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Hematopoietic stem cell transplantation in patients with gain-of-function signal transducer and activator of transcription 1 mutations

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Abstract

Background: Gain-of-function (GOF) mutations in signal transducer and activator of transcription 1 (STAT1) cause susceptibility to a range of infections, autoimmunity, immune dysregulation, and combined immunodeficiency. Disease manifestations can be mild or severe and life-threatening. Hematopoietic stem cell transplantation (HSCT) has been used in some patients with more severe symptoms to treat and cure the disorder. However, the outcome of HSCT for this disorder is not well established.

Objective: We sought to aggregate the worldwide experience of HSCT in patients with GOF-*STAT1* mutations and to assess outcomes, including donor engraftment, overall survival, graft-versus-host disease, and transplant-related complications.

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Methods: Data were collected from an international cohort of 15 patients with GOF-*STAT1* mutations who had undergone HSCT using a variety of conditioning regimens and donor sources. Retrospective data collection allowed the outcome of transplantation to be assessed. *In vitro* functional testing was performed to confirm that each of the identified *STAT1* variants was in fact a GOF mutation.

Results: Primary donor engraftment in this cohort of 15 patients with GOF-*STAT1* mutations was 74%, and overall survival was only 40%. Secondary graft failure was common (50%), and posttransplantation event-free survival was poor (10% by 100 days). A subset of patients had hemophagocytic lymphohistiocytosis before transplant, contributing to their poor outcomes.

Conclusion: Our data indicate that HSCT for patients with GOF-*STAT1* mutations is curative but has significant risk of secondary graft failure and death.

Keywords

Hematopoietic stem cell transplantation; chronic mucocutaneous candidiasis; signal transducer and activator of transcription; Janus kinase; gain of function; graft-versus-host disease; graft rejection; hemophagocytic lymphohistiocytosis

Autosomal dominant gain-of-function (GOF) mutations in signal transducer and activator of transcription 1 (STAT1) cause susceptibility to a range of infections in human subjects, including severe viral and bacterial infections, nontuberculous mycobacterial disease, dimorphic fungal infections, and chronic mucocutaneous candidiasis (CMC), and are associated with development of autoimmunity.[1–9] Some patients with GOF-*STAT1* mutations present with a syndrome that clinically resembles immune dysregulation–polyendocrinopathy–enteropathy–X linked (IPEX) syndrome[10] or with combined immunodeficiency (CID), with variable effects on immunoglobulin levels, antibody production, and lymphocyte development.[11] However, the clinical phenotype can vary from mild to severe.[1,2,4,7–9]

Other than treatment of the infectious manifestations with long-term antifungal, antibacterial, and antiviral therapies, the approach to management of patients with GOF-*STAT1* mutations remains challenging and controversial. Immunosuppression to treat the autoimmune phenomena can cause exacerbation of infections. Ruxolitinib, a Janus kinase (JAK) family tyrosine kinase inhibitor targeting the JAK-STAT1 pathway, has been used to successfully treat CMC and alopecia areata[12] and autoimmune cytopenias and CMC[13] in 2 patients with GOF-*STAT1* mutations. Its mechanism is thought to include reduction of exaggerated cytokine-driven STAT1 phosphorylation,[14] reduction of follicular helper T-cell responses,[13] improved T_H17 cell differentiation,[13] and induced IL-17 production *in vitro.*[15] Granulocyte colony-stimulating factor has also been used to successfully treat CMC in 1 patient with a GOF-*STAT1* mutation.[16]

In patients with severe recalcitrant disease, hematopoietic stem cell transplantation (HSCT) has been attempted with mixed results. Successful transplants were reported in 1 patient with CMC and a confirmed DNA-binding domain GOF-*STAT1* mutation[17] and in 2 patients with severe CMC who were not evaluated for *STAT1* mutations,[18,19] whereas transplantations in 3 patients with confirmed GOF-*STAT1* mutations were unsuccessful.

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[20–22] Because of the need for viable treatment options for patients with GOF-*STAT1* mutations who have severe disease, we set out to obtain a more complete assessment of outcomes of HSCT and the challenges and complications encountered by using this approach.

METHODS

Patients

Patients undergoing transplantation were identified from national and international sites by request to specific transplant centers and by announcements made through the European Society for Blood and Marrow Transplantation Inborn Errors Working Party and the Primary Immune Deficiency Treatment Consortium. To be included, patients were required to have a confirmed heterozygous mutation in *STAT1* that confers GOF activity. Deidentified data for each case were collected by using a questionnaire/spreadsheet filled out by investigators at each institution contributing a patient. All studies involving human subjects were performed in accordance with site-specific Institutional Review Board–approved protocols, as well as the guidelines in the 1964 Declaration of Helsinki and its later amendments.

Patients were categorized based on clinical and laboratory phenotype: IPEX-like, IPEX-like and CID, CID, and severe infections. Patients with an IPEX-like phenotype had evidence of polyendocrinopathy, enteropathy, and autoimmunity; patients with CID had low T-cell and/or B-cell quantities, abnormal lymphocyte proliferation to mitogens or antigens, or both. Patients categorized as those with only severe infections had no evidence of T- or B-cell defects, or immunologic assays had not been performed.

DNA sequencing

DNA was extracted, and full-length sequencing of *STAT1* in genomic DNA was performed, as previously described.[10] In some patients GOF-*STAT1* mutations were identified by means of whole-exome sequencing and confirmed by using Sanger sequencing.[3] In one patient GOF-*STAT1* mutation was identified by means of whole-genome sequencing.[23]

Expression and phosphorylation of GOF-STAT1 mutants determined by means of immunoblotting

Various STAT1 mutants were generated by using site-directed mutagenesis with a pcDNA3-V5–based wild-type (WT) STAT1 expression vector.[24] Immunoblot analysis was performed, as previously described.[4] Briefly, WT or mutant *STAT1*-containing plasmids were transfected into a U3C STAT1-null fibrosarcoma cell line by using Lipofectamine LTX (Thermo Fisher Scientific, Waltham, Mass), according to the manufacturer's protocol. Twenty-four hours later, cells were stimulated with 1000 U/mL IFN-γ (R&D Systems, Minneapolis, Minn) for 20 minutes. Cells were then lysed and subjected to immunoblot analysis. Antibodies to detect Tyr[701] phosphorylated STAT1 (D4A7; Cell Signaling, Danvers, Mass), STAT1 (BD Biosciences, San Jose, Calif), and β-actin (Sigma-Aldrich, St Louis, Mo) were used. Experiments were performed in triplicate to confirm reproducibility.

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Luciferase reporter assay to evaluate transcriptional activation by GOF-STAT1 mutants

WT and the STAT1 mutants generated by means of site-directed mutagenesis (see above) were evaluated by using a Luciferase assay that measures luciferase activity of a reporter gene under the control of the γ-activated sequence (GAS) promoter, as previously described.[3] Reporter plasmids (Cignal GAS Reporter Assay Kit; SABiosciences, Frederick, Md) and WT or mutant *STAT1*-containing plasmids were transferred into U3C cells by means of lipofection. Twenty-four hours after transfection, the cells were stimulated with 10, 10^2, or 10^3 U/mL IFN-γ for 16 hours. The Dual-Glo Luciferase Assay System (Promega, Madison, Wis) was used to analyze firefly and *Renilla* luciferase activities. Experiments were performed in triplicate, and the data were expressed in relative luciferase units.

Cytokine-induced STAT1 phosphorylation

Intracellular staining for phosphorylated STAT1 (pSTATl) was performed on thawed PBMCs (from patients 1 and 2) allowed to recover for 2 hours in complete medium. Cells were then stained with anti-human CD4 antibody (fluorescein isothiocyanate–conjugated mouse clone M-T441; Ancell Immunology Research Products, Bayport, Minn) in sodium azide–free buffer (0.1 % BSA in 1× PBS), washed twice in complete medium, and seeded at 100,000 cells per well in 96-well plates at a volume of 100 μL. After 30 minutes of incubation at 37°C, cells were stimulated with either IL-27 (200 ng/mL) or IL-6 (50 ng/mL) for 7.5, 15, or 30 minutes and fixed for 10 minutes at 37°C with prewarmed paraformaldehyde (at a final concentration of 2%). Cells were then washed twice and permeabilized with prechilled (20°C) BD Phosflow Perm Buffer III (BD Biosciences). After 30 minutes on ice, cells were washed twice with staining buffer (2% FBS in DPBS) and stained for 40 minutes on ice with anti-human phosphorylated STAT1 antibody (Alexa Fluor 647–conjugated mouse clone 4a, BD Biosciences) directed against the phosphorylated tyrosine at position 701 (Y^{701}). Data were collected with a BD LSR II (BD Biosciences). FlowJo software (version 7.2.5/version 10.0.7 (TreeStar, Ashland, Ore) was used for data analysis.

Statistical analysis

Kaplan-Meier survival curves and significance of differences in overall survival (OS) and event-free survival (EFS) were generated and evaluated by using the Online Application for Survival Analysis (OASIS).[25] EFS was determined by analysis of time to first transplant-related complication.

RESULTS

Patients

The clinical characteristics of the 15 patients with GOF-*STAT1* mutations included in this retrospective study are presented in Table I. They were submitted by 12 participating centers from Canada, the United Kingdom, The Netherlands, Japan, Peru, Russia, Spain, Turkey, and the United States and met the criteria for study inclusion. The clinical course of 4 patients (P7, P11, P12, and P15) has been published previously,[11,17,20–22] but those reports lack comprehensive data about HSCT, which have been included in this report. The cohort consists of 9 male and 6 female subjects who ranged in age from 13 months to 33 years at

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the time of transplantation (Table I). Of this cohort, 6 are alive and 9 died of complications related to HSCT (Table II and see Table El in this article's Online Repository at www.jacionline.org).

All 15 patients had heterozygous missense mutations in either the coiled-coil or DNA-binding domain of STAT1 (Table I). Five patients (P2-P5 and P11) from 4 different countries shared the same c.1154C>T, p.T385M missense mutation, and 2 patients (P12 and P14) from Canada and Peru shared the same c.1189A>G, p.N397D missense mutation, both of which were located in the DNA-binding domain. The remaining mutations were identified in only a single patient. All 10 unique mutations conferred GOF activity with enhanced STAT1 phosphorylation in response to IFN-γ (Fig 1) and showed increased GAS-dependent reporter gene transcriptional activity after stimulation with IFN-γ (Fig 2): P1, who was studied before HSCT, demonstrated enhanced and prolonged STAT1 phosphorylation after stimulation with IL-6 or IL-27 (Fig 3, A).

Clinical phenotype and pre-HSCT complications

Infections occurred in all 15 patients before HSCT, with fungal infections being the most common. Twelve patients had CMC affecting the nails, skin, oral mucosa, and intestinal tract. Five had invasive fungal infections, including pulmonary aspergillosis in 3 patients, candidemia in 1 patient, and cryptococcal meningitis in 1 patient. Bacterial infections were also frequent, most commonly affecting the upper and lower respiratory tract, but also causing sepsis in 2 patients and gastroenteritis in 2 patients. When culture results were available, encapsulated organisms were predominant (*Haemophilus influenzae,* n = 3; *Streptococcus pneumoniae,* n = 3), followed by *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis,* and *Clostridium difficile.* Recurrent pneumonia led to bronchiectasis in 2 patients, one of whom has recurrent *Pseudomonas* species pneumonia. Varicella zoster virus and cytomegalovirus were the most common viral infections among this cohort (n = 5 each), but multiple other cutaneous and invasive viral infections occurred (EBV in 3 patients; molluscum contagiosum virus, norovirus, and herpes simplex virus each in 2 patients; and parvovirus, BK virus, JC virus, adenovirus, and human herpes virus 6 each in 1 patient). Disseminated EBV led to hemophagocytic lymphohistiocytosis (HLH) in 1 patient (P12), and parvovirus B19 induced pure red cell aplasia in 1 patient (P9). BK and JC viremia occurred in the context of immunosuppression for treatment of chronic neutropenia and thrombocytopenia in another (P10). Nontuberculous mycobacterial infections were observed in patients 1 and 2, both with lymphadenitis. One patient (P4) had pulmonary tuberculosis.

Autoimmune disorders were observed in 10 patients, 5 of whom had an IPEX-like syndrome (P1-P5). Enteropathy, the hallmark feature of those with IPEX-like syndrome, was often associated with other autoantibody/T cell-mediated manifestations, including type 1 diabetes mellitus, thyroiditis, autoimmune neutropenia, hemolytic anemia, and growth hormone deficiency. Four of the IPEX-like patients had the same missense mutation affecting the DNA binding domain (c.l 154C>T p.T385M). In 6 of the 10 patients without a typical IPEX-like syndrome, a variety of autoimmune symptoms occurred, including vitiligo,

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hypothyroidism, autoimmune cytopenias, antiphospholipid syndrome, pernicious anemia, and autoimmune hepatitis.

Two patients in this cohort had features consistent with HLH that were refractory to medical therapy, contributing to the decision to initiate HSCT (see Fig E1 in this article's Online Repository at www.jacionline.org). Patient 9 had HLH associated with pneumonia at age 7 years. He was successfully treated with prednisolone and high-dose intravenous immunoglobulin, but HLH relapsed a few months later, leading to agranulocytosis unresponsive to granulocyte colony-stimulating factor, prednisone, intravenous immunoglobulin, and cyclosporine. Despite 3 attempted HSCTs, HLH never cleared, and the patient died of multiorgan failure 3 months after the third transplantation and secondary graft failure from the first transplantation.

Patient 12 had HLH at age 10 years in association with EBV viremia while also having microabscesses of the spleen and kidneys and cutaneous herpes zoster infection. She did not respond to dexamethasone, and HLH progressed to involve the central nervous system. Treatment was escalated to include etoposide, cyclosporine, and intrathecal methotrexate but unfortunately failed to induce remission, and the patient died of multiorgan failure within 1 week of transplantation.

Both patients had classic presentations of HLH with very increased inflammatory markers and ferritin and hemophagocytosis visualized in the bone marrow. Assessment of natural killer (NK) cell cytotoxicity was not performed in patient 9 and was normal in patient 12.

Antibody deficiency was present in 9 patients, prompting treatment with immunoglobulin supplementation (P4, P6–P11, P13, and P14). Nine patients (P4, P5, and P7–13) had T-cell lymphopenia, and 3 had abnormal T-cell function when tested (P5, P6, and P11). Eight patients with T-cell defects also had concurrent B-cell and/or NK cell lymphopenia (P4, P6, and P8–13). T-cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs) were quantified in 3 patients (P8–P10) and were less than the limit of detection in all 3. Defects in cellular and humoral immunity occurred independent of the presence of autoimmunity in some and coincided with autoimmunity in others.

Transplantation course and complications

Nineteen HSCTs were performed in this cohort of 15 patients (Fig 4 and Table II). One patient (P9) received 3 transplants, and 2 (P2 and P6) received 2 transplants. Indications for HSCT were severe clinical manifestations, including recurrent infections, autoimmunity, IPEX-like symptoms refractory to medical therapy, HLH, and CID (see Fig E1). Of those patients who survived HSCT, the mean age at HSCT was 8.5 years (range, 4–12 years); of those who died, the mean age was 16.5 years (range, 13 months to 33 years) at the time of transplantation. A variety of graft sources were used, including matched related donors (MRDs; 4 HSCTs), matched unrelated donors (MUDs; 5 HSCTs), mismatched unrelated donors (MMUDs; 1 HSCT), a haploidentical donor (1 HSCT), partially matched unrelated umbilical cord blood (UCB; 3 HSCTs), and fully matched UCB (1 HSCT). Peripheral blood stem cells (PBSCs) were used in 4 transplants. Four of six survivors received unrelated

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grafts (2 MUD-marrow, 1 MUD-PBSCs, and 1 partially matched UCB); the fifth and sixth survivors received haploidentical PBSCs and matched related bone marrow, respectively.

Reduced-intensity conditioning (RIC) regimens were used most frequently (P1, P2 [first HSCT], P3, P4, P6 [second HSCT], P9 [second HSCT], P10, P13, P14, and P15) and were associated with higher OS (P=.11; Fig 5, E) and EFS (P= .07; see Fig E2, E, in this article's Online Repository at www.jacionline.org). The decision to use RIC regimens was institution specific. Patients 1, 3, and 4, 2 of whom survived, received the same RIC regimen consisting of fludarabine, melphalan, and alemtuzumab based on its previous success in patients with IPEX syndrome.[26] Myeloablative regimens were used in 7 HSCTs (haploidentical PBSCs in P2, MMUD in P9, MRD in P8 and P11, partially matched UCB in P6, and MUD in P5 and P12). Five of 7 patients (P5, P6, P8, P9, and P12) who received myeloablative regimens died; however, death was complicated by chronic HLH in 2 patients (P9 and P12). Patients 5 and 8 died before primary engraftment, the latter of which was directly related to cyclophosphamide toxicity. Patient 6 had secondary graft failure and did not survive a second transplantation. Patient 2 received myeloablative conditioning before a second HSCT after secondary graft loss from the first transplantation with RIC. Patient 11 had full immune reconstitution and resolution of disease symptoms and is alive 2 years after HSCT.

A total of 80 posttransplantation events occurred (see Table E1), with infections consisting of viral reactivation and sepsis being the most common. Median event-free time from a posttransplantation complication was 31.5 days. There were also a high number of vascular and cardiac-related transplant complications: catheter-associated venous thrombus formation and supraventricular tachycardia in the same patient and cardiac effusion, thrombotic microangiopathy, and cyclophosphamide-induced cardiac toxicity each in 1 patient. The association of vascular aneurysms and complications in patients with GOF-*STAT1* mutations[8,10,27] could be a risk factor for cardiac- and vascular-related events after HSCT. Other noninfectious complications of HSCT included pancreatitis, acute pulmonary edema, gastrointestinal bleeding, and hepatitis. Overall EFS was low (10% by day +100 after HSCT) and was not affected by age at transplantation, phenotype, genotype, or conditioning regimen (see Fig E2, B–E).

Immune reconstitution

Primary engraftment, which was defined as an absolute neutrophil count of greater than 500 cells/μL for 3 consecutive days, occurred at a median of day + 18 in 14 (74%) of 19 HSCTs and in 13 (80%) of 15 patients with their first HSCT. With the exception of patients 5 and 8, who died soon after transplantation and before engraftment, 12 of 13 patients engrafted with their first transplantation. Primary graft failure occurred in patient 6.

Secondary graft failure occurred in 6 patients (within the first 4 months in 5 patients) after HSCT that had primary engraftment and survived the first 100 days (Fig 4 and Table II and see Fig E3, A, in this article's Online Repository at www.jacionline.org). No isolated risk factors for secondary graft loss, including phenotype, genotype, age at transplantation, conditioning regimen, or donor source, were identified (see Fig E3, B). Of the 6 patients with secondary graft failure: 2 (P2 and P9) underwent subsequent transplant, with 1 (P2) surviving with full immune reconstitution. The other survivor with secondary graft failure

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(P1) is a mixed chimera, has experienced return of symptoms, and has not yet undergone retrains plantation (Fig 4 and Table II). All 5 survivors (P2, P3, P10, P11, and P13) with 95% to 100% donor chimerism established full immune reconstitution and complete reversal of immunodeficiency and resolution of infectious and autoimmune manifestations.

Poor thrombocyte recovery was observed in 3 patients (P4, P7, and P15), resulting in severe transfusion-dependent thrombocytopenia. Patient 7, who received an MRD transplant, engrafted without conditioning but died 3 months after HSCT from bleeding at sites of mycotic brain aneurysms; no bleeding episodes occurred in patients 4 or 15.

Graft-versus-host disease (GvHD) prophylaxis was used in all but 1 patient, P7, who was pancytopenic and received an MRD transplant. Acute GvHD was generally mild or well controlled by immunosuppression. Acute GvHD occurred after 8 of 18 evaluated HSCTs in 8 (57%) patients (P2 [second HSCT], P3, P4, P5, P6 [second HSCT], P11, P12, and P13; Table II). Seven patients had acute GvHD of the skin (grades 1–3), and in all cases there was good response to topical and systemic corticosteroids. Two patients (P6 and P11) had acute GvHD of the gastrointestinal tract.

Outcome

Overall, 6 (40%) patients survived and are presently more than 1 year after transplantation (Fig 4 and Table II). Nine patients died, 7 less than 1 year after HSCT (Fig 4 and Table II and Table El). Of the surviving patients. 5 of 6 had full immune reconstitution, and 1 is a mixed chimera. One survivor had complete secondary graft loss (P2), and 1 (P1) has split donor chimerism with 2% donor myeloid cells and 39% donor lymphoid cells. Patient 2 underwent a second HSCT and had full immune reconstitution and reversal of *in vitro* hyperphosphorylation of STATI (Fig 3, B). Graft loss was gradual in patient 1 and associated with return of infections, enteropathy, continued failure to thrive, and development of lymphopenia and hypogammaglobulinemia. Symptoms remain milder than those in his pre-HSCT condition.

Those patients with IPEX-like syndrome who engrafted and have 95% to 100% immune reconstitution (P2 and P3) resolved enteropathy within the first 100 days, and immunosuppression could be discontinued within 1 year. Patient 3 was able to discontinue parenteral nutrition by day +38. All surviving patients with 95% to 100% donor engraftment (P2. P3, P10, P11, and P13) have complete resolution of autoimmunity and infections and underwent catch-up growth.

Two patients with CID had HLH several months before HSCT (P9 and P12). In both patients HLH was lethal when the patients underwent HSCT in the presence of active disease. HLH-associated death accounted for 22% of the lethal outcomes of HSCT in this cohort. A CID phenotype was associated with a higher frequency of posttransplantation infections (12/22 posttransplantation infections, see Table E1) and negatively affected OS ($P = .24$; Fig 5, B) and EFS ($P = .14$; see Fig E2, B), but neither reached statistical significance.

Death occurred in all patients by the end of this study who did not have some degree of donor engraftment and immune reconstitution. Only patients with 95% to 100% immune

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reconstitution had resolution of infections and autoimmunity (P2, P3, P10, P11, and P13). Younger age ($P = .05$; Fig 5, D), a mutation leading to T385M ($P = .24$; Fig 5, C), lack of CID ($P = .24$; Fig 5, B), and use of RIC ($P = .11$; Fig 5, E) were associated with increased OS, but only age at transplantation reached statistical significance. IPEX-like disease and mutation leading to T385M were favorable factors; however, patients with these characteristics underwent transplantation at a younger age. Four of 5 patients with IPEX-like disease and 3 of 4 with mutations leading to T385M were 12 years or younger at the time of HSCT suggesting that age and not phenotype or genotype most strongly predict OS and EFS.

DISCUSSION

This multinational cohort of 15 patients is the largest aggregation of patients with GOF-*STAT1* mutations who have undergone HSCT yet collected. In 6 patients GOF-*STAT1* mutations were identified retrospectively after HSCT and postmortem in 3. In none of the patients was HSCT elective but rather intended to be lifesaving to reverse severe infections, HLH, or autoimmunity. For this reason, survival rates might have been more dismal and would likely be better in patients undergoing transplantation earlier before the development of severe manifestations.

Data from this cohort suggest that HSCT is a viable and curative treatment option for patients with GOF-*STAT1* mutations; however, disease-related complications are common and can strongly affect outcomes. Numerous questions surrounding appropriate patient selection, timing, donor, and conditioning regimen for HSCT in patients with GOF-*STAT1* mutations still exist. However, our data suggest that HSCT can be considered curative, particularly if performed early in patients with GOF-*STAT1* mutations with severe phenotypes, including those with IPEX-like symptoms, CID, serious life-threatening infections, and severe autoimmunity but not active HLH. Younger age was the strongest positive indicator of OS, suggesting a negative effect of disease-related morbidity on EFS and a higher rate of success when undergoing early transplantation. Because of the effect of age on OS and EFS, early recognition and diagnosis of GOF-*STAT1* is imperative. OS and EFS were not affected by genotype, phenotype, or conditioning regimen. When assessed, abnormal TREC and KREC numbers coincided with immunodeficiency and predisposition to infections. The role of TREC and KREC analysis in early diagnosis and prognosis of patients with GOF-*STAT1* mutations needs more investigation.

Five of 6 patients in this series with IPEX-like symptoms had the common DNA binding domain mutation c.1154C>T, p.T385M, which is known to be associated with IPEX-like disease.[10] Ten patients in this series had mutations (c.1154C>T, p.T385M in P2–P5 and P11; c.494A>G, p.D165G in P6; c.820G>A; R274W in P7, c.821G>A, p.R274Q in P8; and c.1189A>G, p.N397D in P12 and P15) that were previously described in other patients as associated with severe clinical manifestations, including severe infections and autoimmune disease.[11] In agreement with this, these patients also had severe infections, autoimmunity, and immunodeficiency. With the exception of the common DNA binding domain mutation c.1154C>T, p.T385M, which was associated with a higher incidence of IPEX-like symptoms and overall better outcome (Fig 5, C), there was no specific genotype phenotype outcome

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correlation. Within this cohort, patients with GOF-*STAT1* mutations were preferentially observed in the DNA-binding domain (10/15), suggesting that mutations in this region are associated with worse infections, autoimmunity, and immunodeficiency, and patients with these mutations might benefit from early consideration of transplantation.

Although HLH has not been described as a major problem in patients with GOF-*STAT1* mutations,[1–10] the 2 patients reported here had severe HLH without entering remission and died shortly after HSCT during active disease. The mechanism of HLH in patients with GOF-*STAT1* mutations is not well elucidated, but given the proposed role played by IFN-γ in the pathogenesis of HLH, development of HLH associated with GOF-*STAT1* mutations might be related to hyperactivation of IFN-γ–dependent STAT signaling pathways. Defects in NK cell cytotoxicity in patients with GOF-*STAT1* mutations have recently been reported,[29,30] and this might also contribute to an increased risk of HLH in these patients, particularly in the context of viral infections.

IPEX-like symptoms are conventionally treated with long-term immunosuppression, including corticosteroids, calcineurin inhibitors, and rituximab. In the 3 patient with GOF-*STAT1* mutations associated IPEX-like symptoms without CID (P1-P3), various immunosuppressive agents were ineffective in correcting the autoimmune features. HSCT was well tolerated in those with isolated IPEX-like disease. There were no deaths, and two thirds had full immune reconstitution with complete reversal of clinical manifestations, suggesting that HSCT should be considered in patients with GOF-*STAT1* mutations with IPEX-like symptoms, especially if the symptoms are refractory to medical therapy.

Secondary graft loss occurred frequently (6/12 with primary engraftment) and did not discriminate between phenotype, genotype, conditioning regimen, age, or donor source. The reasons behind the high rate of secondary graft loss are unclear, including what role heightened IFN-γ–induced STAT1 signaling plays in patients with GOF-*STAT1* mutations.

IFN-γ receptor deficiency and primary and secondary HLH are examples of primary immunodeficiency diseases in which heightened IFN-γ expression correlates with poor engraftment rates and worse outcome after transplantation.[31,32] Recently, the JAK inhibitor ruxolitinib[33] and anti–IFN-γ mAbs[34] have been effective at suppressing lFN-γ–induced inflammation in murine and human HLH, respectively. *In vitro* exposure of T lymphocytes from patients with GOF-*STAT1* mutations to ruxolitinib resulted in profound suppression of IFN-α and 1FN-β STAT1 phosphorylation,[12,14] and treatment of a patients with a GOF-*STAT1* mutation resulted in improvement of their individual symptoms.[12,13] Ruxolitinib was used as immunosuppressive therapy in 1 patient in this series (P13) for 2 weeks before transplantation. This patient was one of the 6 survivors and had full immune reconstitution with no secondary graft loss. With the exception of acute GvHD, he has no other posttransplantation complications. The success of this specific case might suggest that use of JAK inhibitors, as well as anti-IFN- γ therapies, might be an effective strategy in controlling IFN-γ–induced inflammation in patients with GOF-*STAT1* mutations in the setting of transplantation and could perhaps improve engraftment rate and overall outcome.

Our retrospective data demonstrate that HSCT can be curative for patients with GOF-*STAT1* mutations. With full immune reconstitution, disease manifestations are reversed quickly and permanently. Because those with a severe phenotype have a less favorable outcome, patients should be considered for transplantation earlier to prevent serious complications and improve OS. Without novel strategies to control HLH, HSCT in patients with active disease is likely to fail.

Extended Data

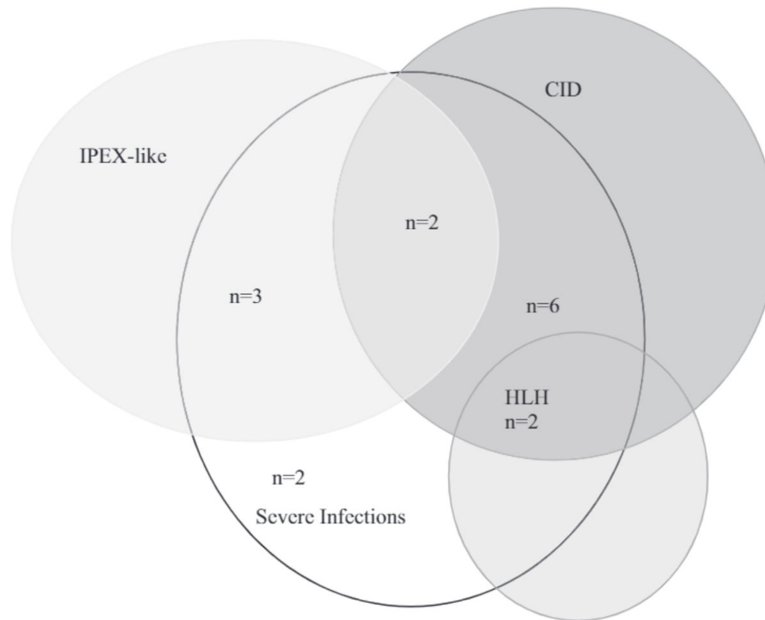

FIG E1.

Indication for HSCT in patients with GOF-*STAT1* mutations. All patients in this cohort had severe infections (n = 15). Indications for HSCT were IPEX-like symptoms (n = 3), CID (n = 6), CID with HLH (n = 2), IPEX-like symptoms and CID (n = 2), or only severe infections (n = 2).

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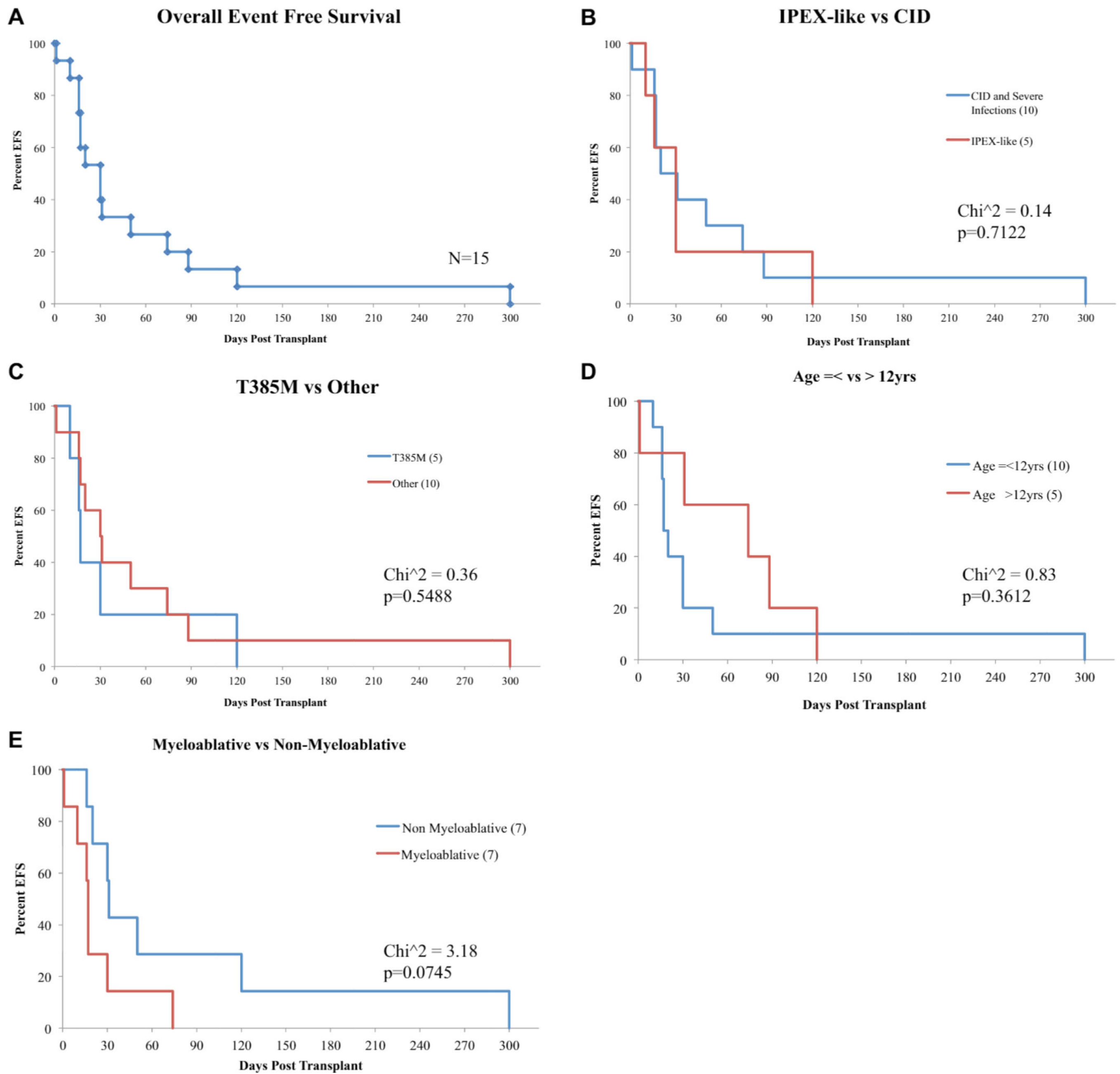

FIG E2.

Overall posttransplantation event-free survival (EFS). **A,** Overall posttransplantation EFS in patients with GOF-*STAT1* mutations. **B,** EFS analysis comparing patients with IPEX-like presentation versus patients with CID, significant infections, or both. **C,** EFS analysis comparing patients with T385M amino acid substitution versus patients with other GOF-*STAT1* mutations. **D,** EFS analysis comparing patients younger or older than 12 years. **E,** EFS analysis comparing patients receiving myeloablative versus nonmyeloablative conditioning regimen. In Fig E2, *B* and *D,* numbers in brackets represent the number of patients in each group.

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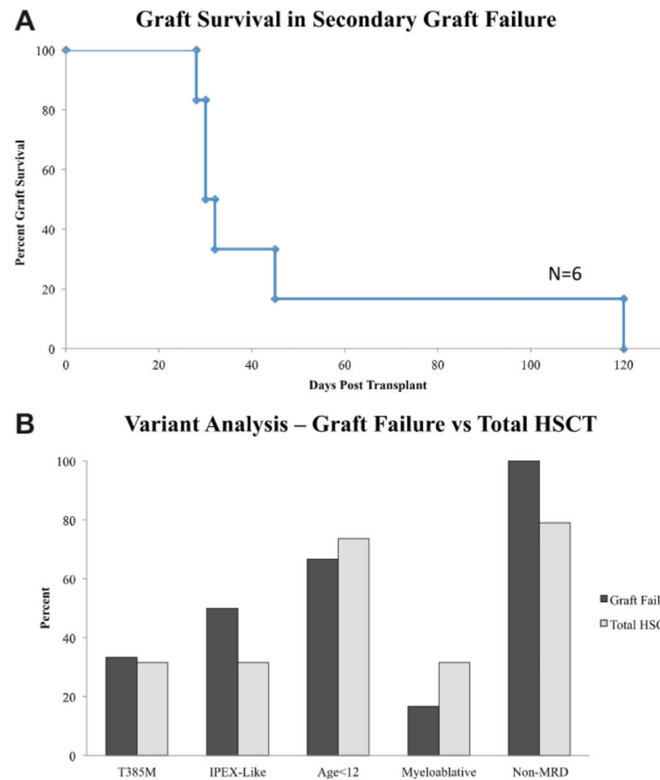

FIG E3.

Secondary graft failure data. A total of 19 HSCTs were performed in 15 patients with GOF-*STAT1* mutations, including 3 patients who underwent 2 HSCTs and 1 patient who underwent 3 HSCTs. A total of 6 events of secondary graft failure were reported, 2 of which occurred in the same patient (patient no. 9). **A,** Survival curve showing the timing of each of the 6 events. **B,** Incidence of different variants in the graft failure group compared with their incidence in all HSCTs performed does not show any statistical significance. Each of the following variants was analyzed: presence of T385M amino acid substitution, clinical phenotype of IPEX-like disease, age less than or equal to 12 years at transplantation, use of a myeloablative conditioning protocol, and use of any stem cell donor other than an MRD (T385M mutation: 33.3% vs 31.58%, $P = .94$; IPEX-like: 50% vs 31.58%, $P = .43$; age < 12 years: 66.67% vs 73.68%, $P = .7512$; myeloablative conditioning: 16.66% vs 31.58%, $P = .49$; non-MRD: 100% vs 78.95%, $P = .24$).

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TABLE E1.

Posttransplantation complications in patients with GOF-*STAT1* mutations

	Day after transplantation	Reversible	Fatal
Patient 1			
Infection			
EBV reactivation	+ 159	Yes	No
Recurrent pneumonia	+ 1464	Yes	No
Bleeding (coagulopathy associated with colitis)	+2765	Yes	No
CV			
Catheter-associated thrombus	+ 159	Yes	No
SVT	+ 159	Yes	No
GI			
Recurrence of colitis	+2756	Yes	No
Heme			
Lymphopenia	+2249	No	No
Hypogammaglobulinemia	+ 1611	No	No
Secondary graft loss	+ 30	No	No
Patient 2 (first transplantation)			
Infection			
S parasanguis bacteremia	−2	Yes	No
CMV viremia	+ 19	Yes	No
Secondary graft loss	+ 33	No	No
Patient 2 (second transplantation)			
Renal/GU			
Drug-associated nephropathy	+ 28	Yes	No
GvHD	+ 30	Yes	No
Patient 3			
GvHD	+ 16	Yes	No
Patient 4			
Heme			
Severe thrombocytopenia	+5	Yes (+490)	No

	Day after transplantation	Reversible	Fatal
Lymphopenia	+ 30	No	no
Hypogammaglobulinemia	+ 304	No	No
Secondary graft loss	+ 120	No	No
Death (sepsis, pneumonia)	+779	No	Yes
Patient 5			
Infection			
CMV	Before transplantation	No	+7
GvHD	+ 10	Yes	No
Death	+ 12		
Patient 6 (first transplantation)			
Infection			
Fungal pneumonia	+ 24	Yes	No
BK virus reactivation	+ 24	No	No
Patient 6 (second transplantation)			
Infection			
Adenovirus reactivation	+ 2	Yes	No
Cryptosporidium gut	+ 20	Yes	No
Fungal pneumonia	+ 74	No	Yes
GvHD	+ 17	Yes	No
Death	+4 mo	No	Yes
Patient 7: Turkey			
Infection			
Mycotic aneurysm	Before transplantation	No	Yes
Bleeding	+ 88	No	Yes
Death	+3 mo		
Patient 8			
CV			
Cardiomyopathy	+ 1	No	Yes
Death	+3		
Patient 9 (first transplantation)			
Infection			

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	Day after transplantation	Reversible	Fatal
S mitis sepsis	+ 22	Yes	No
Hemophagocytosis	+25 (first)	No	No
Secondary graft loss	+45	No	Yes
Patient 9 (second transplantation)			
Infection	+ 14	No	Yes
Adenovirus viremia	+35		
CV			
Cardiac/pleural effusion	+ 21	No	Yes
GI			
Ascites	+21	No	Yes
Renal/GU			
Adenovirus cystitis	+29	No	No
Secondary graft loss	+32	No	Yes
Patient 9 (third transplantation)			
Bleeding	+ 17	No	Yes
GI			
Pancreatitis	+ 13	Yes	No
Death	+ 109	No	Yes
Patient 10: Japan			
CV			
TMA	+20	Yes	No
Lung			
Acute pulmonary edema	+ 4	Yes	No
GI			
Recurrent pancreatitis	−1, +32, +42, +53, +58, +63	Yes	No
Patient 11			
Infection			
Pulmonary aspergillosis	+ 150	Yes	No
GI			
Hemorrhagic bleeding	+30	Yes	No
Renal/GU			

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	Day after transplantation	Reversible	Fatal
Hemorrhagic cystitis	+ 18, +120	Yes	No
Heme			
Lymphopenia	+50	Yes	No
Hypogammaglobulinemia	+50		
GvHD			
Skin	+ 7	Yes	No
GI	+ 17	Yes	No
Patient 12: Canada			
Lung			
Pulmonary hemorrhage	+20	No	Yes
GI			
GI bleeding	+ 13	Yes	No
Renal/GU			
Renal failure	+21	No	Yes
Other			
TEN	+ 16	No	Yes
GvHD	+ 12	No	No
Death	+42	No	Yes
Patient 13: United Kingdom			
GvHD	+50	Yes	No
Patient 14			
Infection			
CMV	+31	Yes	No
Candidiasis	+72	Yes	No
Sepsis	+214	Yes	No
Secondary graft loss	+ 28, +90	No	No
Death (sepsis)	+410	No	Yes
Patient 15			
Infection			
Pneumonia	+52, +300	Yes, no	No, yes

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	Day after transplantation	Reversible	Fatal
GI			
Increased transaminase levels	+ 150	No	No
Heme			
Severe thrombocytopenia	+ 150	No	No
Secondary graft loss	+ 130	No	No
Death	+10 mo	No	Yes

CV, Cardiovascular; *CVM.* cytomegalovirus; *GI,* gastrointestinal; *GU,* genitourinary; *SVT,* supraventricular tachycardia; *TEN,* toxic epidermal necrolysis; *TMA,* thrombotic microangiopathy.

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Acknowledgments

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Abbreviations used

CID	Combined immunodeficiency
CMC	Chronic mucocutaneous candidiasis
EFS	Event-free survival
GAS	γ-Activated sequence
GOF	Gain of function
HLH	Hemophagocytic lymphohistiocytosis
HSCT	Hematopoietic stem cell transplantation
IPEX	Immune dysregulation–polyendocrinopathy–enteropathy–X–linked
JAK	Janus kinase
KREC	Kappa-deleting recombination excision circle
MMUD	Mismatched unrelated donor
MRD	Matched related donor
MUD	Matched unrelated donor
NK	Natural killer
OS	Overall survival
PBSC	Peripheral blood stem cell
R1C	Reduced-intensity conditioning
STAT	Signal transducer and activator of transcription
TREC	T-cell receptor excision circle
UCB	Unrelated umbilical cord blood
WT	Wild-type

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Clinical implications:

HSCT in patients with GOF-*STAT1* mutations can be curative but might be associated with complications that can lead to secondary graft failure and decreased survival.

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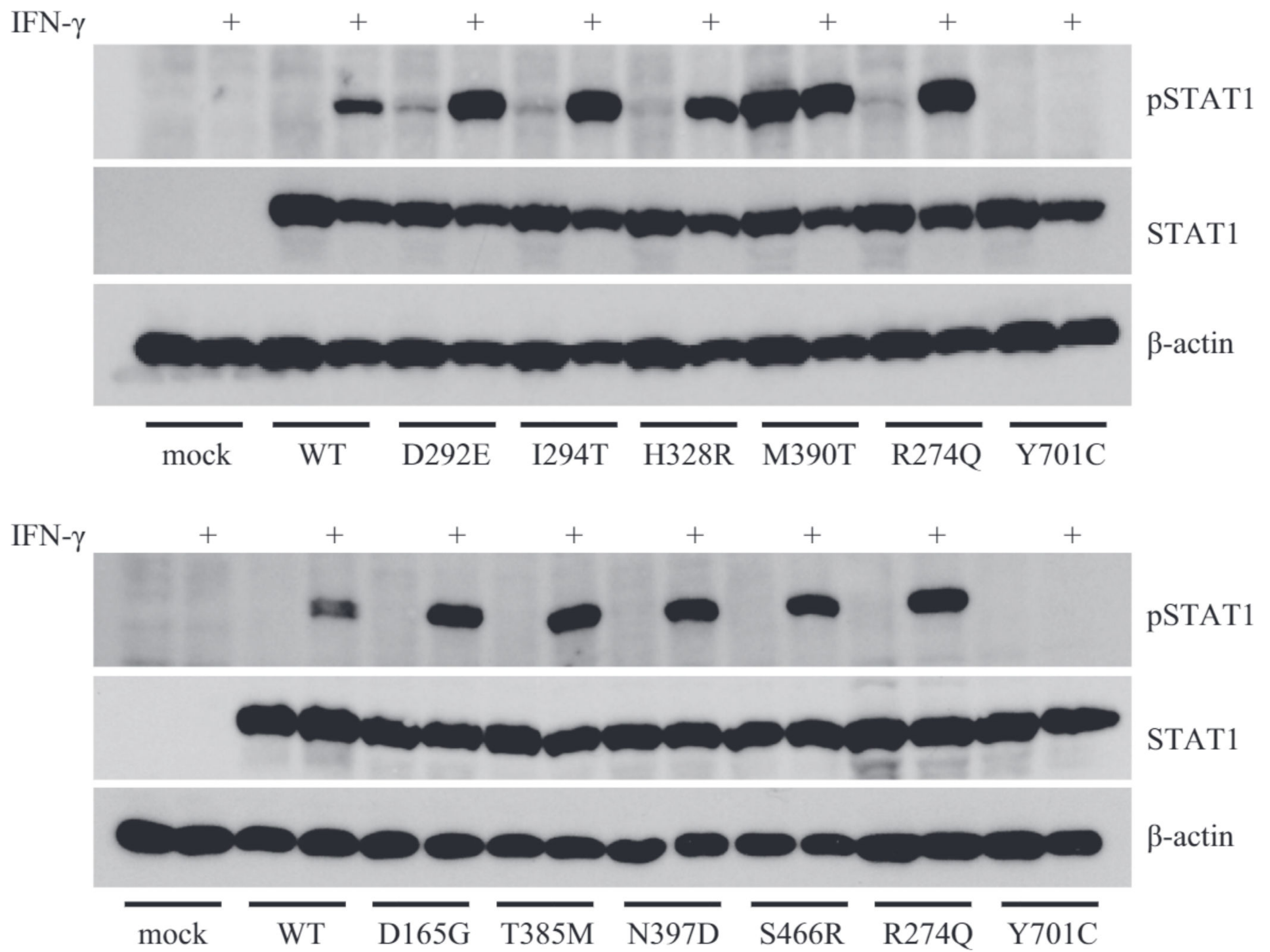

FIG 1.

Evaluation of expression and phosphorylation of GOF-STAT1 mutants by using immunobiotting. GOF-STAT1 mutations led to enhanced phosphorylated STAT1 *(pSTAT1)* expression after stimulation with IFN-γ. U3C cells were transfected with STAT1 mutants or WT and stimulated with IFN-γ for 20 minutes. Western blotting was performed with anti-phosphorylated STAT1, anti-STAT1, and anti-β-actin antibodies. Mutations affecting Y^{701} prevent STAT1 phosphorylation.

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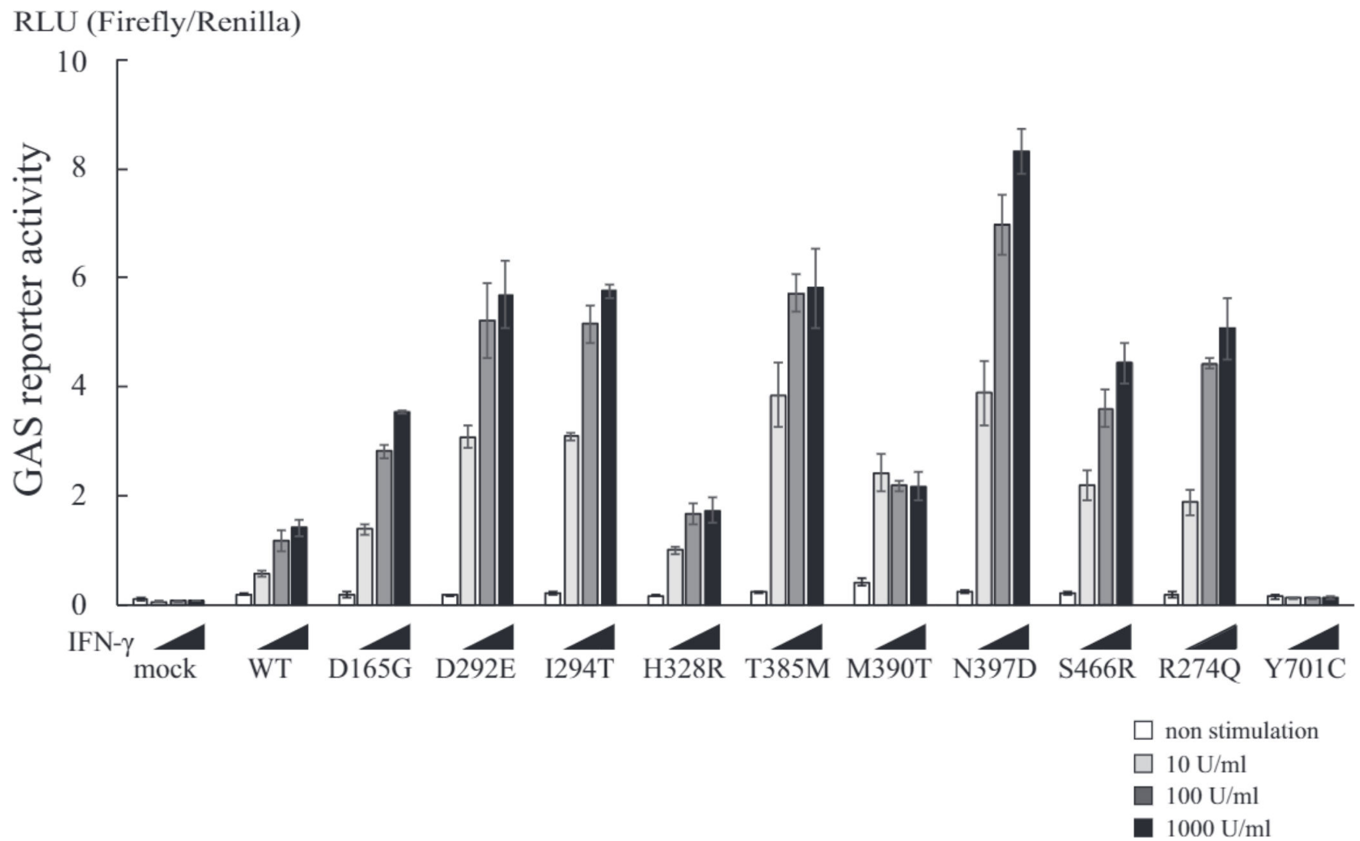

FIG 2.

Luciferase reporter assay to evaluate transcriptional activation by GOF-STAT1 mutants. STAT1 mutants led to enhanced luciferase GAS-induced activity. Transcriptional responses to increasing concentrations of IFN-γ (*white bar,* nonstimulated; *light gray bar,* 10 U/mL; *dark gray bar,* 100 U/mL; *black bar,* 1000 U/mL) were added to U3C cells transfected with STAT1 mutants or WT STAT1 and cultured for 16 hours before GAS-induced activity was measured. Experiments were performed in triplicate, and data are expressed in relative luciferase units *(RLU).* Individual reporter assays were performed 3 times to confirm reproducibility. Y^{701} mutation eliminated transcriptional activity of STAT1.

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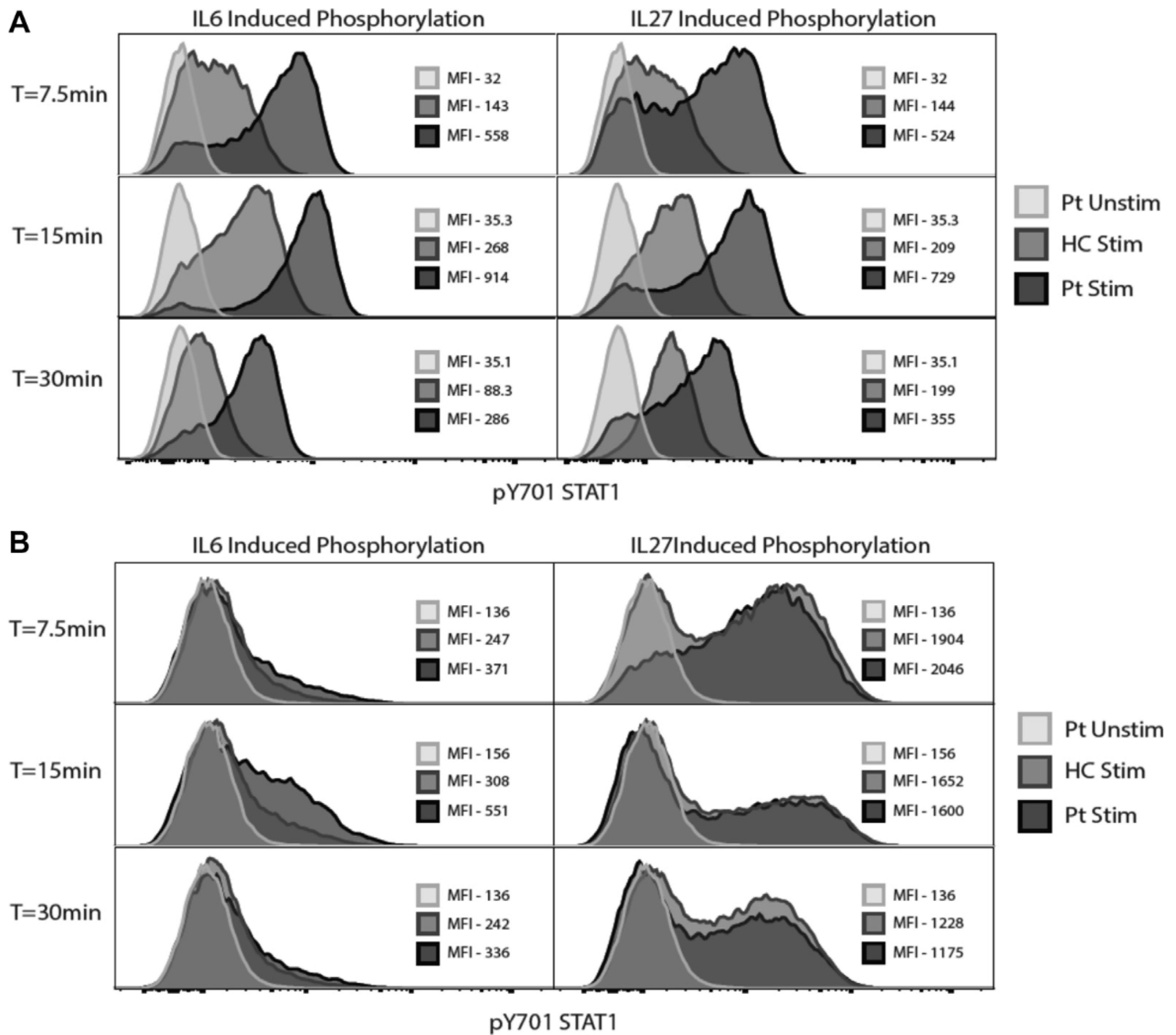

FIG 3.

Cytokine-induced STAT1 phosphorylation. A, Pre-HSCT PBMCs from patient 1 (H328R) led to hyperphosphorylation and delayed dephosphorylation after IL-6 and IL-27 stimulation. B, Post-HSCT PBMCs from patient 2 IT385M) show normal phosphorylation kinetics compared with healthy control subjects *(HC)*.

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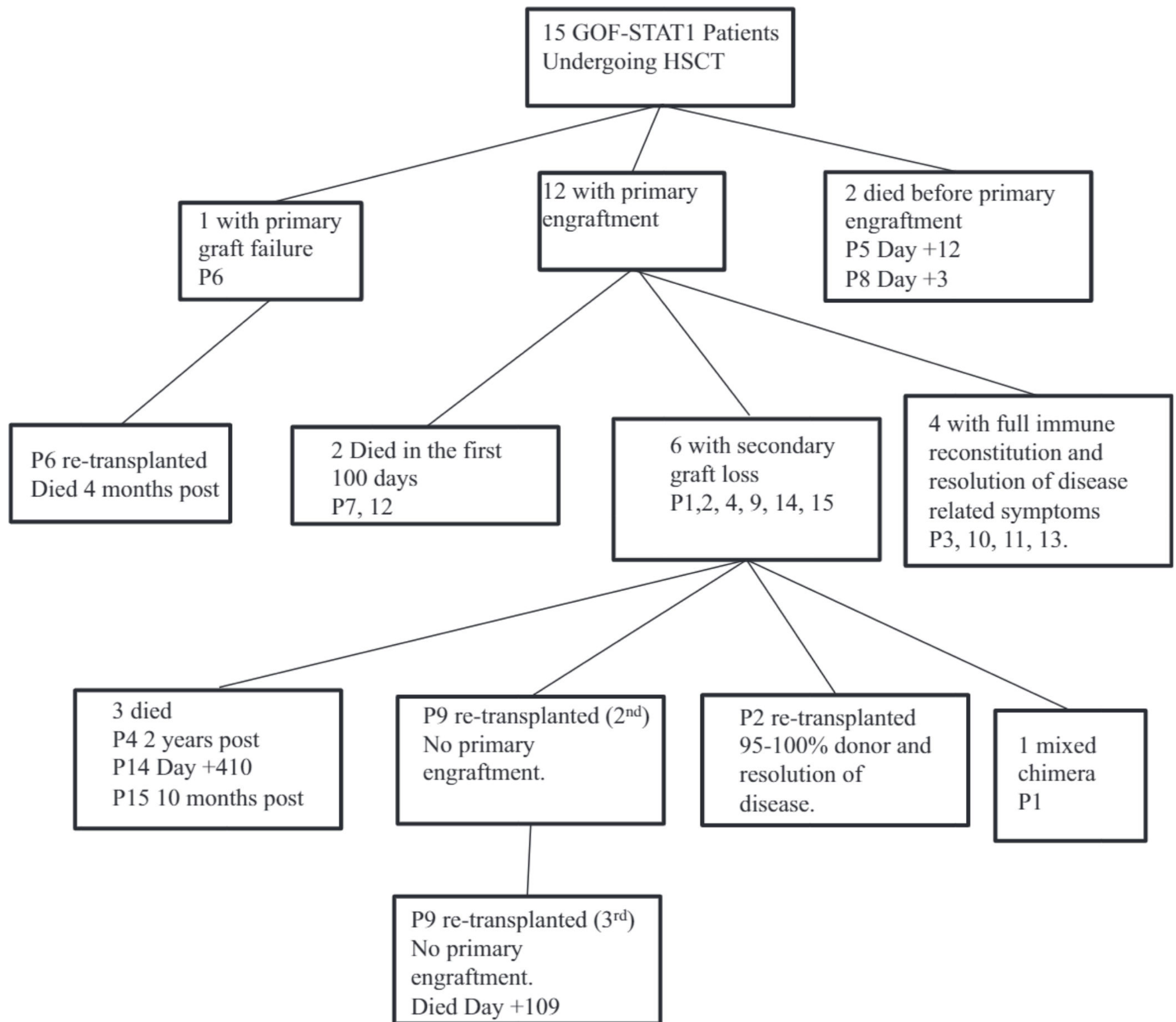

FIG 4.

Outcome of patients with GOF-*STAT1* mutations after HSCT. Fifteen patients with GOF-*STAT1* mutations underwent HSCT. Primary engraftment occurred in 12. Six had secondary graft loss. Six are alive, and 5 have full immune reconstitution and reversal of disease manifestations.

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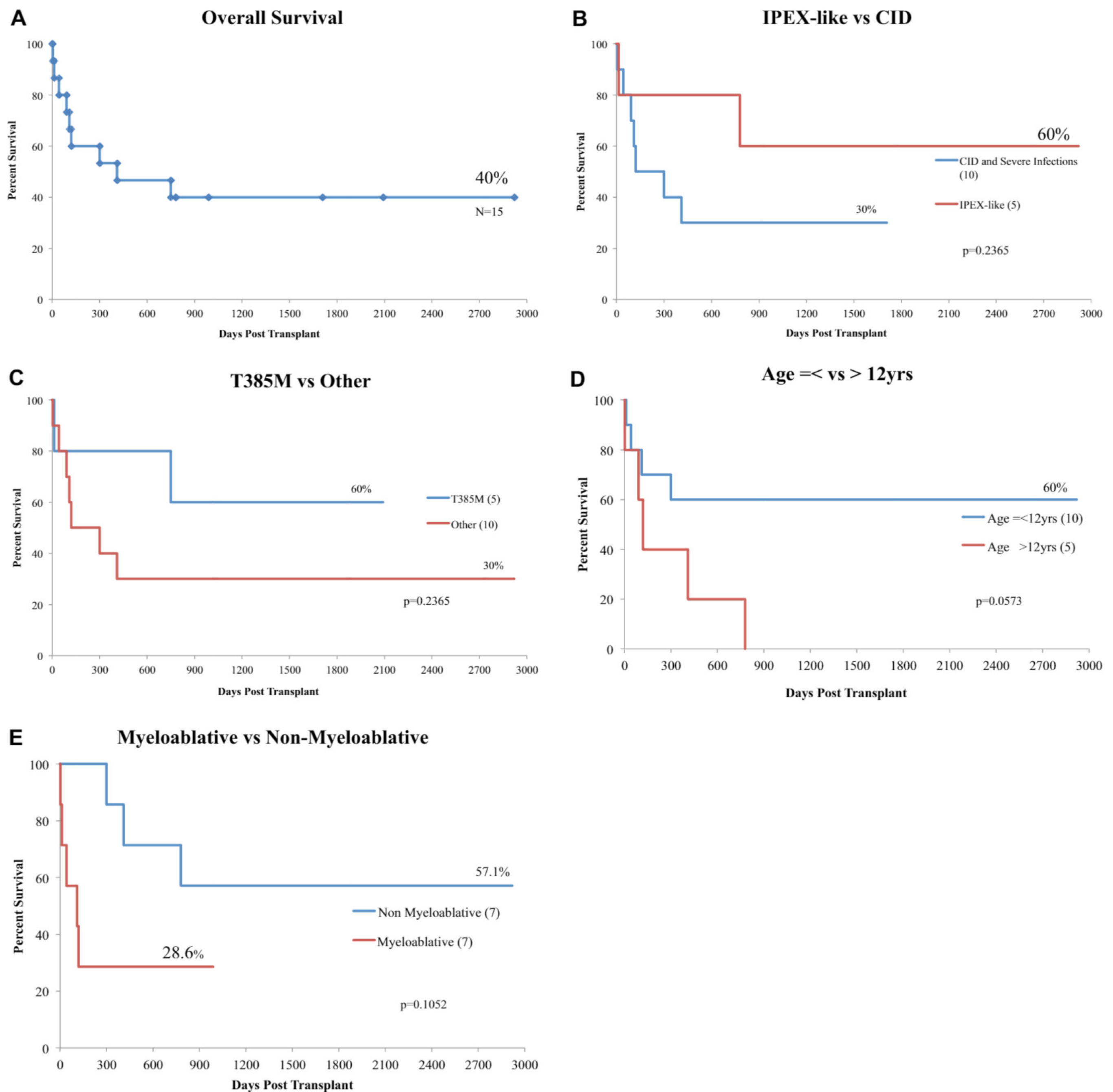

FIG 5.

Overall posttransplantation survival and survival analysis. **A,** Overall posttransplantation survival in patients with GOF-*STAT1* mutations. **B,** Survival analysis comparing patients with an IPEX-like presentation versus patients with CID, significant infections, or both. **C,** Survival analysis comparing patients with T385M amino acid substitution versus patients with other GOF-*SLAT1* mutations. **D,** Survival analysis comparing patients younger or older than 12 years of age. **E,** Survival analysis comparing myeloablative and nonmyeloablative protocols. In Fig 5, *B-E,* numbers in brackets represent the number of patients in each group.

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In patients undergoing more than 1 HSCT, survival is calculated since the last transplantation performed.

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TABLE I.

Clinical phenotype of GOF-*STAT1* mutations

No. and country of origin	Sex	cDNA mutation protein change domain	Mutation analysis done before/after transplantation	Phenotype	Pretransplantation complications			
					Infections	Autoimmunity	Growth	Other
1. United States	Male	c. 983 A>G p.H328R CC	Pre-HSCT Sanger sequencing	IPEX-like	Norovirus enteritis VRE and *Pseudomonas* abscess *Clostridium difficile* enteritis *Mycobacterium fortuitum* mediastinal lymphadenitis	Enteropathy Type 1 diabetes Thyroiditis	FIT	—
2. Russia	Male	c. 1154C>T p. T385M DB	Pre-HSCT Sanger sequencing	IPEX-like	CMC Pulmonary aspergillosis Recurrent pneumonia BCG lymphadenitis	Enteropathy Autoimmune neutropenia Thyroiditis	FTT	Eczema
3. United States	Male	c. 1154C>T p. T385M DB	Pre-HSCT Whole-genome sequencing	IPEX-like	CMC	Enteropathy Thyroiditis Growth hormone deficiency + anti-GAD antibody – hyperglycemia with concurrent steroid use	FTT	—
4. Spain	Male	c. 1154C>T p. T385M DB	Pre-HSCT Sanger sequencing	IPEX-like CID	CMC Cryptococcal meningitis Pneumonia Pulmonary tuberculosis	AIHA Type 1 diabetes IBD Glomerulonephritis Autoimmune hepatitis	Normal	—
5. Canada	Female	c. 1154C>T p. T385M DB	Post-HSCT Sanger sequencing	IPEX-like CID	VZV Systemic CMV Pneumonia	Type 1 diabetes IBD Thyroiditis	FTT	—
6. The Netherlands	Male	c. 494A>G p.D165G CC	Pre-HSCT Sanger sequencing	CID	Pulmonary aspergillosis MCV	Autoimmune hepatitis Thyroiditis	FTT	—
7. Turkey	Male	c.820G>A p.R274W CC	Pre-HSCT Sanger sequencing	CID	CMC Orf cutaneous infection Mycotic cerebral aneurysms Pulmonary CMV Pneumonia (*Haemophilus influenzae, Pseudomonas aeruginosa, Streptococcus pneumoniae*)	AIHA Thyroiditis Autoimmune hepatitis Pernicious anemia Anti-phospholipid syndrome	Normal	—
8. Japan	Female	c. 821 G>A p.R274Q CC	Pre-HSCT Sanger sequencing	CID	CMC Pulmonary aspergillosis VZV Pneumonia (*H influenzae, S pneumoniae*)	None	FTT	Severe gastroesophageal reflux
9. Japan	Male	c.876C>A p.D292E CC	Pre-HSCT Sanger sequencing	CID HLH	CMC Parvovirus B19 Recurrent sinusitis (*H influenza, S pneumoniae*)	Vitiligo Pure red cell aplasia *	FTT	Gray hair depigmentation

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No. and country of origin	Sex	cDNA mutation protein change domain	Mutation analysis done before/after transplantation	Phenotype	Pretransplantation complications			
					Infections	Autoimmunity	Growth	Other
10. Japan	Female	c.881T>C p.I294T CC	Post-HSCT Whole-exome sequencing	CID	CMC MCV VZV CMV Norovirus BK virus JC viremia Chronic sinusitis, otitis media, tonsillitis, bronchitis, pneumonia Bronchiectasis Cutaneous abscess	AIHA Autoimmune Neutropenia[†] Autoimmune thrombocytopenia Colitis	Normal	Malrotated kidney and pancreas divisum laryngeal edema chronic liver dysfunction
11.Canada	Male	c. 1154C>T p.T385M DB	Pre-HSCT Whole-exome sequencing	CID	CMC Pneumonia Otitis media	None	Short stature	—
12. Canada	Female	c. 1189 A>G P.N397D DB	Post-HSCT Sanger sequencing	CID HLH	CMC EBV with microabscessed spleen and kidneys Cutaneous HSV Pneumonia	None	Normal	—
13. United Kingdom	Female	c.1398 C>G p.S466R DB	Pre-HSCT Sanger sequencing	CID	CMC Bronchiectasis EBV and Adenovirus pneumonia and colitis VZV HHV-6 CMV Pyelonephritis Blepharitis Paronychia	None	FTT	—
14. Japan	Male	c. 1169 T>C P.M390T DB	Post-HSCT Sanger sequencing	Severe infections	CMC HSV VZV Chronic EBV Recurrent otitis media *(Staphylococcus aureus)* Recurrent pneumonia (*S aureus, S pneumoniae*) Sepsis (*S aureus*) Urinary tract infection	Hypothyroidism	FTT	—
15. Peru	Female	c. 1189 A>G p.N397D DB	Pre-HSCT Sanger sequencing	Severe infections	CMC CMV Severe diarrhea (*Escherichia coli, Klebsiella pneumoniae*) Sepsis	None	FTT	—

AIHA, Autoimmune hemolytic anemia; *CC*, coiled-coil; *CMV*, cytomegalovirus; *DB*, DNA binding; *FTT*, failure to thrive; *GAD*, glutamic acid decarboxylase; *HHV-6*, human herpes virus 6; *HSV*, herpes simplex virus; *IBD*, inflammatory bowel disease; *MCV*, molluscum contagiosum virus; *VRE*, vancomycin-resistant *Enterococcus*; *VZV*, varicella zoster virus.

*Pure red cell aplasia likely secondary to parvovirus.

†No anti-neutrophil antibody detected.

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TABLE II.

Transplantation course and outcome of patients with GOF-*STAT1* mutations

Patient no.	Phenotype	Age at transplantation	Donor HLA match	CD34 dose	Conditioning
1	IPEX-like	4 y	MUD, 10/10 match	8×10^6/kg	Fludarabine, 150 mg/m^2, days −8 to −4 Melphalan, 140 mg/m^2, day −3 Almetuzumab, 10, 15, or 20, days −21 to −19
2	IPEX-like	(1) 6 y	(1) T cell α/β/CD19$^+$ depleted matched unrelated PBSCs, 10/10 match	(1) 11.21×10^6/kg	(1) Treosulfan, 42 mg/m^2, days −5 to −3 Fludarabine, 150 mg/m^2, days −6 to −2 ATG, 5 mg/kg, days −5 to −4 Rituximab, 375 mg/m^2, day −1
		(2) 6 y	(2) T cell α/β/CD 19$^+$ depleted haploidentical PBSCs	(2) 14.56×10^6/kg	(2) TBI, 6 gray Fludarabine, 30 mg/m^2, days −6 to −2 ATG, 2.5 mg/kg, days −5 and −4 Cyclophosphamide, 60 mg/kg, days −3 and −2 Melphalan, 140 mg/m^2, day − 1 Rituximab, 375 mg/m^2, day − 1
3	IPEX-like	12 y	MUD, 10/10 match	4.58×10^6/kg	Fludarabine, 150 mg/m^2, days −8 to −4 Melphalan, 140 mg/m^2, day −3 Almetuzumab, 10, 15, 20 mg; days −21 to −19
4	IPEX-like CID	29 y	MUD PBSCs, 10/10 match	3.33×10^6/kg	Fludarabine, 50 mg/d, days −6 to −3 Melphalan, 140 mg/m^2/d, day −3 to −2 Alemtuzumab, 12 mg/d, days −10 to −6
5	IPEX-like CID	7 y	MUD, 6/6 match	6.5×10^6/kg	Cyclophosphamide, 50 mg/kg/d, days −5 to −2 Busulfan, 4 mg/kg/d, days −9 to −6
6	CID	(1) 17 y	(1) UCB, 5/6 matched	(1) 0.14×10^6/kg	(1) Busulfan (myeloablative TDM AUC 90 mg*h*I) Fludarabine, 160 mg/m^2 ATG, 10 mg/kg
		(2) 17 y	(2) UCB, 6/6 matched	(2) 0.14×10^6/kg	(2) Treosulfan, 42 g/m^2 Fludarabine, 160 mg/m^2 TBI, 2 × 2 Gy
7	CID	33 y	MRD, 6/6 matched	3.84×10^6/kg	None
8	CID	18 y	MRD, 8/8 matched	8.14×10^6/kg	Busulfan, 4 mg/kg/d, days −9 to −6 Cyclophosphamide, 50 mg/kg/d, days −5 to −2
9	CID HLH	(1) 8 y (2) 8 y (3) 8 y	(1) MMUD, 6/8 matched (2) UCB, 7/8 matched (3) Haploidentical marrow, 5/8 matched	(1) 2.3×10^6/kg (2) 0.77×10^5/kg (3) 2.09×10^6/kg	(1) Fludarabine, 180 mg/m^2, days −9 to −6 Busulfan, 16 mg/kg, days −5 to −2 rATG, 8 mg/kg, days −9 to −6 (2) Fludarabine, 90 mg/m^2, days −4 to −2 Cyclophosphamide, 1 g/m^2, day −2 Etoposide, 100 mg/m^2, day −3 TB1, 4 Gy; day −5 (3) No conditioning
10	CID	12 y	UCB, 5/8 matched	0.126×10^6/kg	Fludarabine, 180 mg/m^2, days −7 to −2 Busulfan, 7.6 mg/kg, days −3 to −2 ATG, 8 mg/kg, days −9 to −6

Patient no.	Phenotype	Age at transplantation	Donor HLA match	CD34 dose	Conditioning
11	CID	7 y	MRD, HLA identical	5.7×10^8/kg nucleated cells	Busulfan, 16 mg/kg Cyclophosphamide, 200 mg/kg
12	CID HLH	10 y	MUD, 10/10 matched	6.32×10^6/kg	Busulfan, 3.6 mg/kg, days −8 to −5 Etoposide, 30 mg/kg/dose, day −4 Cyclophosphamide, 60 mg/kg, days −3 to −2
13	CID	7.5 y	MUD PBSCs	31.2×10^6/kg	Treosulfan, 14 mg/m^2/d, days −7 to −5 Fludarabine, 30 mg/m^2/d, days −6 to −2 Alemtuzumab, 0.2 mg/kg/d, days −8 to −4 Ruxolitinib, 2-wk course (5 mg/d); stopped day −9
14	Severe infections	29 y	MUD, 6/6 matched	3×10^6/kg nucleated cells	Cyclophosphamide, 200 mg/kg, days −10 to −7 Fludarabine, 125 mg/m^2, days −6 to −2 ATG, 25 mg/kg, days −6 to −2 TBI, 3 Gy, day −7
15	Severe infections	13 mo	MRD 6/6, matched	6.56×10^6/kg	Fludarabine, 30 mg/m^2/d, days −8 to −3 Melphalan, 140 mg/m^2/d, day −3 ATG, 5 mg/kg/d, days −3 to −2
Cyclosporine MMF	D+12	EBV reactivation catheter-associated venous thrombus Supraventricular tachycardia	None	Secondary graft loss within 30 d Mixed chimera with 2% donor myeloid cells and 39% donor lymphoid cells Recurrence of *Clostridium difficile* colitis Recurrent sinusitis and pneumonia (bacterial and viral) Abnormal pulmonary diffusion capacity Progressive lymphopenia Hypogammaglobulinemia On IVIG	Alive
(1) Tacrolimus	(1) Day +26 (lymphocytes)	(1) *Streptococcus parasanguis* bacteremia	(1) None	(1) Secondary graft loss in first 30 d	Alive
(2) Tacrolimus	(2) Day +35 (lymphocytes)	CMV viremia (2) Drug-associated nephropathy	(2) Grade I skin	(2) Full immune reconstitution Alive 100% donor Enteropathy resolved Improved growth	
Tacrolimus MMF Methotrexate	D+16	None	Grade III skin	Full immune reconstitution Alive 100% donor Enteropathy resolved Improved growth	Alive
Sirolimus Tacrolimus	D+23	Severe thrombocytopenia Reaction to almetuzumab Pneumonia	Grade I skin	Secondary graft loss over 2 mo Continued lymphopenia Continued hypogammaglobulinemia Continued infections	Dead 2 y after pneumonia and sepsis
Prednisone Cyclosporine	No engraftment	CMV	Grade I skin	Died D+12	Died Multiorgan failure

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Patient no.	Phenotype	Age at transplantation	Donor HLA match	CD34 dose	Conditioning
(1) Cyclosporine Prednisone	(1) Primary graft failure	(1) Fungal Pneumonia	(2) Grade III gut	(1) Primary graft failure	Died
(2) Cyclosporine MMF	(2) D+15	(2) BK virus reactivation Adenovirus reactivation Cryptosporidium *gut* Pneumonia		(2) Full immune reconstitution	4 mo after pneumonia
None	D+25	Severe thrombocytopenia	None	Primary engraftment but not immune reconstituted	Died 3 mo after bleeding from mycotic aneurysms
Tacrolimus Methotrexate	No engraftment	Cardiomyopathy and heart failure secondary to cyclophosphamide	None		Died D+3 from heart failure
(1) Tacrolimus Short methotrexate (2) Tacrolimus Short methotrexate (3) Cyclosporine Dexamethasone	(1) D+19 (2) None (3) None	Refractory HLH *Streptococcus mitis* sepsis Cardiac effusion Ascites Pancreatitis Adenovirus viremia and cystitis	None	(1) Secondary graft loss in first 30 d (2) Refractory HLH (3) Refractory HLH	Died D+109 from multiorgan failure
Tacrolimus Methotrexate Prednisolone	D+33	Acute pulmonary edema TMA Recurrent pancreatitis	None	Full immune reconstitution 100% Donor	Alive
Cyclosporine Methylprednisolone	D+12	Hemorrhagic ulcers with massive GI bleeding Hemorrhagic cystitis Pulmonary aspergillosis	Acute GvHD skin and GI tract	Full immune reconstitution 95% Donor in myeloid and lymphoid lines	Alive
Prednisone Cyclosporine Methotrexate Almetuzumab used as salvage therapy after HSCT	D+16	Refractory HLH GI bleeding Pulmonary hemorrhage Toxic epidermal necrosis Renal failure	Grade II skin	Refractory HLH	Died D+42 from multiorgan failure
Cyclosporine MMF	D+16	None	Grade II skin	Full immune reconstitution 100% Donor	Alive
Tacrolimus Methotrexate	D+17	CMV Candidiasis Sepsis	None	Secondary graft loss in first 90 d	Died D+410 from sepsis
Cyclosporine Methotrexate	D+15	Severe thrombocytopenia Increased transaminase levels	None	Secondary graft loss within first 100 d	Died 10 mo after from fulminant lung infection

ATG, Antithymocyte globulin; *AUC*, area under the blood concentration time curve; *CMV*, cytomegalovirus; *GvHD*, graft-versus-host disease; *MMF*, mycophenolate mofetil; *IVIG*, intravenous immunoglobulin; *rATG*, rabbit anti-thymocyte globulin; *TMA*, thrombotic microangiopathy; *GI*, gastrointestinal; *TBI*, total body irradiation; *TDM*, therapeutic drug monitoring; *UCB*, unrelated cord blood.