065617) to T.A.C and a grant from the Scientific and Technological Research Council of Turkey (1059B191401284) to S.B.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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