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Successful treatment of refractory pure red cell aplasia with eltrombopag after ABO-incompatible allogeneic hematopoietic stem cell transplantation

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Pure red cell aplasia (PRCA) is a well-recognized complication of ABO major mismatched allogeneic hematopoietic stem cell transplantation (allo-HSCT), with a reported incidence of 10%–20% (Zhidong et al., 2012; Busca et al., 2018). It is clinically characterized by anemia, reticulocytopenia, and the absence of erythroblasts in a normal-appearing bone marrow biopsy (Shahan and Hildebrandt, 2015). The mechanism for PRCA has been presumed to be persistence of recipient isoagglutinins, produced by residual host B lymphocytes or plasma cells, which can interfere with the engraftment of donor erythroid cells (Zhidong et al., 2012). Several risk factors of PRCA at presentation are known, such as presence of anti-A isoagglutinins before transplantation, reduced intensity conditioning, absence of acute graft-versus-host disease (GVHD), sibling donors, and cyclosporin A (CsA) as GVHD prophylaxis (Hirokawa et al., 2013). PRCA is not considered to be a barrier to HSCT, as some patients can recover spontaneously or benefit from various approaches including high-dose steroids, erythropoietin (EPO), plasma exchange, immunoadsorption, donor lymphocyte infusion (DLI), treatment with rituximab, bortezomib, or daratumumab, and tapering or discontinuation of immunosuppression (Hirokawa et al., 2013; Bathini et al., 2019). However, there are still some patients who fail to respond even to aggressive treatment; they become red cell transfusion-dependent and iron-overloaded, and their life quality is impaired.

Eltrombopag (EPAG; Revolade, Novartis, Switzerland), a novel oral thrombopoietin receptor agonist (TPO-RA), acts on the thrombopoietin receptor (TpoR; c-myeloproliferative leukemia (c-MPL)) on hematopoietic stem cells (HSCs) and megakaryocytes, showing encouraging efficacy in patients with chronic immune thrombocytopenic purpura (ITP) and refractory severe aplastic anemia (rSAA). Additionally, these investigations reported that red blood cell counts were increased (Olnes et al., 2012; Desmond et al., 2014). However, whether EPAG will improve unilineage erythroid hematopoiesis for PRCA after HSCT is still unknown. Until now, only one case report has been published (Busca et al., 2018). Herein, we describe a patient with refractory PRCA following major ABO-mismatched HSCT from a matched related donor, who eventually responded well to long-course EPAG at a sufficient dosage. Our case might add useful knowledge on the effectiveness, optimal dosage, and ideal treatment duration of EPAG.

A 27-year-old female weighing 45 kg was diagnosed with common B cell-acute lymphoblastic leukemia (ALL) with mixed-lineage leukemia-AF4 (*MLL-AF4*) fusion gene expression. In October 2018, she underwent peripheral blood stem cell transplantation (PBSCT) from her human leukocyte antigen (HLA)-matched sibling brother in first complete remission (CR1) status. The patient did not have any donor-specific antibodies (DSAs) in her plasma. There was a major

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ABO mismatch between the recipient (O) and the donor (A). The conditioning regimen consisted of cytarabine (2 g/(m^2·d) intravenously (IV) on Days −8 to −7), etoposide (VP16, 100 mg/d IV on Days −8 to −6), busulfan (3.2 mg/(kg·d) IV on Days −6 to −4), cyclophosphamide (1.8 g/(m^2·d) IV on Days −3 to −2), and methyl-*N*-(2-chloroethyl)-*N*-cyclohexyl-*N*-nitrosourea (Me-CCNU) (250 mg/m^2 orally on Day −1). CsA and a short course of methotrexate and mycophenolate mofetil were given for GVHD prophylaxis. A total of 1.10×10^9 mononuclear cells per kilogram of patient weight, including 7.41×10^6 $CD34^+$ cells, were transplanted on Day 0. Granulocyte and platelet engraftment were achieved on Day +12.

On Day +34, the patient's hemoglobin (Hb) was 69 g/L, with a normal leukocyte and platelet count. Reticulocytes accounted for approximately 0.2% and the absolute value was 1.00×10^{10} cells/L. Bone marrow smear showed hyperplasia of the megakaryocytic/granulocytic lineages with hypoplasia of erythroid cells (accounting for 5%). Minimal residual disease (MRD) was negative, with less than 0.01% abnormal cells by six-color flow cytometry (FC), and *MLL-AF4* fusion gene was absent. Chimerism was tested by short tandem repeat (STR) analyses every month for the first five months, at the ninth month, and at the first year post-transplantation (Fig. 1). Coombs testing and parvovirus B19 were negative. Levels of folic acid and vitamin B_{12} were in normal ranges. Anti-A isoagglutinin titers were 1:32 (immunoglobulin M (IgM)) and 1:128 (immunoglobulin G (IgG)) on Day +41. According to the above analysis, the patient was diagnosed as PRCA. On Day +34, she was treated with testosterone undecanoate 40 mg bis in die (BID) and recombinant human erythropoietin (rhEPO) 6000 IU once a week. The dose of CsA was tapered rapidly to 50 mg/d per os (PO) and prednisone was given at a dose of 0.5 mg/(kg·d). The patient received red blood cell transfusions on Days +42, +63, +119, and +132. On Days +43, +69, +71, and +79, a series of plasma exchanges were performed, but she remained reticulocytopenic and anemic. DLI was performed twice on Days +77 and +112. The volume of each infusion was 17 mL (8.47×10^7 mononuclear cells/kg, 4.52×10^5 $CD34^+$ cells/kg, 1.27×10^7 CD^{3+} cells/kg). However, there was not a significant increase in Hb level with the value of 49 g/L on Day +119. The patient's anti-A titers had dropped to 1:64 for IgG and 1:2 for IgM on Day +90. Bone marrow smear showed 5%, 2%, 1%, and 1% erythroid cells on Days +31, +64, +94, and +127, respectively. Due to the absence of GVHD, CsA was discontinued within four months. RhEPO and prednisone were withdrawn because of a lack of efficacy. The duration of prednisone was approximately 130 d, and the total dose was 1925 mg. EPAG was initiated on Day +111 at an initial dose of 50 mg/d. After four weeks of treatment with EPAG, it was increased to 75 mg/d without any adverse side effects. On Day +160, after seven weeks of treatment with EPAG, the patient's Hb recovered to 57 g/L. Bone marrow smear also revealed that erythroid cells were at a level of 5.5%. Almost three months after treatment with EPAG, Hb grew to 89 g/L. Moreover, the proportion of reticulocytes rose to 5.9%. EPAG was tapered gradually and eventually discontinued. The patient had a progressive improvement of Hb values; she had taken EPAG 98 d, totaling 258 tablets (6450 mg). On Day +217, bone marrow smear showed an active proliferation of erythroid cells, accounting for 21.5%. One year after the transplant, Hb rose to a normal level and currently was maintained at around 130 g/L. At 9 and 12 months post-HSCT, bone marrow smear showed 16%–20% erythroid cells, indicating complete recovery of normal hematopoiesis in the erythroid lineage. At an 18-month follow-up evaluation after PBSCT, the patient was in excellent physical condition with a normal level of Hb value. The time course of the patient is summarized in Fig. 2.

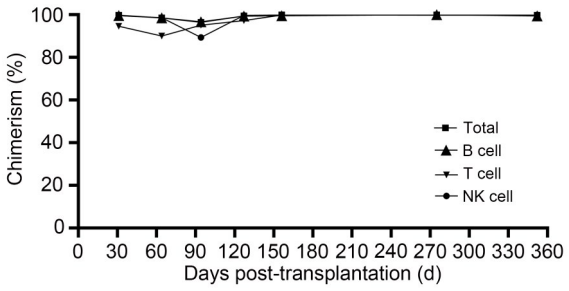

Fig. 1 Chimerism of total cells, B cells, T-cells, and natural killer (NK) cells with short tandem repeat (STR) analysis.

Some stem cell transplantation is performed between ABO-mismatched donors and recipients due to the limitations of donor selection. There is no clear evidence that ABO-incompatibility will influence

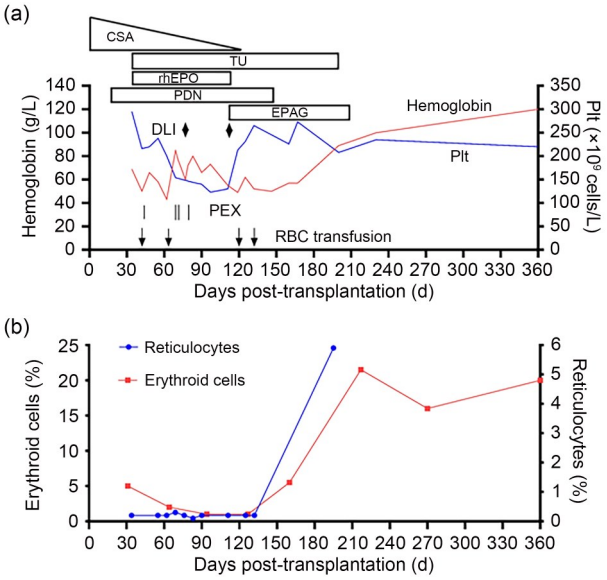

Fig. 2 Clinical courses and changes in hemoglobin and platelet (Plt) levels (a) and the proportions of reticulocytes in peripheral blood and erythroid cells in bone marrow (b). CSA: cyclosporine; TU: testosterone undecanoate; rhEPO: recombinant human erythropoietin; PDN: prednisone; EPAG: eltrombopag; DLI: donor lymphocyte infusion; PEX: plasma exchange; RBC: red blood cell.

the outcome of HSCT in the aspects of incidence and severity of graft rejection or GVHD, the engraftment of myeloid and megakaryocytic lineages, the relapse rate of disease, the non-relapse mortality rate, or overall survival, whereas the probability of PRCA will increase (Yang and Levis, 2014). PRCA following ABO-incompatible allo-HSCT may resolve spontaneously, but some patients still need various treatments. There is no standard treatment modality for PRCA at the moment, and we summarize some studies on successful treatment in Table 1.

EPAG is a synthetic, orally bioavailable, small molecule; it is a nonpeptide agonist of the TpoR which is approved by the US Food and Drug Administration for treatment of immune thrombocytopenia (ITP) and severe aplastic anemia (SAA) (Busca et al., 2018; Alvarado et al., 2019). Olnes et al. (2012) showed that EPAG gave rise to multilineage clinical responses in some patients with rSAA. Some of the treated patients experienced improved Hb levels and no longer needed red cell transfusions. Therefore, we speculate that EPAG may obtain a therapeutic effect in PRCA after ABO-incompatible allo-HSCT. Previously, only one study from Busca et al. (2018) has reported the application of EPAG in treatment of PRCA after transplant. They observed that two patients with PRCA after major ABO-incompatible HSCT achieved complete remission upon treatment with EPAG after failing to respond to many other treatments. During treatment, EPAG was well tolerated by their patients, with only one patient experiencing transient platelet elevation and recovering after dose adjustment. In addition, they speculated that there might be a potential synergy between iron chelation therapy (ICT) and EPAG. The combination of EPAG with other therapeutic approaches for PRCA is worth discussing for its potential to accelerate recovery and reduce iron overload.

The underlying mechanism of EPAG in erythroid reconstitution remains unclear; however, our speculations are as follows. Firstly, interferon-γ (IFN-γ), a pro-inflammatory cytokine that often increases after HSCT, prevents full engagement of thrombopoietin to its receptor c-MPL via steric occlusion of the low-affinity binding site, contributing to perturbation of thrombopoietin-induced signaling pathways and decreasing survival of human hematopoietic stem and progenitor cells (HSPCs). EPAG interacts selectively with c-MPL at a position distinct from the extracellular binding site of thrombopoietin, bypasses the inhibition of IFN-γ, and activates the the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway, which can induce the proliferation and differentiation of HSPCs (Alvarado et al., 2019; Gao et al., 2020). Secondly, EPAG enhances HSPC genome stability and improves HSPC survival and function by promoting DNA repair in human HSPCs (Guenther et al., 2019). Also, Bao et al. (2010) found an improvement in regulatory T cell (Treg) activity and a decrease in proinflammatory soluble CD40 ligand (sCD40L) with a concomitant increase in circulatory transforming growth factor-1 (TGF-1) in patients who received treatment with thrombopoietic agents. The treatment improved peripheral Treg function and had an accompanying decrease in inflammatory state. Finally, EPAG mobilizes intracellular iron via direct chelation, so that the negative effect of excess iron on normal hematopoiesis can be lessened. EPAG can decrease reactive oxygen species (ROS)-mediated cellular damage (Vlachodimitropoulou et al., 2017; Zhao et al., 2018). To sum up, EPAG may be of great help for erythroid reconstitution by activating proliferation and differentiation of HSPCs, improving their survival and function, decreasing the inflammatory state, and reducing cellular damage (Fig. 3).

Table 1 Summary of successful treatments for PRCA in the literature

Reference	Patient number	Diagnosis	Donor type	HSC source	D/R blood type	Isoagglutinin titer pre-transplant	Preconditioning	GVHD prophylaxis	Treatment
Busca et al., 2018	2	AML (1); biphenotypic AL (1)	HLA-matched unrelated (2)	PBSC (2)	A/O (2)	1:512 (1); 1:256 (1)	Myeloablative (1); total body irradiation+ cyclophosphamide (1)	ATG+CsA+ MTX (2)	θ-EPO, PEX, ICT (DFX, DFO), rituximab, EPAG (1); θ-EPO, PEX, ICT (DFX, DFO), rituximab, bortezomib, EPAG (1)
Zhidong et al., 2012	2	CML-CP (2)	HLA-matched sibling (1); HLA-matched unrelated (1)	PBSC (2)	AB/O (1); A/O (1)		Busulfan+ cyclophosphamide (1); busulfan+cytarabine+ cyclophosphamide+ ATG (1)	CsA+MTX (1); CsA+ MTX+ MMF (1)	Taper CsA, EPO, rituximab (2)
Shahan and Hildebrandt, 2015	1	α-β subcutaneous panniculitis-like T cell lymphoma	HLA-matched sibling	PBSC	A/B	Negative	Fludarabine+melphalan	CsA+MTX	Taper CsA, rituximab, pulse-dose dexamethasone, bortezomib
Bathini et al., 2019	1	MDS-RAEB-2	HLA-matched unrelated	PBSC	A/O		Busulfan+fludarabine+ total body irradiation	ATG+ FK506+ MTX	Taper FK506, high-dose corticosteroid, rituximab, bortezomib, daratumumab
Yang and Levis, 2014	1	AML	HLA-matched	PBSC	A/O		Busulfan+ cyclophosphamide	FK506+ MMF	Taper FK506, IVIG, four pulses of high-dose steroid
Sackett et al., 2018	1	Severe congenital neutropenia	HLA-matched unrelated	BM	A/O		Busulfan+fludarabine+ alemtuzumab	CsA+MMF	Taper CsA, rituximab, bortezomib, PEX

The number in the brackets is the number of patients. AML: acute myeloid leukemia; AL: acute leukemia; CML-CP: chronic myeloid leukemia-chronic phase; MDS-RAEB-2: myelodysplastic syndrome with refractory anemia with excess blasts 2; HLA: human leukocyte antigen; PBSC: peripheral blood stem cell; BM: bone marrow; ATG: anti-human thymocyte globulin; CSA: cyclosporine A; MTX: methotrexate; MMF: mycophenolate mofetil; FK506: tacrolimus; EPO: erythropoietin; PEX: plasma exchange; ICT: iron chelation therapy; DFX: deferasirox; DFO: deferoxamine; EPAG: eltrombopag; IVIG: intravenous immunoglobulin; HSC: hematopoietic stem cell; D/R: donor/recipient; PRCA: pure red cell aplasia, GVHD: graft-versus-host disease.

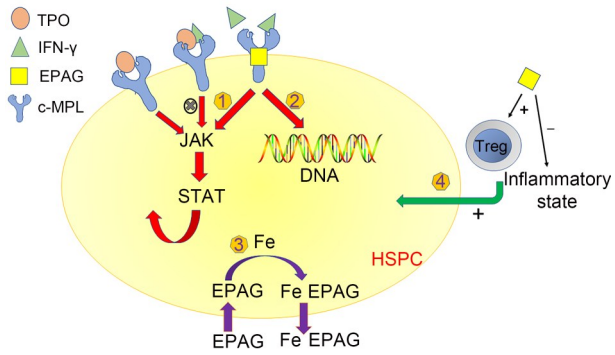

Fig. 3 Underlying mechanism of eltrombopag (EPAG) in erythroid reconstitution. (1) EPAG interacts selectively with thrombopoietin (TPO) receptors at a position distinct from the extracellular binding site of TPO, bypasses the inhibition of interferon-γ (IFN-γ), and activates the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway; (2) EPAG promotes DNA repair; (3) EPAG mobilizes intracellular iron; (4) EPAG improves peripheral regulatory T cell (Treg) function and decreases the inflammatory state. c-MPL: c-myeloproliferative leukemia; HSPC: hematopoietic stem and progenitor cell.

In conclusion, we reported a case of refractory PRCA after allo-HSCT, in which the patient had good recovery of erythropoiesis following treatment with EPAG. Although the possibility of spontaneous recovery cannot be ruled out, we still are of the opinion that EPAG played a key role in this case because spontaneous remission becomes less frequent and treatment is advised when PRCA continues beyond 60 d after HSCT (Bathini et al., 2019). There is little literature on the successful treatment of PRCA with DLI after allo-HSCT. Selleri et al. (1998) showed a case of PRCA after major ABO-incompatible bone marrow transplantation, which achieved full engraftment after two dose-escalating $CD34^+$-enriched DLI. The patient's Hb level, reticulocyte count, and marrow erythropoiesis markedly improved within two months after the second DLI. We cannot completely rule out the role of DLI in our case. However, the patient's Hb increased significantly nearly three months after the last DLI. With this in view, we believe that EPAG played a most important role in our patient's hematopoietic recovery. In the course of treatment, no obvious side effects were noted (such as thrombocytosis, thrombosis, or liver function test abnormalities). EPAG may be effective and safe for unilineage hematopoiesis and can be used for the treatment of PRCA that develops following ABO-incompatible allo-HSCT. Further research is needed to confirm the effect of this medication.

The Clinical Trials website (https://www.clinicaltrials.gov) has not recorded clinical trials of EPAG for PRCA because of the difficulty of multifactorial etiologies, rarity of cases, and the low interest of the pharmaceutical industry in a trial for such a low-volume disease. More clinical trials aim at investigating the treatment of aplastic anemia with EPAG. Hence, the optimal dosage, the time to start and end treatment, and the effectiveness of EPAG for PRCA still need to be refined by accumulated clinical experience.

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Author contributions

Yang GAO contributed to reviewing the patient and writing the manuscript. Fei GAO wrote part of the manuscript and reviewed the literature. Jimin SHI and Huarui FU supported the treatment course of the patient, and provided their comments on this manuscript. He HUANG and Yanmin ZHAO were mainly in charge of the patient, and critically reviewed the patient and the manuscript. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Yang GAO, Fei GAO, Jimin SHI, Huarui FU, He HUANG, and Yanmin ZHAO declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from the patient for being included in the report.

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