Ther Adv Hematol
2020, Vol. 11: 1–13
DOI: 10.1177/2040620720948743

Advances in the understanding of poor graft function following allogeneic hematopoietic stem-cell transplantation

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***Abstract*:** Poor graft function (PGF) following allogeneic hematopoietic stem-cell transplantation (allo-HSCT) is a life-threatening complication and is characterized by bilineage or trilineage blood cell deficiency and hypoplastic marrow with full chimerism. With the rapid development of allo-HSCT, especially haploidentical-HSCT, PGF has become a growing concern. The most common risk factors illustrated by recent studies include low dose of infused $CD34^+$ cells, donor-specific antibody, cytomegalovirus infection, graft *versus* host disease (GVHD), iron overload and splenomegaly, among others. Because of the poor prognosis of PGF, it is crucial to uncover the underlying mechanism, which remains elusive. Recent studies have suggested that the bone marrow microenvironment may play an important role in the pathogenesis of PGF. Deficiency and dysfunction of endothelial cells and mesenchymal stem cells, elevated reactive oxygen species (ROS) levels, and immune abnormalities are believed to contribute to PGF. In this review, we also discuss recent clinical trials that evaluate the safety and efficacy of new strategies in patients with PGF. $CD34^+$-selected stem-cell boost (SCB) is effective with an acceptable incidence of GVHD, despite the need for a second donation. Alternative strategies including the applications of mesenchymal stem cells, N-acetyl-l-cysteine (NAC), and eltrombopag have shown favorable outcomes, but further large-scale studies are needed due to the small sample sizes of the recent clinical trials.

Keywords: allogeneic hematopoietic stem-cell transplantation, mechanism, poor graft function, risk factors, treatment

Received: 1 May 2020; revised manuscript accepted: 14 July 2020.

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Introduction

Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) is important and the only curative treatment for some malignant or nonmalignant hematopoietic diseases. Most patients can achieve hematopoietic recovery after allo-HSCT, but a portion of them develop graft failure (GF), which is caused primarily by graft rejection and poor graft function (PGF) that can be distinguished by the chimerism status.[1] While full chimerism exists for PGF, mixed chimerism is present in graft rejection. This review will focus on PGF.

The criteria for PGF have not been agreed. However, most recent studies have defined PGF based on the severity of cytopenia of at least two cell types: (1) absolute neutrophil count (ANC) $\leqslant 0.5 \times 10^9$/l; (2) platelet (PLT) count $\leqslant 20 \times 10^9$/l; and (3) hemoglobin (HB) $\leqslant 70$ g/l for at least 3 consecutive days beyond day +28 post-HSCT or the requirement of transfusion support with hypoplastic-aplastic bone marrow, excluding severe graft *versus* host disease (GVHD) and relapse.[2–4] Primary PGF refers to incomplete engraftment, while secondary PGF is defined as a loss of initial engraftment. Patients with primary PGF have a lower response rate to treatment and poorer prognosis compared with those with secondary PGF.[5] PGF is a life-threatening complication, and the survival rate is significantly lower

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than patients with good graft function (GGF).[3,4] This is because persistent leukocytopenia and thrombocytopenia increase the risk of infections and bleeding, and, thus, raise the mortality rate. The incidence of PGF is approximately 5–27%[3,5] and has become a growing obstacle after allo-HSCT due to the development of haploidentical-HSCT. However, the underlying mechanism has yet to be elucidated, while treatments are very limited. Recent studies suggested that the bone marrow (BM) microenvironment plays an important role in the pathogenesis of PGF and may provide potential new targets for treatments. Also, the therapeutic strategies such as $CD34^+$-selected stem-cell boost (SCB), mesenchymal stem-cell (MSC) infusion and other new approaches have shown good efficacy and may provide potential new treatments for PGF.

Risk factors

Risk factors for PGF include low dose of infused $CD34^+$ cells, cytomegalovirus (CMV) infection, GVHD, donor-specific antibody (DSA), iron overload, splenomegaly and so on.[2,3,6] In addition, CMV infection and GVHD are more likely associated with secondary PGF, rather than primary PGF.[4]

In previous studies,[7,8] the dose of $CD34^+$ cells is crucial for hematopoietic and immune recovery after allo-HSCT. After comparing two recent studies, we conclude that a higher $CD34^+$ cell dose (5.5×10^6/kg *versus* 2.21×10^6/kg) is linked consistently with a lower risk of developing PGF (2.89% *versus* 5.6%; $p = 0.015$).[3,4] Zhao *et al.* suggested that the $CD34^+$ cell dose $< 5 \times 10^6$/kg [odds ratio (OR) = 5.089; 95% confidence interval (CI): 1.745–14.841, $p = 0.003$] might be an independent risk factor for primary PGF as revealed by multivariate logistic analysis.[3] In addition, a retrospective study focused on secondary PGF also suggested that the low (<median) $CD34^+$ cell dose is an independent risk factor [hazard ratio (HR) = 3.07; 95% CI: 1.207 -7.813; $p = 0.019$].[9]

DSA against the unshared haplotype in the recipient is reportedly associated with an increased risk of engraftment failure in unrelated donor transplantation and haploidentical-HSCT.[10,11] Moreover, a very recent study conducted by our colleagues suggested that DSA is the only risk factor for engraftment failure (OR = 34.0; 95% CI: 2.648–436.545; $p = 0.007$).[12] Sun *et al.* found that DSA-positive patients are more inclined to develop primary PGF (31% *versus* 3.2%, $p = 0.000$) than the DSA-negative patients.[4] Moreover, a prospective study with a total of 345 cases showed that DSA (MFI≥2000) is strongly associated with PGF.[13] In 2018, the European Society for Blood and Marrow Transplantation recommended testing DSA in all haploidentical donor transplant recipients and suggested MFI > 1000 as DSA positivity.[14] In summary, the high DSA level is associated with the occurrence of PGF, and DSA-positive donors should be avoided. However, for patients with limited donor options, with careful DSA assessment and monitoring, the desensitization to weak or negative DSA levels may allow successful transplantation.[15,16] Our previous study indicated that the combination of rituximab and donor platelet can reduce the DSA level and improve engraftment.[12] Recently, Bramanti *et al.* demonstrated that low-level DSA does not increase the risk of PGF development, even without desensitization, but the criteria that make haploidentical-HSCT permissible need to be further identified.[17]

Iron is an essential element for hematopoiesis. However, iron overload can lead to the accumulation of reactive oxygen species (ROS) and exert a suppressive effect on the bone marrow microenvironment and differentiation of human $CD34^+$ cells, thus disrupting hematopoiesis.[18] Moreover, iron is also an important nutrient for bacteria and fungi, and, as such, iron overload makes patients more susceptible to infections.[19] Serum ferritin (SF) is a form of iron storage and has been used widely as a biomarker of iron overload. A recent study, identified the SF level >2000 ng/ml as an independent risk factor for primary PGF (OR = 4.147; 95% CI: 1.452–11.845; $p = 0.008$), and the patients of SF level >2000 ng/ml had a poor 1-year overall survival.[3]

Splenomegaly is reportedly another risk factor of primary PGF,[3,20,21] although the underlying diseases are heterogeneous. The possible mechanism might be that enlarged spleen could trap and destroy hematopoietic cells. Splenomegaly defined as splenic thickness >4 cm or craniocaudal length >12 cm is an independent risk factor for PGF (OR = 3.306; 95% CI:1.062 to 10.289; $p = 0.039$).[3] Alchalby *et al.* suggested that the

persistence of significant splenomegaly (≥10 cm under costal margin) at day +30 after HSCT exhibits a higher incidence of PGF (33% *versus* 12%; $p = 0.05$) in patients with myelofibrosis.[20] Enlarged spleen (>320 cm^3) is linked to low neutrophil and platelet engraftment after allo-HSCT in patients of acute myeloid leukemia and myelodysplastic syndrome.[21] Interestingly, Akpek *et al.* found that, when a $CD34^+$ cell dose $>5.7 \times 10^6$/kg is transplanted, delayed engraftment caused by splenomegaly can be counteracted,[22] indicating that higher doses of $CD34^+$ cells should be considered for patients with an enlarged spleen.

Cytomegalovirus infection is a common complication after allo-HSCT, and occurs in approximately 30–70% patients.[23] CMV can inhibit hematopoiesis directly by infecting bone marrow or suppress hematopoiesis indirectly through the infection of stromal cells.[23,24] Moreover, the treatment of CMV infection, especially with ganciclovir, exhibits bone marrow suppressive effects.[25] Clinical data showed that patients with CMV infection after allo-HSCT are of a higher risk of PGF (OR = 9.146; 95% CI: 1.513–55.276; $p = 0.016$).[26] In secondary PGF, CMV reactivation is also an independent risk factor (HR = 7.827; 95% CI: 2.002–30.602; $p = 0.003$).[9] Human herpesvirus 6 (HHV-6) is closely related to human cytomegalovirus and can affect over 90% of healthy individuals during childhood.[27] It is recognized in 30% to 60% allo-HSCT recipients, especially in those of unrelated cord blood graft.[27,28] Moreover, HHV-6 infection is reportedly associated with delayed engraftment, especially in early reactivating patients.[27–30]

GVHD is an immune-associated complication after allo-HSCT, and the bone marrow microenvironment is impaired in GVHD patients.[31] A previous study showed that patients with GVHD (grades I–IV) have significantly lower platelet counts on day +50 post-HSCT.[6] Peralvo *et al.* demonstrated that PGF is associated with acute GVHD (aGVHD) grade II or more and that aGVHD is the major risk factor ($p = 0.001$) for the development of PGF.[32] Moreover, in patients with PGF, hemopoietic recovery is strongly associated with the resolution of aGVHD. Other risk factors reported by previous studies include elder age, mismatch of donor-recipient blood type, advanced underlying diseases, the intensity of pre-conditioning regimen, HLA-disparity, etc.[5,8,13,21]

The mechanism

Hematopoiesis is a dynamic process throughout the lifetime as blood cells are continually replenished. Self-renewal and directed differentiation are two cardinal properties of hematopoietic stem cells (HSCs). The BM microenvironment, also known as the HSC niche, can maintain and regulate HSC behavior, such as proliferation, maintenance, retention, and quiescence.[33–37] The HSC niche was first proposed by Schofield in 1978, and has been widely studied since then, especially in the past decades.[38] Earlier studies revealed a mostly endosteal location for HSCs, while osteoblastic cells are the first cell type shown to influence hematopoietic stem-cell frequency.[39–41] However, more recently, accumulating studies demonstrated that the majority of HSCs located in the perivascular and highly vascular endosteal region.[34,36,42,43] Because HSCs are located near blood vessels, it is critical to investigate the perivascular microenvironment (including stromal cells, cytokines, etc.). Perivascular stromal cells mainly include mesenchymal stem cells (MSCs), endothelial cells (ECs), adipolineage cells, megakaryocytes, macrophages, regulatory T cells (Tregs), and osteoblasts. Recent studies in the murine models indicated that mesenchymal stem cells, endothelial cells, Treg cells, macrophages, and the cytokines (SCF and CXCL12) secreted by these cells are key components of the niche in the regulation of HSCs.[44–47] Moreover, evidence has been presented that HSCs reside within relatively hypoxic domains of bone marrow.[42,48,49] Although the mechanism underlying PGF has not been elucidated, the BM microenvironment has been recently reported as crucial in the pathogenesis of PGF.

Endothelial cells

Endothelial cells are important stromal cells around the vasculature. Zeng *et al.* suggested that endothelial progenitor cells (EPCs) can accelerate the recovery of BM vasculature and cellularity and facilitate hematopoietic and immune reconstitution in murine models.[50] Recently, Kong *et al.*[51,52] performed two prospective studies and demonstrated that the number of BM ECs was significantly reduced in PGF patients both primarily and

secondarily. Moreover, low count of endothelial progenitor cells is an independent risk factor for secondary PGF (OR = 150.72; 95% CI: 7.85–2893; $p = 0.001$). In addition, BM ECs in subjects with PGF are not only deficient but also dysfunctional, with impaired proliferation, migration, and angiogenesis, and higher levels of ROS and apoptosis.[53] In summary, the impairment of BM ECs may contribute to the occurrence of PGF after allo-HSCT.

Mesenchymal stem cells

Mesenchymal stem cells are crucial components of the perivascular niche to support and regulate HSCs.[35,37,44] An earlier case-control study by Song *et al.* showed that BM MSCs from PGF patients decrease in frequency and exhibit more apoptosis and senescence.[54] In addition, intracellular ROS, p-p53, and p21 levels were elevated in MSCs from PGF patients. Furthermore, the impairment of MSCs results in the deficient ability to sustain hematopoiesis in PGF patients. Therefore, these data indicated that MSCs may be impaired in PGF patients after allo-HSCT and that improvement of BM MSCs may provide a promising therapeutic strategy.

Elevated ROS levels

The BM microenvironment or the HSC niche is normally hypoxic and maintains the essential HSC functions, such as cell cycle control, survival, and metabolism by protecting HSCs against oxidative stresses.[42,55] A series of studies showed that, although similar numbers of donor $CD34^+$ cells were transplanted, with the function of HSCs pre-HSCT being similar, the percentages of BM $CD34^+$ cells in PGF patients were significantly lower compared with those in GGF patients after allo-HSCT.[51,52,56] Furthermore, elevated ROS levels are reportedly associated with the exhaustion of quiescent $CD34^+$ cells in subjects with PGF following allo-HSCT.[56] These findings suggested that elevated ROS might cause exhaustion of quiescent BM $CD34^+$ cells in PGF patients even if $CD34^+$ cells from donors are functionally normal pre-HSCT.

Immune abnormalities

Increasing evidence showed that the BM immune microenvironment is vital for the regulation of hematopoiesis.[57–59] A recent case-control study revealed a significant increase in M1 (classically activated inflammatory macrophages) and a striking reduction in M2 (alternatively activated anti-inflammatory macrophages) in PGF patients compared with those with GGF.[60] The functions of BM macrophages, such as proliferation, migration, and phagocytosis, were impaired in PGF patients. Moreover, when cocultured with BM macrophages from PGF patients, the function of $CD34^+$ cells was impaired through the upregulation of the p38 MAPK pathway. Two recent studies revealed that the dysregulated T cell responses may also contribute to the occurrence of PGF after HSCT.[61,62] Compared with GGF patients and healthy donors, patients with PGF contain significantly higher proportions of Th1 and Tc1 cells (which produce IFN-γ) and reduced proportions of Th2 and Tc2 cells (which produce IL-4), leading to a shift of Th1/Th2 and Tc1/Tc2 ratios towards a type 1 response.[61] Furthermore, Th17 and Tc17 cells, which produce IL-17, are significantly elevated in PGF patients. However, Tregs, which are considered as suppressor T cells and key players in regulating immune responses, are comparable in PGF and other subjects. Thus, the Th17/Treg ratio is elevated dramatically in PGF patients.[62] Also, in patients with prolonged thrombocytopenia after allo-HSCT, the levels of inflammation-associated cytokines, including IGFBP1 (insulin-like growth factor–binding protein 1) and RANTES (regulated on activation, normal T cell expressed and secreted) were elevated, which impaired the megakaryocytic potential of HSCs.[63] Recently, immune mechanisms have been suggested in some donor-type late graft failure with full cherism (secondary PGF), including increased glycosylphosphatidyl inositol-anchored protein-deficient ($GPI\text{-}AP^-$) leukocytes and HLA-allele-lacking leukocytes. Furthermore, in patients with increased $GPI\text{-}AP^-$ cells, hematopoiesis may be restored by anti-thymocyte globulin (ATG) therapy alone without further donor stem-cell infusion, indicating immunosuppressive therapy should be considered in these patients.[64] Together, immune responses are more active in PGF patients, and the BM immune microenvironment might play an important role in PGF.

Treatments

Traditional treatments for PGF patients include mainly the administration of hematopoietic growth factors, donor cell infusion, and second

allo-HSCT, but efficacy is limited. Granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO) can improve the number of neutrophils and hemoglobin levels, but they are usually effective in the short term, and persistent treatment can lead to alloimmunization and transfusion-related iron overload. The second transplantation and donor cell infusion have shown efficacy. However, long-term survival has not been significantly improved due to the high morbidity of severe GVHD.[65,66] To reduce the risk of acute and chronic GVHD, CD34$^+$-selected stem-cell boost (SCB) has become an alternative treatment for patients with PGF.[5,67,68] More recently, with an improved understanding of the mechanisms, MSCs infusion and other new strategies have emerged and demonstrated promising efficacy and good tolerance. Comparisons of recent clinical research in the treatment of PGF are shown in Table 1.

CD34$^+$-selected SCB

Over the past decades, most studies of the treatment of PGF have been focused on the use of CD34$^+$-selected stem-cell boost without preconditioning, leading to favorable outcomes. Stasia *et al.*[67] reported that, after 41 PGF patients were treated with CD34$^+$-selected SCB of different donor types, the overall response rate and trilineage recovery rate were 83% and 75%, respectively, with no significant disparity in different donor subgroups. More importantly, the procedure was safe, with a low risk of grade II acute GVHD (15%) and patients with previously chronic GVHD did not worsen after infusion. A small-scale study by Haen *et al.* included 20 adult patients with PGF after allo-HSCT,[68] which were treated with selected CD34$^+$ SCB by immunomagnetic beads from matched unrelated, mismatched unrelated, or haploidentical donors. They reached rapid engraftment in approximately 90% of all patients, after a median of 14, 13, and 18 days for platelets, leukocytes, and hemoglobin, respectively. In a long-term follow up, the improvement of hematopoiesis (92%) is more favorable compared with previous results. Moreover, the researchers did not observe any SCB-related toxicities and found only very limited complications (e.g. only one patient developed GVHD). These advantages may attribute to CD34$^+$-selected SCB with as low T cell numbers as possible. Another study focused mainly on pediatric patients ($n=50$) also identified good tolerance and efficacy.[70] To generate SCB, donors traditionally must undergo an additional peripheral blood stem-cell (PBSC) collection. However, realistically, many patients might not be able to receive fresh SCB if their donors are unwilling or unable to accept another PBSC collection. Under these circumstances, cryopreserved cells from the donor's previous collection provide an adequate source to create the CD34$^+$-selected SCB. Therefore, a pilot study comparing fresh with cryopreserved stem-cell products for CD34$^+$-selected boost infusions was conducted.[69] This study suggested that cryopreserved products can also be an effective source for SCB because five of the eight recipients receiving SCB created from cryopreserved products achieve complete responses. Although the number of patients was relatively small, it did provide an alternative for fresh SCB. Moreover, this study showed that the addition of plerixafor can increase CD34$^+$ yield over G-CSF alone. Recently, we also applied CD34$^+$-selected SCB without further conditioning to treat a 26-year-old male patient of refractory secondary PGF successfully. The patient was diagnosed aplastic anemia at the age of 17 then developed treatment-related myelodysplastic syndrome, and eventually progressed to acute myeloid leukemia in need of allo-HSCT. On 11 March 2019, the patient underwent haploidentical-HSCT from his elder sister who had a 7/10 loci match with him. During the perioperative period, the patient experienced severe pulmonary infection, but, with careful anti-infection, anti-inflammatory, and supportive treatments, and the adjustment of the pre-conditioning regimen, the patient achieved neutrophil engraftment on day+15 post-HSCT. Unfortunately, he then developed severe GVHD and secondary PGF. We used MSC infusion, eltrombopag, and hematopoietic growth factors, but the efficacy was not significant. Subsequently, on 29 September, the patient received CD34$^+$-selected SCB from his former donor and acquired hematopoietic recovery 3 weeks later (Figure 1). Given the efficacy of CD34$^+$ SCB, Abboud *et al.*[78] sought to employ CD34$^+$ SCB infusion on day 5–6 after allo-HSCT for patients with a high risk of developing graft failure or poor graft function. They found that CD34$^+$-selected SCB as a preventative approach can facilitate the engraftment and reduce graft failure or poor graft function with an acceptable incidence of severe acute GVHD. Although

Table 1. Comparisons of recent clinical research in the treatment for PGF.

	Treatment	Dose	No. of patients	Response rate	Long-term survival rate	Adverse events/ incidence rate
Stasia *et al.*[67]	$CD34^+$-selected SCB	3.4×10^6/kg (median)	41	83%	3-year survival: 63%	aGVHD (15%)
Haen *et al.*[68]	$CD34^+$-selected SCB	4.6×10^6/kg (median)	20	90% in platelets 95% in leukocytes 90% in hemoglobin	2-year survival: 53%	aGVHD (5%)
Ghobadi *et al.*[69]	$CD34^+$-selected SCB	3.1×10^6/kg (G-CSF only) 10.9×10^6/kg (G-CSF plus plerixafor) 1×10^6/kg (cryopreserved products)	26	81%	1-year survival: 65%	aGVHD (23%) cGVHD (31%)
Mainardi *et al.*[70]	$CD34^+$-selected SCB	3.15×10^6/kg (median)	50	78.8%	5-year survival: 38.67%	aGVHD (6%)
Cuadrado *et al.*[71]	$CD34^+$-selected SCB	3.2×10^6/kg (median)	62	75.8%	5-year survival: 54%	aGVHD (11%) cGVHD (8%)
Liu *et al.*[72]	MSC	1×10^6/kg (1–3 times)	20	85%	508 days: 45% (median follow-up time)	Infection (65%) CMV DNA viremia (10%) EBV DNA viremia (35%) EBV-associated PTLD (15%) GVHD (15%)
Servais *et al.*[73]	MSC	$1\text{-}2\times10^6$ /kg (single time)	30	51.8% (day90) 69.2% (day180)	1-year survival: 70%	No severe adverse event
Tang *et al.*[74]	Eltrombopag	Initiated at 25 mg/day for 3 days and then increased to 50 or 75 mg/d	12	83.3%	1-year survival: 83.3%	No severe adverse event
Fu *et al.*[75]	Eltrombopag	initiated at 25 or 50 mg/day and adjusted to a maximum of 50–100 mg/day	15	60.0%		No severe adverse effect
Marotta *et al.*[76]	Eltrombopag	initiated at 50 mg/day, and adjusted to 150 mg	12	58.3%		Skin hyperpigmentation (8.3%)
Olivieri *et al.*[77]	Deferasirox	750 mg bid (–72 day) 625 mg/d (+89 day) and increased to 1250 mg/d	1	100%		Not mentioned

$CD34^+$-selected SCB indicates $CD34^+$ -selected stem-cell boost; CMV, cytomegalovirus; EBV, Epstein–Barr virus; GVHD, graft *versus* host disease; aGVHD, acute GVHD; cGVHD, chronic GVHD; MSC, mesenchymal stem cell; PGF, poor graft function; PTLD, posttransplant lymphoproliferative disorders.

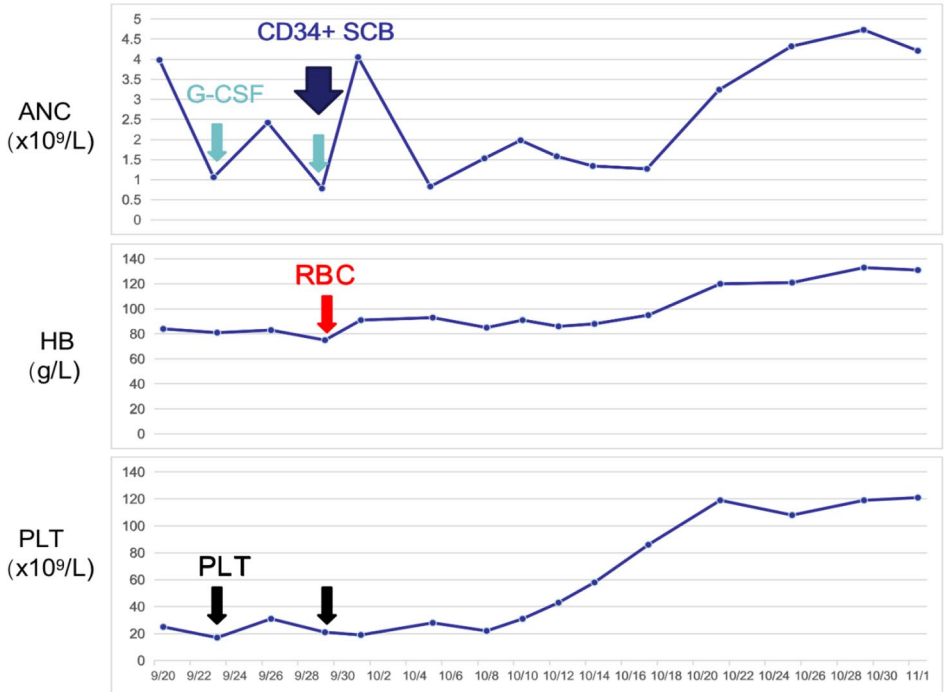

Figure 1. Dynamic changes of blood cells after CD34⁺-selected SCB infusion and supportive treatments.
ANC, absolute neutrophil count; HB, hemoglobin; PLT, platelet; RBC, red blood cell; SCB, stem-cell boost.

numerous studies suggested that the infusion of CD34⁺-selected SCB is an effective way to correct poor graft function, the predictors of efficacy are unclear. A recent study ($n = 62$) showed that in multivariate analysis, CMV seronegative status, the absence of active infection, and matched recipient/donor gender are favorable parameters that are strongly associated with the efficacy for CD34⁺-selected SCB.[71] To sum up, CD34⁺-selected SCB provides an important therapeutic option with limited adverse effects for PGF following allo-SCT.

Mesenchymal stem cells

As indispensable components of the BM microenvironment, mesenchymal stem cells are critical in the maintenance and regulation of hematopoiesis, while MSC infusion has recently been applied to the treatment of PGF. A study by Song *et al.* indicated that dysfunction of BM MSCs might play a role in deficient hematopoiesis in PGF patients, and, as such, the treatment with MSCs might be an effective strategy.[54] MSC treatment is much more convenient than CD34⁺-selected SCB and is without immunogenicity. MSCs are not required to be collected from the original donor necessarily. Instead, they can be collected from a third-party donor. A recent study ($n = 30$) presented at the ASH (American Society of Hematology) meeting in 2019 showed good tolerance and efficacy.[73] The response rates at day 90 and day 180 were 51.8% (95% CI: 33.0–70.7%) and 69.2% (95% CI: 51.5–86.9%), respectively. The overall survival at 1 year after MSC treatment was 70% (95% CI: 53.6–86.3%), and no severe adverse event was reported. In a prospective study of 20 patients with PGF after allo-HSCT treated with third-party MSCs, 17 achieved hematopoietic recovery, whereas 13 developed infections, including bacterial infection, invasive fungal infection, viral infection and mixed infections.[72] Within the first 100 days of post-MSC treatment, five patients developed cytomegalovirus-DNA viremia, and seven developed Epstein–Barr virus (EBV)–DNA viremia. Moreover, three patients in the EBV-DNA viremia group later developed EBV-associated posttransplant lymphoproliferative disorders (PTLD). GVHD occurred in three patients, and there was no short-term toxicity. Based on these findings, we conclude that MSCs might be beneficial in the treatment of PGF patients, but whether the

treatment might increase the risk of infections needs further research.

New strategies

In addition to CD34$^+$ SCB and MSCs, new drugs have also developed rapidly in recent years as new treatments. Because basic research suggested that impaired endothelial cell functions might contribute to PGF after allo-HSCT, a series of studies has been focused on the topic.[53,79] Atorvastatin, a lipid-reducing drug, is used widely to treat dyslipidemia clinically. Furthermore, it reportedly improves endothelial progenitor cells (EPCs) in several diseases. Recent studies showed that atorvastatin treatment can also improve BM EPCs from patients with PGF quantitatively and functionally *in vitro*.[52] The *in vivo* function is yet to be confirmed. In murine models, N-acetyl-L-cysteine (NAC) can facilitate the engraftment of hematopoietic stem cells by reducing the level of ROS.[80] In keeping with these findings, a clinical study suggested that NAC can safely promote impaired BM EC functions in PGF patients.[79] A total of 35 patients with EC $< 0.1\%$ were treated with NAC prophylactically and demonstrated the reconstitution of BM ECs and CD34$^+$ cells with decreased incidence of PGF. Eltrombopag, a novel oral thrombopoietin (TPO) receptor agonist, can stimulate the TPO receptor (c-mpl) in both HSCs and megakaryocytes. Thus, it can promote the production of megakaryocytes and platelets and has been used in the treatment of aplastic anemia and post-transplantation thrombocytopenia. In addition, several case reports declared good efficacy of eltrombopag in treating PGF patients and shed light on subsequent clinical trials.[81,82] Tang *et al.* treated 12 secondary PGF patients with eltrombopag, and 10 patients (83.3%) responded to the treatment, of which 8 achieved complete response.[74] The overall survival rate of 12-month was 83.3%, and no treatment-related mortality was identified. A recent single-center study ($n = 12$) suggested that 7 of 12 patients achieved a hematological response, and complete response could be seen in 6 patients.[76] Moreover, treatment with eltrombopag was discontinued in 6/7 patients, and the response remained stable without further relapse. A study conducted in China has been focused on the treatment with eltrombopag for patients ($n = 38$) of refractory thrombocytopenia after haploidentical-HSCT,[75] among which 15 patients were PGF. Of 15 PGF patients, 9 achieved trilineage response after eltrombopag treatment, and the duration of the response was relatively long after treatment withdrawal. Moreover, the researchers found that the presence of megakaryocytes in the BM before initiation was associated with the response to eltrombopag (68.0% *versus* 23.1%, $p = 0.015$). Five patients developed liver injury during treatment, but they all had clear causes (e.g. hepatitis E, hepatic chronic GVHD, and severe infection). Furthermore, although some patients in the study had GVHD, none of them discontinued eltrombopag, suggesting that eltrombopag can be well tolerated in patients with GVHD. All the studies showed good tolerance and no severe adverse events. Iron overload is reportedly a risk factor of PGF, and iron-cheating, therefore, may facilitate hematopoietic reconstruction. Previous studies indicated that iron chelation therapy can lead to a hematopoietic response in some patients with aplastic anemia and acute leukemia.[83,84] Recently, Olivieri *et al.* demonstrated that a patient diagnosed with PGF, whose underlying disease was severe aplastic anemia, achieved complete hematopoietic recovery after iron chelation treatment with deferasirox.[77]

Conclusion

PGF is a serious complication with high mortality after allo-HSCT, while PGF patients have poor survival without effective treatment. Over the past decades, many studies have sought to identify risk factors pre-HSCT and suggested that low dose of infused CD34$^+$ cells, DSA, CMV infection, GVHD, iron overload, and splenomegaly, among others, are the most important. Physicians should pay attention to risk factors before allo-HSCT, and take measures to prevent the occurrence of PGF and eventually improve survival. Although there are discrepancies in the criteria used to define PGF in previous studies, we should nonetheless be cautious about the potential risk factors, especially those overlapping among different studies. Moreover, large-scale studies with uniform diagnosis criteria are needed to verify the findings. Although the mechanism of PGF has not been well elucidated, various studies indicated that the BM microenvironment abnormalities might play a crucial role in the pathogenesis of PGF, and the treatments aimed at improving the BM microenvironment can, therefore, facilitate hematopoietic recovery. These findings have

also prompted the development of new treatment strategies. New advances in the treatment of PGF after allo-HSCT in recent years have led to three main conclusions. Firstly, $CD34^+$ SCB (fresh or cryopreserved) without further conditioning is safe and effective in the treatment of PGF patients, and CMV seronegative status, absence of active infection and matched recipient/donor gender are favorable factors for efficacy. Secondly, mesenchymal stem cells can also improve the prognosis of PGF, but whether they can increase the risk of infections needs further studies. Finally, new strategies including NAC, eltrombopag, deferasirox, and atorvastatin, have shown good tolerance and efficacy. However, the sample size of these studies was relatively small, and some of the treatments only have produced results in the laboratory settings but not clinical settings. In the future, large-scale randomized and controlled clinical trials are needed to confirm the results. Furthermore, the potential efficacy of the therapies combining these new strategies to improve graft functions should be investigated.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was funded by grants from the National Megaproject on Key Infectious Diseases (grant number 2017ZX10202102); the CAMS Initiative for Innovative Medicine (grant number 2016-I2M-1-017; 2016-I2M-1-018; 2019-I2M-1-006), Tianjin Municipal Natural Science Foundation (grant number 18JCZDJC34400) and the Nonprofit Central Research Institute Fund of Chinese Academy of Medical Sciences (grant number 2018PT32034).

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