Successful treatment of severe refractory autoimmune hemolytic anemia after hematopoietic stem cell transplant with abatacept

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BACKGROUND: After hematopoietic stem cell transplantation (HSCT) autoimmune hemolytic anemia (AIHA) is a known and fairly common complication. It is often refractory to conventional therapies including corticosteroids, intravenous immunoglobulin, splenectomy, and the more recently described use of monoclonal antibodies. The high morbidity associated with these severe persistent cases elucidates the gaps in alternative therapies available for treatment.
STUDY DESIGN AND METHODS: We described the successful use of abatacept for severe refractory AIHA after HSCT in three patients.
RESULTS: Three pediatric patients with refractory AIHA after allogeneic stem cell transplantation were observed to be unresponsive to multitude immunosuppressive therapies, resulting in persistent transfusion dependency. Treatment with abatacept, a fusion protein that inhibits T-cell activation by binding to CD80/CD86 on antigen-presenting cells (APCs), thus blocking the required CD28 interaction between APCs and T cells, resulted in the resolution of hemolysis.
CONCLUSION: Abatacept may provide significant clinical benefit in the management of AIHA after HSCT.

ABBREVIATIONS: AIHA = autoimmune hemolytic anemia; CTLA-4 = cytotoxic T-lymphocyte antigen 4; HSCT = hematopoietic stem cell transplantation.

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Autoimmune hemolytic anemia (AIHA) is a recognized complication of hematopoietic stem cell transplantation (HSCT).[1] The incidence of AIHA after HSCT is between 4 and 6%.[2–4] Unrelated donor transplants and chronic graft-versus-host disease (GVHD) have been implicated as risk factors, although the etiology underlying this complication remains unclear.[5] The development of autoreactive lymphocytes in the context of failed central and peripheral immune tolerance is thought to play a role.[6] Clinical outcomes are variable; however, there is a risk for significant morbidity and mortality either as a consequence of severe hemolysis or from infections due to frequently utilized multiagent immunosuppression.[1] While the optimal treatment regimen remains uncertain, accepted or standard treatments include systemic corticosteroids, intravenous immunoglobulin (IVIG), splenectomy, plasma exchange, rituximab, or cyclophosphamide.[6–8] Bortezomib, a proteasome inhibitor has recently been reported as effective in treating AIHA after HSCT.[9] However, AIHA in the posttransplant setting can be variably resistant to immunosuppressive agents and there is no established standard of care.

Abatacept (Orencia) is a fusion protein formed by linking extracellular domain of cytotoxic T-lymphocyte antigen 4 (CTLA-4) with the Fc region of immunoglobulin G (IgG).[10] Abatacept works by inhibiting T-cell (T-lymphocyte) activation through competitive binding of CD80 and CD86 on antigen-presenting cells (APCs), thus blocking the required CD28 costimulatory interaction between APCs and T cells.[11] It is Food and Drug Administration approved for the treatment of autoimmune rheumatoid arthritis, psoriatic arthritis, and juvenile idiopathic arthritis. It is used off-label for the treatment of uveitis.[12,13] Abatacept is associated with adverse effects including increased risk of infection. When compared to the general population, an increased risk of lymphoma and lung cancer was noted in clinical trials. In addition there have been reported rare cases of hypersensitivity and anaphylactic reactions.[14] Recently, it has shown efficacy in prophylaxis against acute GVHD in HSCT recipients.[15] In the context of hemolytic anemia, disruption of T-cell–mediated regulation of the humoral immune system

can impact self-tolerance and promote AIHA. Abatacept, similar to the CTLA-4 on CD4+CD25+foxp3+ regulatory T cell (Tregs), can induce expression of indoleamine 2,3-dioxygenase in modified dendritic cells resulting in further activation and induction of CD4+CD25+Tregs.[16] We postulated that the restoration and up regulation of functional Tregs with abatacept might help mitigate the processes that drive AIHA in HSCT recipients. In this case series, we describe the successful use of abatacept in the treatment of refractory AIHA after allogeneic HSCT in three consecutive pediatric patients.

MATERIALS AND METHODS

Patients

The Phoenix Children's Hospital Medical Center Institutional Review Board approved this study. A retrospective chart review was performed to identify and review patients who developed AIHA after allogeneic HSCT that received abatacept in 2017. All three patients had pre-HSCT ABO, Rh typing, and red blood cell (RBC) antibody screening tests performed on recipient samples before and whenever patients required transfusion of blood products as standard of care. The direct antiglobulin test (DAT) was performed in pretransfusion compatibility testing and in patients with clinical suspicion of hemolytic anemia, on RBCs collected in ethylenediaminetetraacetic acid using polyspecific antihuman globulin (anti-IgG, -C3d [murine monoclonal], Gammaclone, Immucor) according to manufacturer's recommendations with standard tube methods.[17] When the polyspecific DAT was positive, further testing with specific anti-IgG (anti-IgG [murine monoclonal], Gammaclone, Immucor) and anti-C3d, -C3b (anti-C3b, -C3d [murine monoclonal], Gammaclone, Immucor) reagents was performed if ordered or a transfusion was requested. RBC eluates were prepared and tested by acid elution (Gamma Elukit II, Immucor). Eluates and sera of patients with positive screening tests or positive DAT were tested for specificity utilizing a standard panel method. Allogeneic adsorption techniques were performed to exclude an underlying alloantibody if the patient had received a transfusion in the prior 30 days and 1) an adsorption had not yet been performed, 2) there was increase in strength of the DAT, or 3) there was evidence of increasing hemolysis (increased lactate dehydrogenase [LDH], decreased haptoglobin, or decrease in hemoglobin [Hb] and/or hematocrit [Hct]). Patients who developed autoantibodies or clinically significant antibodies to RBC antigens received RBCs that were phenotypically similar for the D, C, E, c, e, Kell, Duffy, and Kidd RBC antigens. Institutional reference ranges for laboratory values referred to below are as follows: Hb 11.5 to 14.5 g/dL (female range) and 12 to 16 g/dL (male range), Hct 33% to 43% (female range) and 35% to 47% (male range), reticulocyte percentage 0.5% to 2%, bilirubin less than 0.8 mg/dL, LDH 81 to 234 U/L, and haptoglobin 30 to 200 mg/dL.

Case 1

A 9-year-old boy with severe HbSS disease received an unmanipulated allogeneic marrow transplant from a male HLA-matched unrelated donor. A clinical history of RBC alloimmunization prompted pretransplant treatment with 375 mg/m^2 rituximab weekly × 4 doses. The donor's blood group was O+; the recipient's blood group A+. The plasma reduced allogeneic, marrow, stem cell graft contained 2.4×10^8 total nucleated cells (TNCs)/kg and 1.48×10^6 CD34+ cells/kg. He received a reduced-intensity conditioning regimen including distal alemtuzumab (given on Days −21 to −19) followed by 5 days of 30 mg/m^2/day fludarabine and 1 day of 140 mg/m^2 melphalan. Tacrolimus, methotrexate (7.5 mg on Days 1, 3, and 6), and methylprednisolone (1 mg/kg on Days 7-28) were given for prevention of GVHD.[18] His immediate posttransplant course was uncomplicated. However, he developed severe AIHA and reticulocytopenia on Day +170. Laboratory findings demonstrated a severe normocytic, hypochromic anemia with a Hb level of 3.1 g/dL, Hct of 8.5%, and reticulocyte count of 0.2%. Biochemical evidence of hemolysis was supported by a bilirubin level of 4.6 mg/dL, a LDH level of 414 IU/L, and a haptoglobin level less than 10 mg/dL. The polyspecific and IgG DAT were strongly positive with an eluate demonstrating pan reactivity with all cells consistent with warm autoantibodies against IgG and eliciting complements C3 and C3d on the RBC surface. He was treated with 2 mg/kg/day methylprednisolone without clinical response. He received 1 g/kg IVIG on Days 176 and 177 and 375 mg/m^2 rituximab weekly for four doses starting on Day 183. He remained refractory to therapy and received 1.3 mg/m^2 bortezomib weekly starting on Day 234 for 4 weeks. The AIHA persisted, he remained transfusion dependent, and the DAT remained strongly positive. Sirolimus (3 mg/m^2) on Day 264 followed by maintenance doses of 1 mg/m^2 daily were added to his regimen, but discontinued after 1 week secondary to neutropenia, markedly elevated transaminases, and failure to improve his transfusion requirement. Abatacept (10 mg/kg) was initiated on Day 286. Rapid improvement was noted within 1 week with stabilization of Hb to greater than 10 g/dL. Most notably, he has required no further RBC transfusions. Abatacept was maintained monthly for 4 months and stopped without any side effects or rebound hemolysis. The patient is now 9 months after initiation of abatacept. His DAT remains positive, but he remains asymptomatic and transfusion independent with Hb levels ranging from 12 to 14 g/dL.

Case 2

A 4-year-old female with central nervous system hemophagocytic lymphohistiocytosis received a haploidentical unmanipulated marrow stem cell transplant from her mother.

The donor's blood group was A+ the recipient's blood group was O+. The RBC-depleted stem cell graft contained 4.8×10^8 TNCs/kg and 5.02×10^6 CD34+ cells/kg. She received a myeloablative conditioning regimen consisting of 4 days of 0.25 mg/kg/day alemtuzumab, 10 mg/kg thiotepa × 1 day, 4 days of 40 mg/m^2/day fludarabine, and 0.8 mg/kg/dose busulfan × 16 doses. Cyclophosphamide (50 mg/kg/day Days 3 and 4), tacrolimus, and mycophenolate were given for prophylaxis of GVHD.

She achieved neutrophil engraftment on Day 17. Her immediate posttransplant course was complicated by MRSA bacteremia and pneumonia, HHV-6 viremia, adenovirus enteritis, and cytomegalovirus viremia successfully treated with cytotoxic T lymphocytes. On Day 302, she was diagnosed with *Pneumocystis jiroveci* pneumonia and on Day 304, she developed warm autoantibody AIHA. Her Hb level acutely dropped from 10 to 7 g/dL, with a reticulocyte count of 7.1%. Her LDH was 478 IU/L with a haptoglobin level of less than 10 mg/dL. The DAT was strongly positive with warm antibodies against IgG and presence of C3d. She was treated with 1 g/kg IVIG on Days 307 and 308, followed by 2 mg/kg/day methylprednisolone for 7 days starting on Day 325. She remained refractory to treatment. Rituximab and bortezomib were considered but ultimately not used given concern for associated hypogammaglobinemia in the setting of active *P. jiroveci* pneumonia. Rather, she was treated with abatacept 10 mg/kg every 2 weeks × 4 doses starting on Day 345. She demonstrated clinical resolution of hemolysis without further RBC transfusion requirement after two doses of abatacept. Her DAT remained positive. She was 5 months after initiation of abatacept and remained asymptomatic with a Hb level greater than 11 g/dL.

Case 3

A 14-year-old male with hyper-IgE syndrome secondary to DOCK8 deficiency received an unmanipulated allogeneic, marrow transplant from a male HLA-matched unrelated donor. The donor's blood group was AB+, and the recipient's blood group was O+. The RBC-reduced marrow stem cell graft contained 5×10^8 TNCs/kg and 3.08×10^6 CD34+ cells/kg. He received a myeloablative preparatory regimen consisting of 4 days of 40 mg/m^2/day fludarabine and 0.8 mg/kg/dose busulfan × 16 doses. Antithymocyte globulin (2.5 mg/kg on Day −1), methotrexate (5 mg/m^2 on Days 1, 3, 6, and 11), and tacrolimus were administered for prophylaxis of GVHD. His immediate course was complicated by persistent elevation of incompatible isohemagglutinin titers ranging between 64 and 512 and necessitating plasmapheresis just before transplant through Day 23.

He achieved neutrophil engraftment on Day 17 and platelet engraftment on Day 34. Delayed RBC engraftment was expected due to ABO-incompatible graft and he remained RBC transfusion dependent at weekly intervals. On Day +45, he underwent a marrow biopsy and aspiration. Marrow biopsy showed normal myeloid and megakaryocytic lineages with absent erythroid cells and normal karyotype. He was diagnosed with an acquired RBC aplasia. He remained transfusion dependent, with Hb levels persistently ranging from 6.1 to 8.3 g/dL with an associated reticulocytopenia. His DAT remained negative. He sustained complete donor chimerism of 100% based on short tandem repeat analysis. He also demonstrated continued persistence of anti-A and anti-B isohemagglutinin titer ranging between 32 and 512. On Day 70 he presented with an Hb level of 2.5 g/dL in the setting of septic shock. His anti-A titer was 256 and anti-B titer was 512. The DAT was strongly positive with warm autoantibodies against IgG and negative for complements (C3 and C3d). All cells present in the antibody screen and ID panel were equally panreactive. He required 4 units of RBCs and again underwent plasmapheresis. Active hemolysis was attributed to an anti-E from a RBC transfusion he received the week prior was identified after referral to a reference laboratory for allogeneic adsorption. He was treated with 2 mg/kg/day methylprednisolone starting on Day 71. He received 375 mg/m^2 rituximab weekly × 4 starting on Day 77. His hemolysis remained refractory to treatment. On Day 110 he was started on 10 mg/kg abatacept every 2 weeks × 4 doses. He demonstrated a significant response with clinical resolution of hemolysis and transfusion independence after the initial dose of abatacept. There was a remarkable reduction in isohemagglutinin titers with both anti-A and anti-B titers decreasing from 512 before treatment with abatacept to 256 and 16 at 30 and 60 days after treatment with abatacept therapy. His DAT remained positive. He was 3 months after initiation of abatacept and remained asymptomatic with a Hb level greater than 11 g/dL and reticulocyte count of 1.8%.

DISCUSSION

This is the first report of the successful use of abatacept for the treatment of refractory immune hemolytic anemia after allogeneic HSCT in three consecutive cases (Fig. 1). AIHA may occur in the setting of Treg dysfunction.[19–21] Tregs constitute a subset of lymphocytes that have the capability of suppressing immune responses in vivo and in vitro both directly by cell–cell contact and indirectly through the production of anti-inflammatory cytokines. They constitute a small subset of T lymphocytes—CD4+CD25+foxp3+ cells.[20] As noted in the patient in Case 3, after the administration of abatacept, he demonstrated an immediate increase in proportion of CD4+CD25+foxp3+ cells (Fig. 2). This observation is similar to one noted by Lee and coworkers[13] and could suggest a restoration of Treg-cell function as FOXP3 is a Treg-cell function regulator.[22] This type of upregulation limits the T-cell activation and may not lead to as much immune suppression thereby a potential intervention that may limit the risk of infection. p values were calculated by

comparing mean values of each cell subpopulation between before and after use of abatacept using an unpaired t test. It should be noted that given an extremely small sample size, with human subjects, the result might not be reproducible. The patients treated with abatacept did not appear to have any statistical significant effect on lymphocyte subsets, CD4 ($p = 0.6$), CD8 ($p = 0.6$), CD19 ($p = 0.7$), or NK cell ($p = 0.2$; Fig. 3). We observe in the patient in Case 2 that the CD 4 and CD 8 levels decreased by 50% after abatacept as seen in Fig. 3. It is possible that the reason the other two patients did not demonstrate such a change is because before they got abatacept, they had been treated with other immune modulators that may have had some effect directly or indirectly on the lymphocyte subsets. Abatacept has been anecdotally reported to treat pancytopenia and associated life-threatening autoimmunity otherwise refractory to multiple immunosuppressive medications in patients with CTLA-4 haploinsufficency.[13,23] All patients exhibited decrease in or resolution of hemolysis after two to three doses of abatacept.

Our initial use of abatacept in the setting of AIHA occurred in Patient 1 in an attempt to provide GVHD prophylaxis after discontinuing sirolimus for toxicity. There is considerable evidence from murine models and now human trials to suggest that abatacept might be an active compound against the immune activation that occurs during GVHD.[24] Studies have demonstrated that an abatacept-containing immunosuppressive regimen could significantly protect against the development of primate GVHD.[25] These results, along with the clinical evidence for efficacy of abatacept in both autoimmunity and solid organ transplantation have provided the rationale for the development of a first-in-disease feasibility trial of abatacept for GVHD prevention (ClinicalTrials.gov NCT01012492). In Case 1, it was serendipitously noted that the patient's hemolysis was under control. Abatacept was not used for GVHD prevention in the other two patients, but rather primarily as a treatment for AIHA. None of the patients had GVHD. For Patients 2 and 3, we gave the drug 10 mg/kg intravenously every 2 weeks × 4 doses based off pharmacokinetic studies of an abatacept half-life of 16.7 (12-23) days in healthy subjects and 13.1 (8-25) days in patients with rheumatoid arthritis.[26]

In conclusion, AIHA after allogeneic HSCT is a rare but well-recognized complication that presents a significant risk for morbidity and a reported mortality of greater than 50%. Although many patients with hemolytic anemia respond to standard treatments, effective third-line agents are not established. However, our observations indicate that abatacept may provide significant clinical benefit in the management of AIHA after HSCT. The drug was well tolerated, allowed limitation of steroid use, and was not associated with an increased risk of infection. Prospective studies using abatacept as a second-line treatment in patients with AIHA

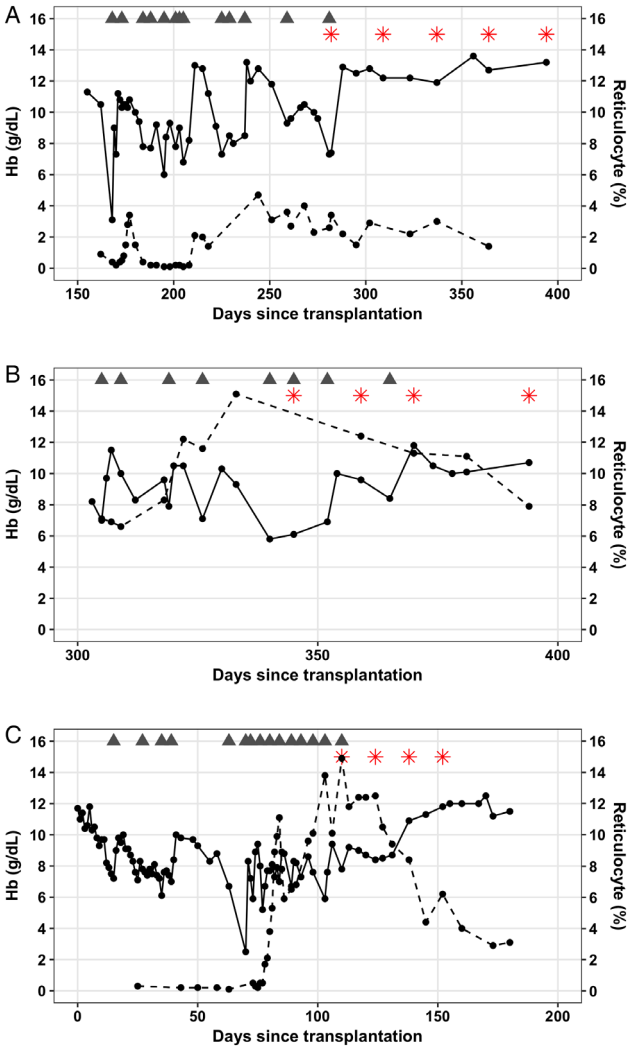

Fig. 1. (A) Case 1; (B) Case 2; (C) Case 3. Graph of Hb (—) and reticulocyte percentage (– – –) with timing of RBC transfusions (▲) and abatacept doses (✳).

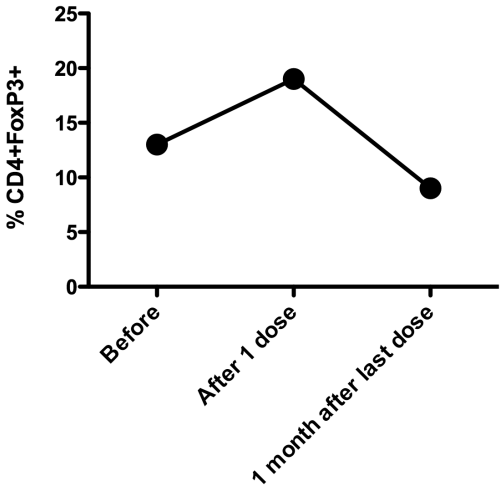

Fig. 2. Effects of abatacept on regulatory T cell (CD4+FOXP3+) population as observed in Case 3.

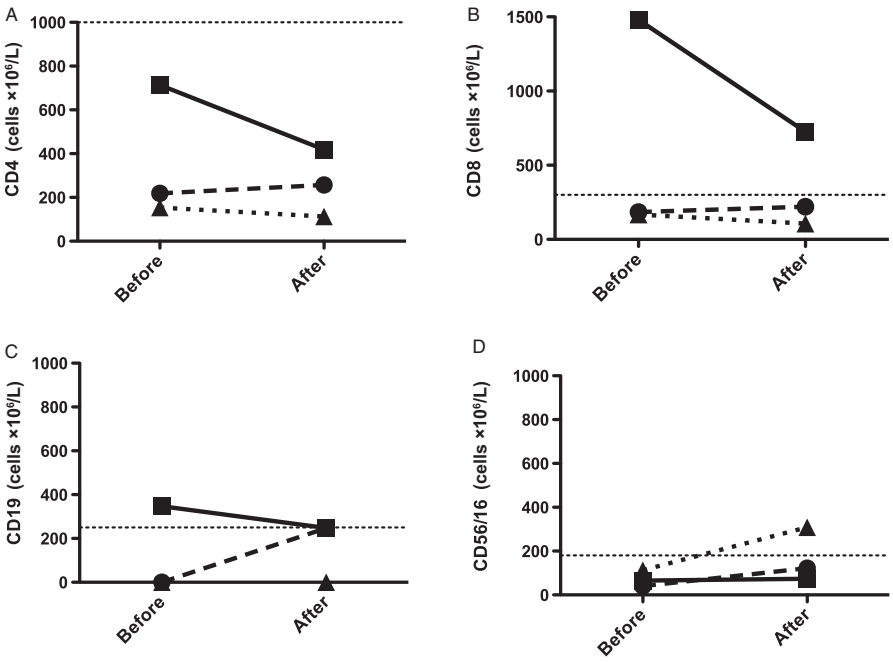

Fig. 3. Abatacept had no significant effect on lymphocyte subset. Lymphocyte subsets were evaluated before abatacept administration and at completion of therapy. (A) CD4+ T cells; (B) CD8+ T cells; (C) CD19+ B cells; (D) CD56+/16+ cells. (●) Case 1; (■) Case 2; (▲) Case 3. (■ ■ ■) Lower threshold of normal values.

after HSCT are currently under way at our institution with a goal of further delineating safety and efficacy.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Wang M, Wang W, Abeywardane A, et al. Autoimmune hemolytic anemia after allogeneic hematopoietic stem cell transplantation: analysis of 533 adult patients who underwent transplantation at King's College Hospital. Biol Blood Marrow Transplant 2015;21:60-6.
2. Holbro A, Abinun M, Daikeler T. Management of autoimmune diseases after haematopoietic stem cell transplantation. Br J Haematol 2012;157:281-90.
3. Ahmed I, Teruya J, Murray-Krezan C, et al. The incidence of autoimmune hemolytic anemia in pediatric hematopoietic stem cell recipients post-first and post-second hematopoietic stem cell transplant. Pediatr Transplant 2015;19:391-8.
4. O'Brien TA, Eastlund T, Peters C, et al. Autoimmune haemolytic anaemia complicating haematopoietic cell transplantation in paediatric patients: high incidence and significant mortality in unrelated donor transplants for non-malignant diseases. Br J Haematol 2004;127:67-75.
5. Chang TY, Jaing TH, Wen YC, et al. Risk factor analysis of autoimmune hemolytic anemia after allogeneic hematopoietic stem cell transplantation in children. Medicine (Baltimore) 2016;95: e5396.
6. Holbro A, Passweg JR. Management of hemolytic anemia following allogeneic stem cell transplantation. Hematology Am Soc Hematol Educ Program 2015;2015:378-84.
7. Hill QA, Stamps R, Massey E, et al. Guidelines on the management of drug-induced immune and secondary autoimmune, haemolytic anaemia. Br J Haematol 2017;177:208-20.
8. Silva VA, Seder RH, Weintraub LR. Synchronization of plasma exchange and cyclophosphamide in severe and refractory autoimmune hemolytic anemia. J Clin Apher 1994;9:120-3.
9. Hosoba S, Jaye DL, Cohen C, et al. Successful treatment of severe immune hemolytic anemia after allogeneic stem cell transplantation with bortezomib: report of a case and review of literature. Transfusion 2015;55:259-64.
10. Dall'Era M, Davis J. CTLA4Ig: a novel inhibitor of costimulation. Lupus 2004;13:372-6.
11. Bruce SP, Boyce EG. Update on abatacept: a selective costimulation modulator for rheumatoid arthritis. Ann Pharmacother 2007;41:1153-62.
12. Pimentel-Quiroz VR, Ugarte-Gil MF, Alarcon GS. Abatacept for the treatment of systemic lupus erythematosus. Expert Opin Investig Drugs 2016;25:493-9.
13. Lee S, Moon JS, Lee CR, et al. Abatacept alleviates severe autoimmune symptoms in a patient carrying a de novo variant in CTLA-4. J Allergy Clin Immunol 2016;137:327-30.

14. Weinblatt M, Combe B, Covucci A, et al. Safety of the selective costimulation modulator abatacept in rheumatoid arthritis patients receiving background biologic and nonbiologic disease-modifying antirheumatic drugs: a one-year randomized, placebo-controlled study. Arthritis Rheum 2006;54: 2807-16.
15. Koura DT, Horan JT, Langston AA, et al. In vivo T cell costimulation blockade with abatacept for acute graft-versus-host disease prevention: a first-in-disease trial. Biol Blood Marrow Transplant 2013;19:1638-49.
16. Fallarino F, Grohmann U, Hwang KW, et al. Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol 2003;4:1206-12.
17. Fung MK. Technical manual. 19th ed. Bethesda: AABB Press; 2017.
18. Shenoy S, Eapen M, Panepinto JA, et al. A trial of unrelated donor marrow transplantation for children with severe sickle cell disease. Blood 2016;128:2561-7.
19. Ward FJ, Hall AM, Cairns LS, et al. Clonal regulatory T cells specific for a red blood cell autoantigen in human autoimmune hemolytic anemia. Blood 2008;111:680-7.
20. Sakaguchi S, Ono M, Setoguchi R, et al. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 2006;212:8-27.
21. Ahmad E, Elgohary T, Ibrahim H. Naturally occurring regulatory T cells and interleukins 10 and 12 in the pathogenesis of idiopathic warm autoimmune hemolytic anemia. J Investig Allergol Clin Immunol 2011;21:297-304.
22. Devaud C, Darcy PK, Kershaw MH. Foxp3 expression in T regulatory cells and other cell lineages. Cancer Immunol Immunother 2014;63:869-76.
23. Lo B, Zhang K, Lu W, et al. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science 2015;349:436-40.
24. Via CS, Rus V, Nguyen P, et al. Differential effect of CTLA4Ig on murine graft-versus-host disease (GVHD) development: CTLA4Ig prevents both acute and chronic GVHD development but reverses only chronic GVHD. J Immunol 1996;157: 4258-67.
25. Miller WP, Srinivasan S, Panoskaltsis-Mortari A, et al. GVHD after haploidentical transplantation: a novel, MHC-defined rhesus macaque model identifies CD28- CD8+ T cells as a reservoir of breakthrough T-cell proliferation during costimulation blockade and sirolimus-based immunosuppression. Blood 2010;116:5403-18.
26. Moreland LW, Alten R, van den Bosch F, et al. Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4Ig and LEA29Y eighty-five days after the first infusion. Arthritis Rheum 2002;46: 1470-9.