Title

Unrelated Hematopoietic Cell Transplantation in a Patient with Combined Immunodeficiency with Granulomatous Disease and Autoimmunity Secondary to RAG Deficiency

Journal

Journal of Clinical Immunology, 36(7)

ISSN

0271-9142

Authors

John, Tami
Walter, Jolan E
Schuetz, Catherina
et al.

Publication Date

2016-10-01

DOI

10.1007/s10875-016-0326-x

Peer reviewed

HHS Public Access

Author manuscript

J Clin Immunol. Author manuscript; available in PMC 2017 February 01.

Published in final edited form as:

J Clin Immunol. 2016 October ; 36(7): 725–732. doi:10.1007/s10875-016-0326-x.

Unrelated Hematopoietic Cell Transplantation in a Patient with Combined Immunodeficiency with Granulomatous Disease and Autoimmunity Secondary to RAG Deficiency

Tami John[1], **Jolan E. Walter**[2], **Catherina Schuetz**[3], **Karin Chen**[4], **Roshini S. Abraham**[5], **Carmem Bonfim**[6], **Thomas G. Boyce**[7], **Avni Y. Joshi**[5], **Elizabeth Kang**[8], **Beatriz Tavares Costa Carvalho**[9], **Arash Mahajerin**[10], **Diane Nugent**[10], **Geetha Puthenveetil**[10], **Amit Soni**[10], **Helen Su**[8], **Morton J. Cowan**[11], **Luigi Notarangelo**[12], and **David Buchbinder**[10]

[1]Division of Hematology/Oncology, CHOC Children's Hospital, 1201 W. La Veta Avenue, Orange, CA 92868, USA

[2]Division of Immunology, MassGeneral Hospital for Children, 55 Fruit Street, Boston, MA 02114, USA

[3]Department of Pediatrics and Adolescent Medicine, University Medical Center Ulm, Ulm, Germany

[4]Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, University of Utah School of Medicine, 81 Mario Capecchi Drive, Salt Lake City, UT, USA

[5]Allergy and Immunology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

[6]Bone Marrow Transplantation Unit, Federal University of Paraná, Rua XV de Novembro, 1299 – Centro, Curitiba, PR 80060-000, Brazil

[7]Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

[8]NIAID, National Institutes of Health, Building 10CRC, Room 5-3940, 10 Center Drive, MSC 1456, Bethesda, MD 20892-9806, USA

[9]Disciplina de Alergia, Imunologia Clínica e Reumatologia –UNIFESP, Sao Paulo, Brazil

[10]Division of Hematology, CHOC Children's Hospital, 1201 W. La Veta Avenue, Orange, CA 92868, USA

[11]Department of Pediatrics, University of California, San Francisco, Box 1278, UCSF, San Francisco, CA 94143, USA

[12]Division of Immunology, Children's Hospital Boston, Karp Building, Room 10217, 1 Blackfan Circle, Boston, MA 02115, USA

Abstract

Correspondence to: Tami John.

Compliance with Ethical Standards: Written consent was obtained from the patient for participation in an NIAID IRB-approved research protocol. Written consent was obtained from the patient for participation in CHOC Children's Hospital IRB-approved research protocols.

Conflict of Interest: The authors declare that they have no competing interests.

The use of HLA-identical hematopoietic stem cell transplantation (HSCT) demonstrates overall survival rates greater than 75 % for T-B-NK+ severe combined immunodeficiency secondary to pathogenic mutation of recombinase activating genes 1 and 2 (RAG1/2). Limited data exist regarding the use of HSCT in patients with hypomorphic RAG variants marked by greater preservation of RAG activity and associated phenotypes such as granulomatous disease in combination with autoimmunity. We describe a 17-year-old with combined immunodeficiency and immune dysregulation characterized by granulomatous lung disease and autoimmunity secondary to compound heterozygous *RAG* mutations. A myeloablative reduced toxicity HSCTwas completed using an unrelated bone marrow donor. With the increasing cases of immune dysregulation being discovered with hypomorphic *RAG* variants, the use of HSCT may advance to the forefront of treatment. This case serves to discuss indications of HSCT, approaches to preparative therapy, and the potential complications in this growing cohort of patients with immune dysregulation and RAG deficiency.

Keywords

RAG deficiency; primary immunodeficiency; immune dysregulation; autoimmunity; bone marrow transplantation

Introduction

Based on recent reports of newborn screening, recombinase activating genes 1 and 2 (RAG1/2) are the second most prevalent genes associated with severe combined immunodeficiency (SCID) with a predicted prevalence of 1 in 330,000 (in the USA) and the most common genes associated with leaky SCID [1]. Curative treatment with hematopoietic stem cell transplantation (HSCT) is the standard of care in this otherwise fatal disease. HSCT using an HLA-identical donor for recombinase-related disorders including T-B-NK+ SCID demonstrates excellent overall survival of greater than 75 % [2, 3]. Next-generation sequencing has supported the expansion of the phenotypic heterogeneity of RAG deficiency. Hypomorphic RAG gene variants with partially preserved RAG activity are associated with a spectrum of immunologic and clinical phenotypes ranging from Omenn syndrome to leaky SCID with or without expansion of T cell receptor γδ+ T cells, CD4 T cell lymphopenia, and combined immunodeficiency with granulomatous disease and/or autoimmunity (CID-G/AI) [4–6]. In the era of whole-exome sequencing, RAG deficiency is now being associated with previously unexpected phenotypes, such as agammaglobulinemia, IgA deficiency, selective antibody deficiency, and even chronic recurrent osteomyelitis, with variable outcomes ranging from mild to fatal disease [7, 8].

In these newly discovered phenotypes with more preserved overall RAG activity (>10 %), the clinical outcome may not be predicted with confidence, as genotype correlation is less robust and wide intrafamilial variability has been reported [9, 10]. The uncertainty of predicting clinical outcomes based on the genetic mutation alone hinders proper timing of definitive treatment, such as HSCT (Table 1). In fact, very limited data exist with respect to the tailoring of HSCT in patients with RAG deficiency characterized by a CID-G/AI phenotype and beyond (Table 2) [4, 6, 9, 11–14]. Many of these recently discovered RAG-

deficient patients pose a clinical challenge to transplanting physicians secondary to unique immunological profiles (e.g., greater numbers of T cells) and pre-HSCT morbidity (e.g., age, chronic infections, signs of immune dysregulation). We discuss the management of a 17-year-old patient with a CID-G/AI phenotype in association with compound heterozygous *RAG1* gene mutations who underwent an unrelated bone marrow transplant using a myeloablative reduced toxicity conditioning regimen [5]. This case report and review of current literature serves to discuss the clinical decision-making that leads to HSCT among patients with CID-G/AI phenotype, tailoring of the preparative therapy, as well as to highlight the spectrum of post-HSCT complications that may be expected in this unique cohort of patients.

Methods

We have previously reported the case of a patient who was diagnosed with common variable immunodeficiency. Using whole-exome sequencing, an ultimate genetic diagnosis of combined immunodeficiency with compound heterozygous *RAG1* gene mutations c. 256_257delAA; p.K86VfsX33 and c.1835A>G; and p.H612R was documented [5]. By 18 years of age, she had suffered from recurrent sinopulmonary disease (*Penicillium*, *Corynebacterium propinquum*, *Pseudomonas aeruginosa*), autoimmunity (cytopenias, vitiligo, duodenitis), and exacerbations of granulomatous lymphocytic interstitial lung disease (GLILD) despite a variety of immunomodulatory therapies including immunoglobulin replacement, corticosteroids, and anti-tumor necrosis factor alpha agents (infliximab, adalimumab), sirolimus, and abatacept. Thus, she was evaluated for consideration for unrelated donor HSCT.

The HSCT preparative regimen included alemtuzumab (0.5 mg/kg/day) from day –12 to day –10; melphalan (70 mg/m^2/day) from day –9 to day –8; thiotepa (10 mg/kg/day) on day –7; and fludarabine (40 mg/m^2/day) from day –6 to day –2. This was followed by a day of rest on day –1 and an infusion of 3 × 10e^6 CD34+ cells/kg from a 10/10 HLA allele matched unrelated bone marrow (bidirectional ABO incompatibility with recipient blood type of A+ and donor blood type of B+) from a 28-year-old CMV seropositive male donor (recipient was also CMV seropositive). Graft-versus-host disease (GVHD) prophylaxis was initiated with mycophenolate mofetil and tacrolimus. Tacrolimus levels were monitored to maintain a trough between 5 and 10 ng/mL. Ursodiol served as prophylaxis for sinusoidal obstruction syndrome. Prophylaxis against *Pneumocystis jiroveci*, herpes simplex virus (HSV), and fungal organisms included trimethoprim/sulfamethoxazole, acyclovir, and micafungin (subsequently transitioned to fluconazole), respectively. Empiric treatment for a single acid-fast bacillus colony isolated from a pre-HSCT sinus culture was started with rifampin, isoniazid, and pyrazinamide, which subsequently was changed to ethambutol. The culture of the single colony of acid-fast bacillus did not grow and could not be further specified; therefore, the anti-mycobacterial regimen was switched to prophylaxis with azithromycin. Intravenous immunoglobulin (IVIG) continued at a dose of 0.5 g/kg/dose every 4 weeks before and after HSCT. Pre-emptive screening for cytomegalovirus (CMV), Epstein-Barr (EBV) virus, and adenovirus was completed.

Results

On day +4, she developed severe gastrointestinal toxicity characterized by abdominal pain and diarrhea (up to 3.9 L/day), requiring supportive care including narcotic analgesics, parenteral nutrition, and a continuous infusion of octreotide. Mycophenolate mofetil was discontinued on day +5 given previous descriptions of mycophenolate mofetil-associated enterocolitis. Infectious evaluation documented a positive norovirus PCR on day +6, likely contributing to the early colitis. Because of fever and worsening abdominal pain, CT exams of her sinuses, chest, abdomen, and pelvis were performed on day +12, which showed worsening of her chronic sinusitis and bibasilar consolidations of the lungs. On day +19, a bronchoalveolar lavage was obtained which revealed *Stenotrophomonas maltophilia*, adenovirus via viral culture, and rhinovirus/enterovirus detected via PCR. Upper and lower endoscopic exams were completed with biopsies that demonstrated histopathologic evidence of adenovirus-associated colitis. In addition, a urine culture was positive for adenovirus. Disseminated adenovirus was initially treated with cidofovir (5 mg/kg/dose) with probenecid (6 g divided in three doses) and transitioned on day +34 to brincidofovir (100 mg twice weekly); however, the brincidofovir was discontinued on day +82 due to persistent nausea. A one-time treatment with high-dose IVIG (1.5 g/kg total dose) was administered immediately following the diagnosis of disseminated adenovirus and then continued at 0.5 g/kg/dose weekly until discharge. By day + 47, her serum, urine, and stool samples were negative for adenovirus via PCR. Her transplant course was also complicated by pancreatitis (peak lipase of 1629 units/L on day +12) detected during evaluation of persistent nausea. At this time, tacrolimus was stopped and cyclosporine started for continued GVHD prophylaxis. In addition, she developed *Granulicatella adiacens* bacteremia requiring intravenous antibiotic therapy. Finally, BK viruria (>50,000,000 copies/mL) and viremia (detected but <5000 copies/mL) were documented in association with mild symptoms (abdominal pain, dysuria) of transient cystitis on day +58. Her absolute neutrophil count exceeded 500 cells/μL for three consecutive days on day +24. Secondary to ongoing cytopenias developed post-HSCT, a bone marrow evaluation was completed on day +24, which demonstrated 20 % cellularity, trilineage hematopoiesis, and 100 % donor chimerism in all cell lines assessed (T cells, B cells, monocytes, and neutrophils). She was discharged on Day +72 and showed no evidence of acute or chronic GVHD. Secondary to persistent nausea and global decrements in her pulmonary function testing at day +93, repeat CT exams of her chest, abdomen, and pelvis were performed but showed only mild colitis. A repeat bronchoalveolar lavage documented only *Stenotrophomonas maltophilia* on culture and rhinovirus/enterovirus by PCR. A repeat upper and lower endoscopic exam demonstrated no histopathologic evidence of infectious colitis or GVHD. She developed asymptomatic EBV viremia (peak EBV DNA level of 8047 copies/mL on day +112), which resolved with weaning of cyclosporine. On day +148, she was diagnosed with shingles; acyclovir prophylaxis had been stopped.

At day +237, she remains on subcutaneous immunoglobulin supplementation (0.1 g/kg every 2 weeks) with normal immunoglobulin levels. Lymphocyte immunophenotype was assessed pre-HSCT and 6 months post-HSCT and is depicted in Table 3. Post-HSCTevaluation also documented absence of T-cell receptor excision circles (TRECs) and abnormal T-cell

receptor diversity (14 of 28 TCR Vb families/subfamilies demonstrate an oligoclonal repertoire). Her Karnofsky score is 90, although she continues to show evidence of asymptomatic cytopenias as demonstrated by a white blood cell count of 3000/μL (absolute neutrophil count 1920/μL), hemoglobin 10.9 g/dL, and platelet count of 104,000/μL while on eltrombopag (50 mg daily). She has no clinical evidence of ongoing GLILD or autoimmunity.

Discussion

Patients with CID-G/AI phenotype secondary to hypomorphic *RAG* variants present a challenge to clinicians. As a result of partially preserved RAG activity (>10 %), patients tend to present with initially milder but progressively worsening immunophenotype and distinct clinical characteristics of immune dysregulation. As exemplified in our case, the progression of immunological decline and only partial response to immune modulation may prompt the clinician to consider curative treatment such as HSCT. The identification of these patients with pathogenic RAG mutations allows the clinician not only to justify HSCT but also to arrange for proper donor selection. Excluding family members with the same genetic mutations but milder phenotype is an important part of this process [9].

Based on the current literature, we identified 13 patients with CID-G/AI phenotype who received HSCT (Table 2) [4, 6, 9, 11–14]. With the introduction of newborn screening for SCID, we have reported a case of HSCT for an asymptomatic infant of RAG deficiency with immune dysfunction and strong family history of fatal complications and autoimmunity. The other 12 patients were transplanted for worsening immune dysregulation, and the age at the time of transplant ranged from 29 months to 19 years. Our patient is among the oldest HSCT recipients with hypomorphic *RAG* mutations. Three (25 %) of these patients died, two secondary to transplant-related complications and one from an accident. The benefit of a successful HSCT is to reduce the risk for fatal infections and prevent escalation of autoimmunity. The selection of donors and of myeloablative regimens for previously reported patients varied. The indication and proper timing for HSCT in CID-G/AI phenotype is still unclear, especially for those with pathogenic mutations but otherwise mild symptoms. Unfortunately, limited data exist to provide clinicians with information regarding the ideal approach to HSCT and the subsequent course of these patients.

Unrelated donor HSCT in the setting of RAG deficiency with a CID-G/AI phenotype, as demonstrated in our patient, is not without risks. The infectious complications may have developed secondary to the intense T cell depletion we utilized and the requirement of ongoing post-HSCT immunosuppression to prevent GVHD. Treatment with investigational agents proved to be efficacious, sparing the need for adoptive immunotherapy with virus-specific T cells. We anticipate that some early immune recovery was achieved including specific cytotoxic T cell function as demonstrated by the clearance of her adenovirus infection. Given the high mortality associated with disseminated adenovirus infection, her clearance of adenovirus is a striking finding. Her immune recovery has been limited; however, the duration of follow-up is short. Low T cell counts as well as absent TRECs may also be associated with underlying thymic dysfunction [15]. It is unknown whether HSCT earlier in life could allow for greater capacity for immune reconstitution for this patient,

although we have a report of an asymptomatic patient with T cell lymphopenia and RAG1 compound heterozygous mutation (RAG1 mutation/activity level R841Q 0 %, F947R 56 %) associated with CID-AI/G phenotype, who was transplanted at 3 months of age and had full reconstitution and no complications [14]. Hence, early transplant with no prior exposure or infection with viruses seems to improve outcomes.

Despite improvement in her GLILD (by chest CTand clinical exam) and autoimmunity, our patient continues to have evidence of asymptomatic cytopenias in the context of 100 % donor engraftment across all cell lines assessed. One possible explanation is that survival of long-lived autoreactive plasma cells are in niches not cleared by myeloablation. In fact, autoantibody production is frequently seen post-HSCT in patients with primary immunodeficiency disorders. Despite this, no laboratory evidence of autoantibodies has been detected (e.g., negative direct antiglobulin test). The graft dysfunction may be related to concurrent early adenoviral infection; however, the cytopenias persisted even after the resolution of infection. In RAG deficiency, patients tend to be inflammation-prone, partly related to an overactive innate system, including NK cells prone for hyper-responsiveness but with decreased “fitness” to survive after virus proliferation [16]. In adequate pre-transplant immunosuppression or an inadequate number of infused CD34+ cells are associated with graft dysfunction; however, the immunosuppression we utilized appears to have been adequate based on post-HSCT engraftment studies, and the number of CD34+ cells infused was >2.0 × $10e^6$ CD34+ cells/kg [17, 18]. Treatment options for graft dysfunction and aforementioned poor immunereconstitution include a stem cell boost from the same donor or consideration of a second HSCT [19, 20]. At this time, the benefit of a second transplant (given the asymptomatic nature of her cytopenias and improvement in infectious and autoimmune complications thus far) may not outweigh the risk of GVHD, lethal infection, or other transplant-related morbidity. Despite this, the use of re-transplantation has been utilized successfully.

The tailoring of conditioning regimens to the needs of each individual patient is often needed. HSCT for SCID secondary to V(D)J recombination defect seems to have a less favorable outcome, including resistance to engraftment and poor B cell reconstitution, when compared to patients with pure T cell defects (such as common gamma chain) [3, 21, 22]. The likely reason for this difference is the accumulation of early T cell precursors in the thymus and B cell elements in the bone marrow that may limit the environment for proper engraftment of donor-derived lymphocytes; therefore, it has been established that SCID secondary to impaired V(D)J recombination require myeloablation. For RAG defects with SCID, full myeloablation is recommended whereas defects in other non-homologous end-joining complex might require reduced intensity treatment secondary to impaired DNA repair [21–23]. Conditioning was important in our patient in order to ensure proper stem cell engraftment and B cell reconstitution. Given the significant pre-HSCT morbidity including interstitial lung disease, a myeloablative reduced toxicity regimen consisting of fludarabine, melphalan, thiotepa, and alemtuzumab was chosen to minimize transplant-associated toxicity. Other regimens were considered including busulfan-based regimens targeting submyeloablative dosing. However, we felt that avoidance of busulfan was best given the risk of pulmonary toxicity in this susceptible patient. This regimen did appear to offer adequate immunoablation, as graft rejection did not occur, and lowered the risk of GVHD

mediated by donor T cells. Treosulfan-based regimens which offer myeloablation and reduced toxicity have also been successfully utilized in patients with primary immunodeficiency disorders [24–26].

In summary, this report and review of current literature suggests that HSCT using a matched unrelated bone marrow donor may represent a reasonable option for patients with hypomorphic *RAG* mutations and associated RAG deficiency with a progressive CID-G/AI phenotype. This approach may be extended to older patients with newly identified RAG deficiency as well as those with significant co-morbidities such as autoimmune cytopenias or GLILD. Careful attention must be given to the selection of a conditioning regimen that maximizes immunoablation while limiting organ-specific toxicities. Aggressive monitoring and intervention are necessary to limit infectious morbidity. Despite this, challenges including autoimmune cytopenias may still arise or recur. We found that allogeneic HSCT using a matched unrelated bone marrow donor following an immunoablative combination of fludarabine, melphalan, thiotepa, and alemtuzumab is a safe and less toxic approach to HSCT in these unique patients; however, additional modifications are likely to be necessary to ensure effective HSCT procedures.

Acknowledgments

Funding This work was supported in part by funds from the Intramural Research Program of the National Institute of Allergy and Infectious Disease (NIAID), National Institute of Health (NIH).

References

1. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA. 2014; 312(7):729–38. [PubMed: 25138334]
2. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. Annu Rev Immunol. 2004; 22:625–55. [PubMed: 15032591]
3. Dvorak CC, Hassan A, Slatter MA, Honig M, Lankester AC, Buckley RH, et al. Comparison of outcomes of hematopoietic stem cell transplantation without chemotherapy conditioning by using matched sibling and unrelated donors for treatment of severe combined immunodeficiency. J Allergy Clin Immunol. 2014; 134(4):935–43. e15. [PubMed: 25109802]
4. Walter JE, Rosen LB, Csomos K, Rosenberg JM, Mathew D, Keszei M, et al. Broad-spectrum antibodies against self-antigens and cytokines in RAG deficiency. J Clin Invest. 2015; 125(11): 4135–48. [PubMed: 26457731]
5. Buchbinder D, Baker R, Lee YN, Ravell J, Zhang Y, McElwee J, et al. Identification of patients with RAG mutations previously diagnosed with common variable immunodeficiency disorders. J Clin Immunol. 2015; 35(2):119–24. [PubMed: 25516070]
6. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with RAG mutations and granulomas. N Engl J Med. 2008; 358(19):2030–8. [PubMed: 18463379]
7. Reiff A, Bassuk AG, Church JA, Campbell E, Bing X, Ferguson PJ. Exome sequencing reveals RAG1 mutations in a child with autoimmunity and sterile chronic multifocal osteomyelitis evolving into disseminated granulomatous disease. J Clin Immunol. 2013; 33(8):1289–92. [PubMed: 24122031]
8. Geier CB, Piller A, Linder A, Sauerwein KM, Eibl MM, Wolf HM. Leaky RAG deficiency in adult patients with impaired antibody production against bacterial polysaccharide antigens. PLoS One. 2015; 10(7):e0133220. [PubMed: 26186701]

9. Schuetz C, Pannicke U, Jacobsen EM, Burggraf S, Albert MH, Honig M, et al. Lesson from hypomorphic recombination-activating gene (RAG) mutations: why asymptomatic siblings should also be tested. J Allergy Clin Immunol. 2014; 133(4):1211–5. [PubMed: 24331380]
10. Norarangelo LD, Kim MS, Walter JE, Lee YN. Human RAG mutations: biochemistry and clinical implications. Nat Rev Immunol. 2016; 16(4):234–46. [PubMed: 26996199]
11. Chen K, Wu W, Mathew D, Zhang Y, Browne SK, Rosen LB, et al. Autoimmunity due to RAG deficiency and estimated disease incidence in RAG1/2 mutations. J Allergy Clin Immunol. 2014; 133(3):880–2. e10. [PubMed: 24472623]
12. Avila EM, Uzel G, Hsu A, Milner JD, Turner ML, Pittaluga S, et al. Highly variable clinical phenotypes of hypomorphic RAG1 mutations. Pediatrics. 2010; 126(5):e1248–52. [PubMed: 20956421]
13. De Ravin SS, Cowen EW, Zarember KA, Whiting-Theobald NL, Kuhns DB, Sandler NG, et al. Hypomorphic Rag mutations can cause destructive midline granulomatous disease. Blood. 2010; 116(8):1263–71. [PubMed: 20489056]
14. Henderson LA, Frugoni F, Hopkins G, de Boer H, Pai SY, Lee YN, et al. Expanding the spectrum of recombination-activating gene 1 deficiency: a family with early-onset autoimmunity. J Allergy Clin Immunol. 2013; 132(4):969–71. e1–2. [PubMed: 23891352]
15. Marrella V, Poliani PL, Notarangelo LD, Grassi F, Villa A. Rag defects and thymic stroma: lessons from animal models. Front Immunol. 2014; 5:259. [PubMed: 25076946]
16. Karo JM, Schatz DG, Sun JC. The RAG recombinase dictates functional heterogeneity and cellular fitness in natural killer cells. Cell. 2014; 159(1):94–107. [PubMed: 25259923]
17. Dominietto A, Raiola AM, van Lint MT, Lamparelli T, Gualandi F, Berisso G, et al. Factors influencing haematological recovery after allogeneic haemopoietic stem cell transplants: graft-versus-host disease, donor type, cytomegalovirus infections and cell dose. Br J Haematol. 2001; 112(1):219–27. [PubMed: 11167808]
18. Larocca A, Piaggio G, Podesta M, Pitto A, Bruno B, Di Grazia C, et al. Boost of CD34 + –selected peripheral blood cells without further conditioning in patients with poor graft function following allogeneic stem cell transplantation. Haematologica. 2006; 91(7):935–40. [PubMed: 16818281]
19. Haen SP, Schumm M, Faul C, Kanz L, Bethge WA, Vogel W. Poor graft function can be durably and safely improved by CD34 + –selected stem cell boosts after allogeneic unrelated matched or mismatched hematopoietic cell transplantation. J Cancer Res Clin Oncol. 2015; 141(12):2241–51. [PubMed: 26272482]
20. Wolff SN. Second hematopoietic stem cell transplantation for the treatment of graft failure, graft rejection or relapse after allogeneic transplantation. Bone Marrow Transplant. 2002; 29(7):545–52. [PubMed: 11979301]
21. Lev A, Simon AJ, Bareket M, Bielorai B, Hutt D, Amariglio N, et al. The kinetics of early T and B cell immune recovery after bone marrow transplantation in RAG-2-deficient SCID patients. PLoS One. 2012; 7(1):e30494. [PubMed: 22295088]
22. Buckley RH, Win CM, Moser BK, Parrott RE, Sajaroff E, Sarzotti-Kelsoe M. Post-transplantation B cell function in different molecular types of SCID. J Clin Immunol. 2013; 33(1):96–110. [PubMed: 23001410]
23. Schuetz C, Neven B, Dvorak CC, et al. SCID patients with ARTEMIS vs RAG deficiencies following HCT: increased risk of late toxicity in ARTEMIS-deficient SCID. Blood. 2014; 123(2): 281–9. [PubMed: 24144642]
24. Morillo-Gutierrez B, Beier R, Rao K, Burroughs L, Schulz A, Ewins AM, et al. Treosulfan based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicenter experience. Blood. 2016
25. Slatter MA, Boztug H, Potschger U, Sykora KW, Lankester A, Yaniv I, et al. Treosulfan-based conditioning regimens for allogeneic hematopoietic stem cell transplantation in children with non-malignant diseases. Bone Marrow Transplant. 2015; 50(12):1536–41. [PubMed: 26259076]
26. Slatter MA, Rao K, Amrolia P, Flood T, Abinun M, Hambleton S, et al. Treosulfan-based conditioning regimens for allogeneic hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience. Blood. 2011; 117(16):4367–75. [PubMed: 21325599]

Table 1

Clinical and immunological characteristics of previous reported cases of RAG mutations causing the CID-G/AI phenotype that later underwent HSCT [4, 6, 9, 11–14]

Number	Gender	Mutation	Clinical features	Immunophenotype	Immunomodulatory therapy pre-HSCT
1 Schuetz, 2008 Schuetz, 2014	F	*RAG1* a. pR314W b. pR507W/R737H	Papulonodular skin lesions, presumed T-cell lymphoma, tonsillar diffuse large B-cell lymphoma	Naïve CD4 and B cell lymphopenia, hypogammaglobulinemia, abnormal specific Ab production, diminished T cell function	Topical corticosteroids followed by chemotherapy for T cell lymphoma; Rituximab for B cell lymphoma
2 Schuetz, 2008 Schuetz, 2014	F	*RAG1* a. pR778Q b. pR975W	Varicella with hepatitis and bacterial superinfection, ulcerative skin lesions, nodular tongue lesions, granulomatous changes in adenoids and the lung, (pneumonias, bronchiectasis, and atelectasis)	Naïve CD4 and B cell lymphopenia, hypogammaglobulinemia, abnormal specific Ab production, diminished T cell function	subcutaneous immunoglobulin
3 Schuetz, 2008 Schuetz, 2014	F	*RAG2* a. pT77N b. pG451A	Varicella, bronchopneumonia, spleen and lung granulomas, autoimmune cytopenias	Naïve CD4 and B cell lymphopenia, hypogammaglobulinemia, abnormal specific Ab production, diminished T cell function	Rituximab
4 Avila, 2010	M	*RAG1* a. pM435V b. pR699W	Autoimmune cytopenias, vitiligo, psoriasis, Guillain-Barre, thrush, sinopulmonary infections, bronchiectasis, bacterial overgrowth	Naïve CD4 and B cell lymphopenia, hypogammaglobulinemia, abnormal specific Ab production, diminished T cell function	Corticosteroids, infliximab, cyclophosphamide
5 De Ravin, 2010	M	*RAG1* a. pW522C b. pL541C	Upper respiratory tract infections, Candidal paronychia, adenitis, granulomas and soft tissue destruction associated with speech and swallowing difficulty	Naïve CD4 and B cell lymphopenia, hypogammaglobulinemia, abnormal specific Ab production, diminished T cell function	IVIG, thymectomy, dapsone, methotrexate, infliximab, corticosteroids, cyclophosphamide, rituximab
6 Henderson, 2015	F	*RAG1* a. pR841Q b. pF974L	No infections or autoimmunity	Naïve CD4 and B cell lymphopenia, hypogammaglobulinemia, abnormal specific Ab production, diminished T cell function	TMP/SMX and IVIG
7 Chen, 2014	F	*RAG1* a. pR474C b. pK983Nfs*9	Autoimmune hemolytic anemia (AIHA), recurrent viral infections, nephrotic syndrome, encephalopathy related to influenza infection	Diminished T cell function, naïve CD4 lymphopenia	Rituximab for AIHA, 1 year prior to diagnosis. TMP/SMX and IVIG after diagnosis
8 Chen, 2014	F	*RAG1* a. pR474C b. pK983Nfs*9	Recurrent bacterial and viral respiratory infections, AIHA, neutropenia with anti-neutrophil antibody, thrombocytopenia	Naïve CD4 and B cell lymphopenia, marrow with expanded gamma delta TCR population	Rituximab and prednisone (for autoimmune cytopenias), IVIG initially started for autoimmunity, then continued after RAG deficiency diagnosis made, TMP/SMX
9 Schuetz, 2014	M	*RAG1* a and b. p.H612R	Skin granuloma, varicella infection, scabies, recurrent bronchitis, AIHA, ITP	Naïve CD4 and B cell lymphopenia, hypogammaglobulinemia, abnormal specific Ab production, diminished T cell function	Rituximab

Number	Gender	Mutation	Clinical features	Immunophenotype	Immunomodulatory therapy pre-HSCT
10 Schuetz, 2014	M	*RAG1* a and b p.S480G	Enteropathy, bronchopneumonia, glomerulonephritis, AIHA, generalized urticaria	Naïve CD4 and B cell lymphopenia, hypogammaglobulinemia, abnormal specific Ab production, diminished T cell function	Rituximab
11 Walter, 2015	F	*RAG1* a and b. p.C176F	Granulomas, recurrent otitis media, bronchiectasis, pneumonia, chronic sinusitis, ulcerating skin granulomas, mycobacterial infection with lymphadenopathy		Steroid, infliximab, methotrexate, adalimumab, dapsone and topical tacrolimus
12 Walter, 2015	M	*RAG1* a and b. p.S117fs	AIHA, granulomas, pneumonias, liver abscess, mycobacterial osteomyelitis, herpeszoster, diarrhea from rotavirus, viral encephalitis after yellow fever vaccine, varicella		IVIG
13 Walter, 2015	F	*RAG1* a. p.K86VfsX33 b. p.H612R	Recurrent sinopulmonary disease (*Penicillium, Corynebacterium propinquum, Pseudomonas aeruginosa*), autoimmunity (cytopenias, vitiligo, duodenitis) and exacerbations of granulomatous lymphocytic interstitial lung disease	T lymphopenia, hypogammaglobulinemia, abnormal specific Ab production, diminished T cell function	IVIG Corticosteroids, infliximab, adalimumab, sirolimus, abatacept

IVIG intravenous immunoglobulin, *TMP/SMX* trimethoprim/sulfamethoxazole, *AIHA* autoimmune hemolytic anemia, *Ab* antibody, *ITP* idiopathic thrombocytopenia purpura

Table 2

HSCT characteristics of previously reported cases of RAG mutations causing the CID-G/AI phenotype that underwent HSCT [4, 6, 9, 11–14]

Number	Age at HSCT (years)	Conditioning	Donor source	HLA match	GVHD Ppx	GVHD	Other complications	Chimerism	Outcome	Post HSCT follow-up
1	6	Radioimmunotherapy 20 Gy Fludarabine 150 mg/m^2 ATG 10 mg/kg	UR	M				Mixed donor	Alive and well, on regular IVIG, obese	10 years
2	8.5		UR	M		Chronic GVHD			Deceased from complications of chronic GVHD	1 year
3	14	Radioimmunotherapy 27 Gy Treosulfan 36 g/m^2 Fludarabine 150 mg/m^2 Alemtuzumab 0.5 mg/kg	UR	M		Chronic GVHD		Full donor	Alive and well with vitiligo after chronic GVHD	7 year
4	6		Sib	M					Engrafted	
5	19	Busulfan 5 mg/kg ATG 40 mg/kg 300 cGy TBI	UR	M	Sirol	No GVHD	No complications	Full donor	Deceased due to accident	30 days
6	0.4	Busulfan (AUC1026 mmol*min) Cyclophosphamide ATG	UR	M		No GVHD			Alive and well with full immune reconstitution	14 months
7	8	Busulfan Fludarabine ATG	Sib	M	CSA MTX	No GVHD	CMV viremia (donor CMV+). Post transplant infections included: respiratory adenovirus, bacteremia with *S. aureus*/*E. faecalis*/*Strep pneumoniae*, norovirus	1 year post: whole-blood 97 % donor, T cell 97 % donor, B cell 100 % donor 3.5 years post: whole-blood 100 % donor	Alive, well	3.5 years
8	**1** 2.5 **2** 4	**1** Busulfan, fludarabine, ATG **2** Alemtuzumab, fludarabine, thiotepa, melphalan	**1** UR **2** UR	**1** M **2** M	**1** CSA, MTX **2** CSA, MTX, prednisone	**1** Grade 3 GI at 6 months **2** None	**1** Delayed neutrophil engraftment with persistent anti-neutrophil antibodies, then presumed HLH with graft failure requiring 2nd transplant **2** Multiple episodes of viremia and other viral infections	**1** Gradual loss with graft failure **2** 100 % donor, B cell 100 % donor, T cell 100 % donor	Alive, well with full immune reconstitution 2.5 years after 2nd transplant. With growth deficiency requiring growth hormone treatment	2 years (post 2nd HSCT)
9	7.5	Radioimmunotherapy 17.7 Gy Treosulfan 36 g/m^2 Fludarabine 150 mg/m^2 ATG 10 mg/kg	UR	M				Full donor	Alive and well	7 years
10	9.5	TBI 12 Gy Fludarabine 160 mg/m^2 ATG 15 mg/kg	Father	H		Chronic GVHD	Aspergillosis		Died in association with chronic GVHD, aspergillosis	9 months
11	12.5	Campath Fludarabine Melphalan	UR	M		GI GVHD	Adrenal insufficiency, short stature, and delayed puberty, non-traumatic fractures but limited osteoporosis, ulcerating granulomatous lesion of lower extremity requiring debridement. Neutropenia and thrombocytopenia (+anti-platelet and anti-neutrophils Abs). CMV, BKV, and EBV viremia s/p privirus-specific CTLS and Cytogam. BK viremia (treated with weekly IVIG) but asymptomatic	Chimerism started to fall >1 month after Once off IS is 100 %	Alive	18 months
12	8	Cyclophosphamide 30 mg/kg Fludarabine 150 mg/m^2 TBI 200 Gy	Sib	M	Cyclophosph50-mg/kg D +3 and +4, CSA MMF	No GVHD	Mycobacteriosis reactivation and cutaneous nodules	Donor 89 % T cell 98 %, B cells 100%, myeloid 83 %	Alive and well, no need for IVIG, attending school, hearing aid secondary to encephalitis	2 years
13	18	Alemtuzumab 0.5 mg/kg/day D −12 to −10 Melphalan 70 mg/m^2/day D −9 to −8 Thiotepa 10 mg/kg/day D −7 Fludarabine 40 mg/m^2/day D −6 to D −2	UR	M	Tacro MMF	No GVHD	GI toxicity (diarrhea, pancreatitis), infection (norovirus, *Stenotrophomonas maltophilia*, rhino/enterovirus, disseminated adenovirus, shingles), pancytopenia	Full donor	Alive	6 months

Gy gray, *D* day, *ATG* anti-thymocyte globulin, *TBI* total body irradiation, *UR* unrelated, *Sib* sibling, *M* matched, *H* haploidentical, *Ppx* prophylaxis, *CSA* cyclosporine, *MTX* methotrexate, *Siro* sirolimus, *Tacro* tacrolimus, *MMF* mycophenolate mofetil, *GI* gastrointestinal, *IS* immunosuppression, *CMV* cytomegalovirus, *EBV* Epstein-Barr virus, *CTL* cytotoxic T lymphocytes

Table 3

Immunologic profile of our patient with RAG deficiency resulting in the CID-G/AI phenotype both prior to HSCT and 6 months after HSCT

	Pre-HSCT	Post-HSCT (6 months)	Normal
Marker	%	%	%
CD3+ T cells	64	55 (L)	58–86
CD4+ T cells	49	13 (L)	32–64
CD4+CD45RA+ T cells	2 (L)	3	3–59
CD4+CD45RA+CD62L+T cells	1 (L)	0 (L)	11–824
CD4+CD45RO+ T cells	98 (H)	97 (H)	15–69
CD8+ T cells	13 (L)	39	18–40
CD8+CD45RA+ T cells	13	20	6–84
CD8+CD45RA+CD62L+ T cells	0 (L)	3	2–78
CD8+CD45RO+ T cells	86 (H)	80 (H)	4–49
CD19+	NA	15	8–24
NK (CD16+CD56+)	NA	27	3–28

NA not available, (*L*) low, (*H*) high