

# Clinical features, pathophysiology, and therapy of poor graft function post-allogeneic stem cell transplantation

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Poor graft function (PGF), defined by the presence of multilineage cytopenias in the presence of 100% donor chimerism, is a serious complication of allogeneic stem cell transplant (alloSCT). Inducers or potentiators of alloimmunity such as cytomegalovirus reactivation and graft-versus-host disease are associated with the development of PGF, however, more clinical studies are required to establish further risk factors and describe outcomes of PGF. The pathophysiology of PGF can be conceptualized as dysfunction related to the number or productivity of the stem cell compartment, defects in bone marrow microenvironment components such as mesenchymal stromal cells and endothelial cells, or immunological suppression of post-alloSCT hematopoiesis. Treatment strategies focused on improving stem cell number and function and microenvironment support of hematopoiesis have been attempted with variable success. There has been limited use of immune manipulation as a therapeutic strategy, but emerging therapies hold promise. This review details the current understanding of the causes of PGF and methods of treatment to provide a framework for clinicians managing this complex problem.

## Introduction

“Engraftment” refers to 2 key measures post-allogeneic stem cell transplantation (alloSCT): the restoration of peripheral blood counts and confirmation of exclusive donor hematopoiesis via assessment of chimerism. Poor graft function (PGF) is the clinical syndrome of persistent cytopenias despite evidence of complete donor chimerism post-alloSCT. This is in contrast to graft failure due to graft rejection because of retained recipient immune-effector mechanisms manifested by loss of donor chimerism.<sup>1</sup> The quoted incidence of PGF is 5% to 27%, however, understanding the true burden of PGF and its pathophysiology has been difficult due to the inclusion of patients with graft failure in previous studies.<sup>2,3</sup> The clinical course of PGF varies from spontaneous recovery to death due to complications of cytopenias and thus presents a challenge to treating clinicians. This review will summarize the current knowledge regarding the clinical features, pathophysiology, and therapy of PGF.

## PGF is distinct from graft rejection

There are multiple engraftment syndromes post-alloSCT that can be classified depending on the level of donor chimerism: (1) graft rejection (<5% donor chimerism), (2) cytopenias with mixed chimerism, and (3) PGF (>95% donor chimerism). The European Society for Blood and Marrow Transplantation (EBMT) was the first transplant society to officially define PGF as “two or three cytopenias, >2 weeks after day +28 in the presence of >95% donor chimerism.”<sup>4</sup> However, despite the EBMT definition and other

consistent published definitions,<sup>5,6</sup> PGF was included under the umbrella of graft failure in prior publications.<sup>2</sup> Nomenclature surrounding the attainment of engraftment following allogeneic transplantation is problematic. In our view, PGF, in which there is clear evidence of donor cell survival in the recipient, should be considered a subtype of graft failure distinct from graft failure due to graft rejection. Graft rejection occurs when residual recipient immunity, including cytotoxic T cells, natural killer cells, and/or donor-directed antibodies, eradicates incoming donor hematopoietic stem cells (HSCs).<sup>1</sup> In addition to complications of cytopenias, the absence of donor chimerism in graft rejection compared with PGF increases the risk of relapse of the underlying condition for the recipient.<sup>7</sup> Cytopenias in the setting of mixed donor chimerism is also described post-alloSCT and appears to occur with mixed T-cell chimerism and complete donor myeloid chimerism.<sup>8</sup> Whether the mechanisms underlying PGF are the same as cytopenias in the setting of mixed chimerism is unclear.

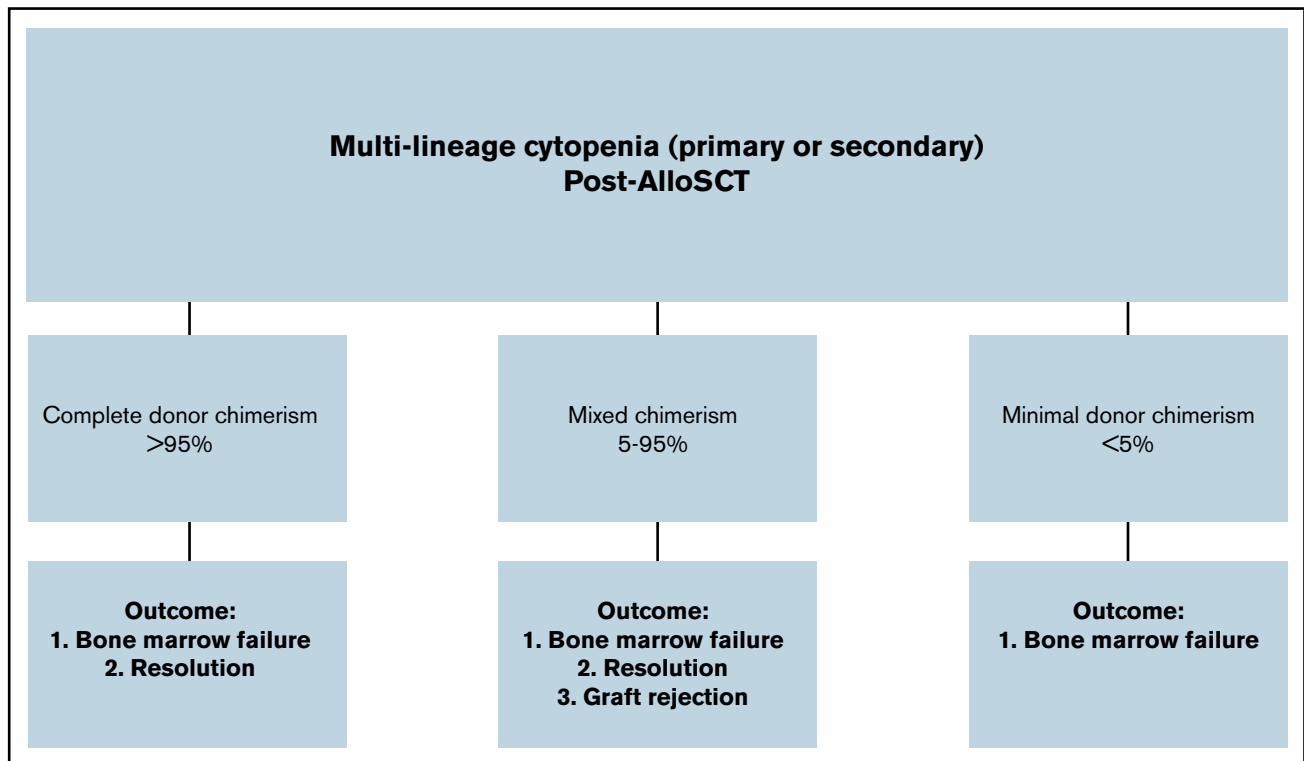
The focus of this review is PGF as defined by multilineage cytopenias in the setting of complete donor chimerism as illustrated in Figure 1, which classifies the different engraftment syndromes post-alloSCT.

### Risk factors for the development of PGF and clinical outcomes post-PGF

Publications assessing risk factors and outcomes associated with PGF are summarized in Table 1. Variability in PGF definitions between studies has resulted in the identification of different risk factors and outcomes linked to PGF. One aspect of variability is the depth and duration of cytopenias required to fulfill PGF definitions. Of note, the

EBMT does not specify cytopenia thresholds for their definition. Thresholds are critical elements in identifying which patients will have cytopenias that will persist as opposed to those that will be transient or display recovery kinetics. The presence or absence of neutrophil recovery (secondary vs primary PGF) also influences outcome. Increased nonrelapse mortality (NRM) would be expected in PGF without neutrophil recovery because of the risk of infective complications. Although risk factors associated with PGF appeared varied between publications, risk factors such as age, cytomegalovirus (CMV) viremia, hyperferritinemia, ABO incompatibility, early intensive care unit admission, blood culture positivity, nonsibling donor, and graft-versus-host disease (GVHD) are associated with either increased or potentiation of alloimmunity and may suggest an immunologic basis to PGF.

Regarding outcomes, although many publications presented demonstrate heterogeneous outcomes following establishment of PGF, a recent study by our group showed that patients who did not recover marrow function had a 2-year overall survival (OS) of 6%.<sup>9</sup> Outside of PGF-specific publications, there are numerous retrospective studies evaluating risk factors for “graft failure” defined by a variety of terms including neutropenia or thrombocytopenia irrespective of level of donor chimerism.<sup>2,3</sup> This definition of “graft failure” would encompass patients with PGF; splenomegaly, CMV infection, multiorgan dysfunction due to sepsis, as well as low stem cell dose have been found as possible associations.<sup>10</sup> Outcomes in these “graft failure” studies are poor, with 5-year survival of 15% to 35%. Unfortunately, patients with PGF alone cannot be separated in these data. Clearly, further studies are required to understand the risk factors and outcomes of patients



**Figure 1. Approach to engraftment syndromes post-alloSCT.** A method of classifying engraftment syndromes post-alloSCT based on level of donor chimerism present.

**Table 1. Recent publications evaluating risk factors and outcomes of PGF**

Author and year	Type of study	Definition	Population	Risk factors for PGF	OS	Rates of relapse, %	NRM, %
Xiao et al, 2014 <sup>66</sup>	Retrospective study	2 cytopenic lines at day 30; platelets < 30*; neutrophils < 1.0*, Hb < 100*	124 alloSCT recipients 15 with PGF	Older age ABO mismatch CMV infection	NR	NR	NR
Sun et al, 2015 <sup>87</sup>	Retrospective case control study	2 cytopenic lines; 3 consecutive days after day 28; platelets ≤ 20; neutrophils ≤ 0.5; Hb ≤ 70; hypoplastic bone marrow, no concomitant GVH	484 haploidentical transplant recipients 26 with primary PGF	No significant variables on multivariate analysis	34% at last follow-up	3	62
Alchalby et al, 2016 <sup>42</sup>	Retrospective study	2 cytopenic lines for 2 consecutive weeks after day 14; platelets ≤ 30, neutrophils ≤ 1.5, Hb ≤ 85; hypoplastic marrow, no GVHD, no CMV	100 alloSCT recipients with myelofibrosis 17 with PGF	Age Splenomegaly	3-y OS 64%	N/A	12
Zhao et al, 2019 <sup>14</sup>	Retrospective nested case control study	Primary PGF as per Sun et al	Population of 830 alloSCT patients 24 with PGF	Cell dose < 5 × 10 <sup>6</sup> /kg Hyperferritinemia Splenomegaly	20% at a median of 7.8 mo	17	63
Reich-Slotky et al, 2020 <sup>88</sup>	Retrospective study	Platelets < 20; neutrophils < 0.5; Hb < 100 or red cell transfusion dependence at day 100 No minimum duration of cytopenias Complete donor chimerism not required, however, most patients were donor chimeric	104 patients who received T-cell deplete 54 with PGF	ABO mismatch CMV viremia Acute GVHD II-IV	2-y OS 66% for entire cohort	NR	45
Prabhan et al, 2021 <sup>9</sup>	Retrospective study	2 cytopenic lines for 30 d from day 30; Hb < 90 g/L, neutrophils < 1.0, platelets < 100	819 patients who received alloSCT from single center; 106 with PGF	Nonsibling donor ICU admission Positive blood cultures first 30 d Acute GVHD CMV viremia	2-y OS of 56% in PGF cohort 2-y OS of 6% in those without count recovery	NR	44

CMV, cytomegalovirus; GVHD, graft-versus-host disease; Hb, hemoglobin; ICU, intensive care unit; N/A, not applicable; NR, not reported; NRM, nonrelapse mortality; OS, overall survival.  
\*Platelets and neutrophils reported as × 10<sup>9</sup>/L; Hb reported as g/L.

who develop PGF exclusively. Studies aimed at validating current definitions of PGF are also required.

Deciding when to intervene in a patient who is unlikely to recover is also an important consideration in determining outcome following the diagnosis of PGF. In our study, we demonstrated that most patients recovered blood counts at a median of 230 days with a large range of 23 to 1146 days. This is consistent with another study evaluating the role of CD34 selected infusions in the treatment of PGF, in which the maximal cumulative incidence of recovery (40%) was reached only at 24 months post-alloSCT in the arm of the study that did not receive any treatment. The depth of the cytopenias with associated complications, such as infections or bleeding, as well as the need for supports such as transfusions may be more relevant to the decision to intervene than the duration of cytopenias. Indeed, in our study, we demonstrated that a platelet count of ≤ 60 × 10<sup>9</sup>/L or hemoglobin (Hb) < 80 g/L was associated with mortality after establishment of PGF.<sup>9</sup> To resolve the question of whether depth vs duration of cytopenias is important in deciding when to treat PGF, further prospective studies evaluating clinical or biomarker-based associations with poor outcome would be useful to determine who will not improve without intervention.

## Proposed pathophysiology of PGF: seed, soil, and climate model

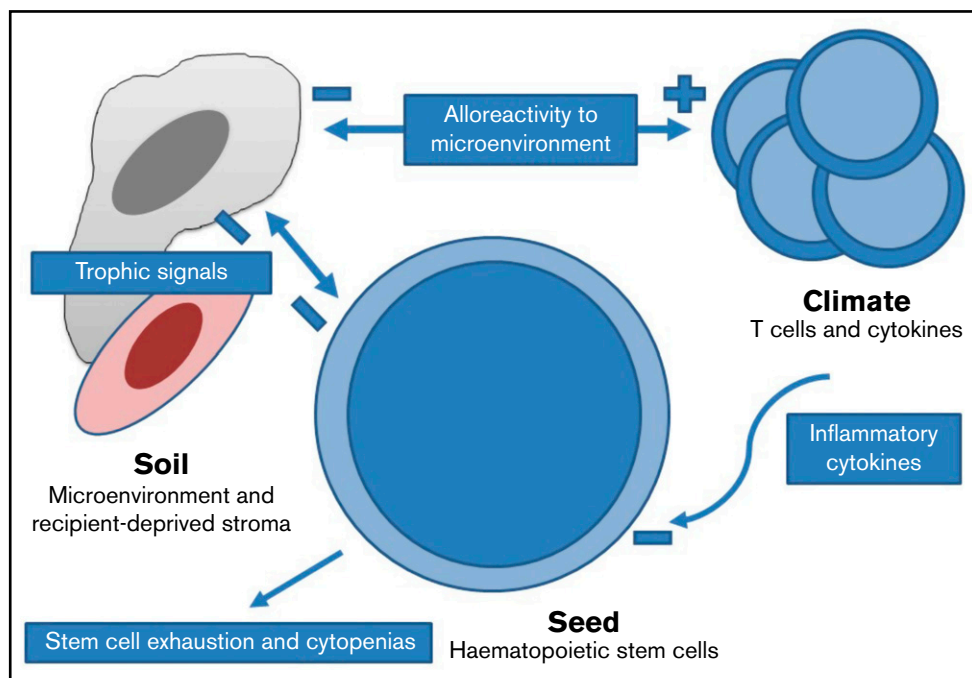
Figure 2 describes the mechanisms resulting in PGF. Table 2 provides the evidence for these proposed mechanisms as well as therapeutic strategies targeted against these mechanisms.

### HSCs (seed)

HSCs are the progenitor cell type from which mature blood cells arise.<sup>11</sup> Based on current literature, quantitative and qualitative HSC abnormalities may have a causative role in PGF.

Low stem cell dose in the graft may be associated with PGF. In a retrospective analysis of factors influencing count recovery after alloSCT with predominantly bone marrow grafts, a total nucleated cell dose of < 4.1 × 10<sup>8</sup>/kg was associated with thrombocytopenia at day 50 (74 × 10<sup>9</sup>/L vs 102 × 10<sup>9</sup>/L) and up to a year posttransplant.<sup>12</sup> Anemia and neutropenia at day 28 were associated with a CD34<sup>+</sup> dose of < 6.4 × 10<sup>6</sup>/kg in a subsequent study involving mainly peripheral blood stem cell grafts.<sup>13</sup> In the first study, 94% of patients were donor chimeric whereas in the second, chimerism data were not available; PGF was not assessed as an outcome in either study. A recent study of risk factors for primary PGF demonstrated that a lower CD34<sup>+</sup> cell dose, 4.42 × 10<sup>6</sup>/kg vs 6.99 × 10<sup>6</sup>/kg in comparators, was associated with development of PGF.<sup>14</sup> A lower number of transfused and hence engrafted HSCs may be more susceptible to the attrition by external forces and thus contribute to the development of PGF.

Another potential factor that may be associated with the development of PGF is use of alternate donor sources such as umbilical cord blood and cryopreserved grafts. There are no studies evaluating PGF as an outcome of receiving cord blood as a stem cell source. It is well established that time to neutrophil and platelet engraftment is longer in cord blood transplants.<sup>15</sup> Furthermore, treatment-related mortality appears higher in cord blood transplants compared with matched unrelated donors and haploidentical donors, primarily due to infection.<sup>16,17</sup> Whether infection causing treatment-related mortality is accompanied



**Figure 2. Seed, soil, and climate model of PGF.** Proposed pathophysiology of PGF based on the interplay of key components of the bone marrow. Seed (HSCs), soil (microenvironment), and climate (T cells and cytokines). T cells target and suppress microenvironment cells and actively suppress HSC function through inflammatory cytokines and loss of microenvironment trophic signals.

**Table 2. Evidence for seed, soil, and climate: proposed therapeutic interventions and potential therapies**

Cell type/Proposed mechanism	Evidence and citation	Proposed therapies
<b>HSC (seed)</b>		
Acquired HSC dysfunction	Case-control studies GGF vs PGF <sup>89</sup>	CD34-selected cell infusions
Reduced number of infused HSC	Retrospective cohort studies <sup>10,13</sup>	TPO agonism
Loss of bone marrow microenvironment regulation by critical HSC progeny such as megakaryocytes and neutrophils	Animal models <sup>24,90-92</sup>	
<b>Nonhematopoietic stromal cells/bone marrow microenvironment (soil)</b>		
Loss of stromal signals due to cellular dysfunction	Case-control studies GGF vs PGF <sup>6,27,33,93</sup>	NAC Atorvastatin
Stromal dysfunction due to previous hematologic malignancy	Animal models <sup>94</sup>	Mesenchymal stem cell infusion
<b>Adaptive immunity (climate)</b>		
Proinflammatory T-cell and innate response directed at key NHSC	Animal models <sup>43</sup> Retrospective studies <sup>45</sup> Case-control studies of GGF vs PGF <sup>46,48,62</sup>	ATG JAK inhibition IFN- $\gamma$ blockade
HSC suppression by inflammatory cytokines such as IFN- $\gamma$	Animal models <sup>51,52</sup>	Adoptive cellular therapy (T-regs)
Impaired thymopoiesis and generation of T-regs	Retrospective studies <sup>67</sup>	

ATG, antithymocyte globulin; GGF, good graft function; IFN- $\gamma$ , interferon- $\gamma$ ; NAC, *n*-acetyl cysteine; NHSC, non-hematopoietic stromal cell; TPO, thrombopoietin; T-reg, regulatory T cell.

by PGF in these studies is unclear. Similarly, there are no studies evaluating whether PGF specifically occurs more frequently in frozen vs fresh donations. However, the data regarding rates of unspecified graft failure are conflicting between studies, which may be accounted for by differences in population demographics such as graft source and indications for alloSCT. Most data with heterogeneous transplant populations biased toward unrelated donors with predominantly peripheral blood stem cell grafts suggest no differences in neutrophil and platelet recovery as well as rates of graft failure with frozen grafts.<sup>18</sup> A recent Center for International Blood and Marrow Transplant Research (CIBMTR) study of 52 recipients who received mainly cryopreserved bone marrow grafts for aplastic anemia (AA) demonstrated higher rates of unspecified graft failure and 1-year mortality in recipients of frozen grafts.<sup>19</sup> There was significant attrition in the total nucleated cell count postthawing in the recipients of bone marrow grafts, which may be an explanation for the findings in the study. A recent study demonstrated that thawing affected CD34<sup>+</sup> cell viability but neutrophil recovery did not appear to be impacted even in those with <50% CD34<sup>+</sup> cell recovery.<sup>20</sup> However, the long-term effect on graft function of this variable recovery is unknown. The question of engraftment quality and PGF associated with frozen vs fresh HSCs is particularly relevant, especially with disruptions related to the COVID-19 pandemic, and requires further evaluation.

Intrinsic HSC dysfunction has also been associated with PGF. An *ex vivo* study demonstrated increased quantities of intracellular reactive oxygen species (ROS), DNA damage, and expression of apoptotic proteins in PGF HSCs compared with those with good graft function (GGF). Stored CD34<sup>+</sup> cells from donors whose recipients developed PGF were xenografted to mice and no deficits were found in the cells' capacity to repopulate the marrow.<sup>21</sup> This suggests that the deficits are acquired after transplant. Experimental models demonstrate that HSCs and their progeny play a role in maintenance of the bone marrow microenvironment and thus their absence may lead to a domino effect of worsening bone marrow function. HSCs secrete autocrine factors, such as vascular endothelial growth factor, that can prevent HSC apoptosis<sup>22</sup>; the progeny of HSCs, such as megakaryocytes, have a role in the maintenance of HSC quiescence through the secretion of CXCL4, and assist in HSC expansion postchemotherapy with the production of fibroblastic growth factor. Ablation of megakaryocytes in mice results in increased stem cell cycling and subsequent HSC exhaustion.<sup>23</sup> Neutrophils appear to have a role in promoting HSC quiescence and aiding in postablative recovery.<sup>24</sup> The loss of these growth and regulatory signals because of HSC dysfunction and failure to produce key mature cell types, such as megakaryocytes and neutrophils, may set up a vicious cycle of progressive stem cell loss through excessive cell cycling. Intrinsic HSC dysfunction causes persistence and progression of PGF, but as this dysfunction is acquired in the recipient, it may be a manifestation of another inciting cause.

## Bone marrow microenvironment (soil)

The supporting cell types within the bone marrow microenvironment are critical in regulating the balance between HSC replication and quiescence.<sup>25</sup> Quiescence is required for self-renewal and allows HSCs to limit genotoxic stress leading to exhaustion, thus causing hematopoietic failure.<sup>26</sup> Other cellular types of the bone marrow microenvironment such as osteoblasts, osteoclasts, neuronal tissue, and adipocytes and their role in HSC physiology have been reviewed

elsewhere.<sup>25</sup> Dysfunction of 2 nonhematologic stromal cells, mesenchymal stromal cells (MSCs) and endothelial cells (ECs), has been associated with PGF.<sup>6,27</sup>

MSCs are present in the sinusoidal and perivascular areas and ECs line the vascular endothelium.<sup>28</sup> Both ECs and MSCs express molecules, such as CXCL12 and stem cell factor, needed for stem cell maintenance and engraftment post-alloSCT.<sup>29-31</sup> MSCs may also coordinate the bone marrow microenvironment, as subcutaneously implanted MSCs can generate a heterotopic HSC niche.<sup>32</sup> A case-matched study demonstrated low numbers of perivascular MSCs, ECs, and osteoblasts in the PGF bone marrow microenvironment, which corresponded to a reduction in HSCs. In a related study, *ex vivo* MSCs cultured from patients with PGF demonstrated abnormal morphology, increased intracellular ROS, expression of apoptotic proteins, and poor supportive ability of HSCs in coculture.<sup>33</sup> These findings were mirrored in ECs cultured from patients with PGF.<sup>27</sup> p38 MAPK, a critical protein that reduces the numbers of endothelial progenitor cells in patients with coronary artery disease, was also found to be highly expressed in the dysfunctional PGF ECs.<sup>34</sup> Stromal cells are critical for HSC function and the abnormalities described in these PGF studies offer explanation for the subsequent markers of cellular exhaustion seen in HSCs derived from patients with PGF. However, the inciting cause of stromal cell dysfunction has not been demonstrated.

Another consistent abnormality demonstrated in components of the PGF bone marrow microenvironment is that of elevated ROS. Intracellular ROS is a by-product of aerobic metabolism in the mitochondrion, and a balance exists between ROS production and multiple antioxidant systems that prevent its accumulation. In hematopoiesis, levels of ROS within the bone marrow microenvironment are strictly controlled as low levels promote HSC quiescence, whereas high levels induce replication and differentiation.<sup>35</sup> Extracellular mechanisms, such as regulatory T-cell (T-reg) secretion of adenosine, maintain a low level of ROS in the HSCs.<sup>36</sup> Disruption of the CXCL4/CXCL12 axis, which defines many stromal and HSC interactions related to quiescence, increases production of ROS.<sup>37</sup> Elevated ROS in the HSCs of patients with PGF may be related to disruptions in bone marrow microenvironment/HSC interactions, causing increased stem cell cycling. The finding of elevated ROS in the PGF bone marrow microenvironment may offer a potential therapeutic target in the management of PGF.

Aside from acquired abnormalities in the cellular microenvironment components, there are also disease-intrinsic abnormalities that predispose patients to PGF post-alloSCT. Patients with myelofibrosis (MF) are present at a higher frequency in studies of PGF relative to proportions in a transplant population<sup>38-40</sup> and this may be related to alterations in the bone marrow microenvironment intrinsic to MF. MF causes bone marrow microenvironment dysfunction through progressive fibrosis and proinflammatory cytokines such as interleukin 2 (IL-2) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ).<sup>41</sup> In an alloSCT context, this creates an adverse environment for engrafting HSCs. Furthermore, splenomegaly associated with MF as well as other malignant conditions has been identified as a risk factor for PGF due to the hypothetical sequestration of both mature cells and CD34<sup>+</sup> progenitors, resulting in peripheral cytopenias.<sup>42</sup> The adverse impact of an altered recipient bone marrow microenvironment because of MF demonstrates how important supporting cells are to the functioning of HSCs. Clinicians evaluating patients with MF should be cautious and consider PGF as another potential complication of alloSCT.

## Immune dysfunction (climate)

A graft-versus-bone marrow response directed at recipient-derived components of the bone marrow microenvironment, such as stromal cells, offers a unifying hypothesis for the development of PGF. Evidence supporting this graft-versus-bone marrow response comes from mismatched transplantation mouse models and case-control studies of patients with PGF. Major histocompatibility complex (MHC)-incompatible lymphocyte transfer mouse models result in bone marrow suppression accompanied by reduction in stromal elements in recipient bone marrow.<sup>43</sup> This suggests that these cells may be the inducers and targets of the alloreactive response.<sup>44</sup> In the clinical setting, a graft-versus-bone marrow response post-alloSCT was highlighted in a cohort of patients with chronic GVHD, unexplained cytopenias, and corresponding reduction in bone marrow osteoblasts.<sup>45</sup> Case-control studies suggest that the main instigators of graft-versus-bone marrow response are T cells, with assistance from macrophages. In 1 study, patients with PGF had a higher ratio of T-helper 1 (Th1)/Th2 and T-cytotoxic (Tc1)/Tc2 cells consistent with a skewed inflammatory T-cell response.<sup>46</sup> In a related study, proinflammatory M1 macrophages, which are primed by lipopolysaccharide in concert with interferon  $\gamma$  (IFN- $\gamma$ ), TNF- $\alpha$ , and granulocyte macrophage colony-stimulating factor,<sup>47</sup> were more prevalent in patients with PGF.<sup>48</sup> Ex vivo-cocultured PGF macrophages caused increased apoptosis in CD34<sup>+</sup> cells. Further evidence for immune-mediated suppression of HSCs as the underlying pathophysiology of PGF comes from case reports. Expansion of certain T-cell clones has been demonstrated in a case report of PGF following post-alloSCT vaccination.<sup>49</sup> Clonal selection of HSCs, as manifested by the production of glycosylphosphatidylinositol-anchored protein-deficient HSCs, possibly due to immune-selection pressures akin to AA, has been demonstrated in PGF.<sup>50</sup> Based on current evidence, a graft-versus-bone marrow response induced by recipient stromal cells, mediated by donor T cells and macrophages, may be the inciting cause of PGF. This subsequently results in the loss of non-haematopoietic stromal cells ability to support HSC function, leading to cytopenias that define PGF.

In addition to the loss of critical NHSC trophic signals, inflammatory cytokines produced during a graft-versus-bone marrow response also impair HSC function. Inflammatory suppression of HSCs by reactive T cells has been demonstrated in a complex murine cotransplantation experiment. Bone marrow failure was induced by transplanting lethally irradiated B6DF1 mice with lymphocytes from B6 donors. Bone marrow cells from the B6DF1 (H2 b/d) mice with induced bone marrow failure were combined with BM cells from a B6 mice and transplanted into lethally irradiated CbyB6F1 mice. This transplant could not rescue irradiated recipients, and HSC suppression was mediated by a cytokine “bystander effect,” rather than MHC alloreactivity.<sup>51</sup> This response could be reversed by IFN- $\gamma$  blockade, indicating that IFN- $\gamma$  is a critical cytokine in this response. Another experiment demonstrated the adverse effect of excessive IFN- $\gamma$ , whereby IFN- $\gamma$  receptor-deficient C57BL/6 mice recipients were transplanted with wild-type donors. This subsequently caused marked marrow hypoplasia due to the high exposure of the donor cells to IFN- $\gamma$  and lack of physiologic sinks. This was also accompanied by suppression of cycling genes *CCND1* and *MYC*,<sup>52</sup> consistent with prior evidence that prolonged exposure to IFN- $\gamma$  eventually induces stem cell exhaustion.<sup>53,54</sup> External to the HSC, IFN- $\gamma$  heterodimerizes a key proliferative cytokine, thrombopoietin (TPO), making it unable to stimulate its receptor MPL, impairing HSC proliferation.<sup>55</sup> Specific to PGF, a

case-control study demonstrated similar levels of IFN- $\gamma$  in the bone marrow plasma of patients with PGF compared with GGF, however, given the low cell counts in patients with PGF, there may be a higher relative exposure of the remaining HSCs, T cells, and stromal cells to the same amount of IFN- $\gamma$ .<sup>46</sup> Other cytokines such as TNF- $\alpha$  and type 1 interferons and their effect on HSC biology have been reviewed elsewhere, however, their role in PGF has not been investigated.<sup>56</sup> The suppression of HSCs outside of MHC alloreactivity because of IFN- $\gamma$  provides an explanation for impaired hematopoiesis in PGF in the setting of complete donor chimerism.

In addition to the direct effects on HSC proliferation, IFN- $\gamma$  may also potentiate alloreactivity that sustains PGF. IFN- $\gamma$  induces the upregulation of MHC class II on ECs and MSCs.<sup>57,58</sup> Upregulation of HLA-DR increases T-cell alloreactivity and is a causative mechanism in transplant-associated thrombotic microangiopathy.<sup>59</sup> In addition to being inducers of T-cell alloreactivity, MSCs produce proinflammatory cytokines, such as IL-6, and IL-1 $\beta$  when exposed to IFN- $\gamma$ , possibly sustaining the PGF response.<sup>60</sup> Under the influence of IFN- $\gamma$ , stromal tissue may contribute to inflammation, resulting in dysfunction of the bone marrow microenvironment, HSC suppression, and development of PGF, but this requires further investigation.

## Immune dysregulation

The inability to control this proinflammatory T-cell response suggests a failure of immune regulation in PGF. T-regs are important in regulating immune responses through the production of anti-inflammatory cytokines such as TGF- $\beta$  and IL-10.<sup>61</sup> It has been established that HSCs exist near T-regs, suggesting that these cells provide an immune-privileged environment for HSC function.<sup>36</sup> Despite normal overall proportions of T-regs in PGF vs GGF, patients with PGF have higher Th17 cells compared with T-regs, resulting in an abnormal Th17/T-reg ratio.<sup>62</sup> Th17 cells have been associated with autoimmune conditions, such as inflammatory bowel disease and rheumatoid arthritis, and an abnormal Th17/T-reg ratio has been implicated in autoimmunity.<sup>61</sup> Aside from T-reg frequency, T-reg functional impairment may contribute to immune dysregulation. T-regs in AA, another condition that results in HSC suppression, have reduced migration capacity and an inability to suppress effector T-cell function, but this has not been assessed in PGF.<sup>63</sup> T-regs require intact thymic function to be produced.<sup>64</sup> Loss of thymopoiesis in aging and GVHD reduces T-reg numbers, further worsening GVHD.<sup>65</sup> T-cell receptor excision circles are surrogate markers of thymic function and are reduced by GVHD and CMV reactivation.<sup>66</sup> In a cohort study involving patients with sibling donors, recipients with acute GVHD had lower levels of thymic function.<sup>67</sup> Sepsis, another common complication of alloSCT, has been shown to impair T-cell lymphopoiesis in the bone marrow and cause thymic atrophy in mice models, but has not been studied in the transplant setting.<sup>68</sup> Common complications of alloSCT, such as GVHD, sepsis, and CMV, cause thymic dysfunction, leading to lower numbers of T-regs impairing their ability to suppress immune responses such as those involved in PGF. Qualitative and quantitative dysfunction within T-regs has not been extensively reviewed in PGF and should be an area of further research in future studies involving PGF.

## Viral reactivation

Viral reactivation of latent DNA viruses, particularly CMV and Epstein-Barr virus, is a common posttransplant complication that may initiate PGF. Like the immune profile in PGF, viral infection is associated

with a type I immune response resulting in increased IFN- $\gamma$  expression.<sup>69</sup> Stromal cells such as fibroblasts and ECs can be targets of CMV infection, which in turn may cause their dysfunction as targets of an immune response, impairing hematopoiesis.<sup>70</sup> Non-CMV viral infections such as adenovirus, human herpesvirus 6, Epstein-Barr virus, and BK virus can also cause PGF potentially through similar mechanisms.<sup>71</sup> Compounding the effect of viral infection, antiviral treatments such as valganciclovir, ganciclovir, and cidofovir cause myelosuppression.<sup>72</sup> Viral infections have been demonstrated as a risk factor for cytopenias post-alloSCT, but the association with PGF has only been reported in abstract form.<sup>71</sup> Viral infection may precipitate PGF by inciting a graft-versus-bone marrow response that is exacerbated by antiviral therapy, however, further studies are required to confirm the association.

### Areas of further research

It should be noted that most immunologic assessment in PGF has been limited to small patient numbers and single timepoints, with most methods of analysis consisting of flow cytometry with cytokine analysis. Larger longitudinal-assessment studies comparing those with persisting PGF vs those with resolution are required. Utilization of other novel techniques such as digital spatial profiling applied to bone marrow trephines<sup>73</sup> and gene-expression profiling will give further insight to the immunobiology of PGF.

## Current and future therapies for PGF

A summary of therapies evaluated in PGF are presented in Table 3.

### Stem cell-directed therapy

Re-establishment or augmentation of hematopoiesis using CD34-selected stem cell reinfusions by providing HSCs without an alloreactive T-cell component, has been used as therapy in PGF with variable efficacy and toxicity (Table 3). In addition to the logistic challenges of donor cell availability and the potential need for further immunosuppressive conditioning, there remains no established means of evaluating the mechanisms of the responses seen with CD34-selected HSC infusion. Potential mechanisms include restoration of adequate HSC numbers for hematopoiesis and/or the infusion of non-T-reg immune populations such as mature myeloid cells and myeloid-derived suppressor cells. What effect granulocyte colony-stimulating factor (G-CSF) mobilization as well as cryopreservation have on the effectiveness of subsequent infusions is also unknown. CD34-selected HSC infusions may be an effective therapy in PGF but need further validation in prospective studies.

The TPO mimetic eltrombopag has been used as a treatment of immune-mediated thrombocytopenia but has broader effects on hematopoietic progenitors as demonstrated by its ability to stimulate multilineage recovery in patients with AA.<sup>74</sup> The key ligand of endogenous TPO is c-MPL, which is expressed on HSCs. Aside from direct stimulation of MPL on HSCs, eltrombopag may also improve stem cell renewal and increase the amount of functional stem cells due to reduction in intracellular iron by eltrombopag-mediated iron chelation.<sup>75</sup> Furthermore, eltrombopag can bypass IFN- $\gamma$ -induced inhibition of endogenous TPO.<sup>55</sup> Eltrombopag has been used as an HSC-directed treatment in PGF and prolonged thrombocytopenia post-alloSCT. A recent review detailing current available evidence utilizing eltrombopag and romiplostim (another TPO mimetic) as a treatment of post-alloSCT thrombocytopenia noted that overall responses

ranged from 70% to 80% and treatment was largely safe; however, most evidence supporting this conclusion was derived from retrospective and case studies.<sup>76</sup> A recent phase 1/2 study has assessed the use of romiplostim in post-alloSCT thrombocytopenia, while not strictly involving patients with PGF, this therapy was effective and safe to deliver, with only 25% of patients reporting adverse events. Most of these were unlikely attributable to the drug, reflecting the inherent complexity in delivering treatments to post-alloSCT patients. The authors also note that they were unable to determine whether some marrow recovery was spontaneous and unrelated to the drug given the lack of a comparator arm. Aside from improving HSC function under inflammatory stimulation, TPO agonism may also result in production of important immune and bone marrow microenvironment regulatory cells such as mature myeloid cells, myeloid-derived suppressor cells, megakaryocytes, and T-regs. TPO agonism has preclinical and clinical evidence of improving HSC function. This may be more favorable to deliver than CD34-selected HSC infusions due to the ongoing risk of GVHD with that treatment and logistic consideration. Further prospective studies to confirm efficacy and assess durability of response are required.

### Bone marrow microenvironment and stromal cell-directed therapy

MSCs have been investigated as a treatment of PGF (Table 3). Potential clinical utility aside, current evidence suggests that MSCs within the post-alloSCT bone marrow microenvironment are recipient-derived, suggesting that donor MSCs are not required for engraftment.<sup>77</sup> Furthermore, MSCs appear to be sequestered in the lung and liver postinfusion,<sup>78</sup> and therapeutic effect (if any), is likely mediated by paracrine signaling. Further studies are required to test the efficacy of MSCs in PGF, but evidence that donor-derived MSCs are not required for hematopoiesis or do not infiltrate the bone marrow microenvironment suggests that they may not be effective.

### ROS

Antioxidant therapy with *n*-acetyl cysteine (NAC) has been used to target ROS in PGF and has been used in a prospective trial of 74 patients aimed at preventing PGF post-haplo-alloSCT (Table 2). Patients at risk of PGF based on the amount of pretransplant bone marrow ECs were treated prophylactically with NAC. The primary end point was the cumulative incidence of PGF at +2 months, which was reduced in the treatment cohort, compared with untreated patients in a previous study. This was a small study with short follow-up but it may give an indication of the beneficial effect of a low-risk drug in the prevention of PGF.<sup>79</sup>

### Immune-directed therapy

Successful use of antithymocyte globulin (ATG) in 2 of 3 PGF patients in a case series who developed glycosylphosphatidylinositol-anchored protein-deficient leukocyte clones is the only evidence of immune manipulation as a therapy in PGF.<sup>50</sup> There are emerging targeted immune modulators that may have efficacy in PGF. Emapalumab, a humanized antibody to IFN- $\gamma$ , has shown promise in hemophagocytic lymphohistiocytosis as well as graft failure but is yet to be tested in PGF.<sup>80,81</sup> Indirectly inhibiting IFN- $\gamma$ , baricitinib acts by inhibiting downstream molecules JAK1/JAK2, thus improving marrow aplasia caused by experimental GVHD.<sup>82</sup> Improving immune regulation via cellular therapies, specifically adoptive T-regs may be effective in PGF. In the alloSCT context, infused T-regs have only been used in the

**Table 3. Therapies evaluated for the treatment of PGF**

Reference	Study type	Group size	Intervention	Comparator	Efficacy	Factors affecting recovery	Acute GVHD	OS	NRM
<b>Stem cell–directed therapy: CD34 infusions</b>									
Larocca et al, 2006 <sup>5</sup>	Retrospective cohort	54 adult patients with PGF 3 treatment arms				Use of CD34 <sup>+</sup> selected cells Patients who had previously achieved neutrophil recovery were more likely to recover	5-y OS		
Group A				No infusion <sup>20</sup>	Trilineage recovery at day 100 postinfusion: 40%		None reported	45%	55%
Group B				G-CSF mobilized Unmanipulated infusion <sup>14</sup>	Trilineage recovery at day 100 postinfusion: 36%		21%	29%	64%
Group C			G-CSF–mobilized CD34 <sup>+</sup> selected infusion without conditioning <sup>20</sup>		Trilineage recovery at day 100 postinfusion: 76%		None reported	65%	20%
Klyuchnikov et al, 2014 <sup>39</sup>	Retrospective cohort	32 adult patients with PGF	CD34 <sup>+</sup> selected infusions without conditioning	No comparator arm	Response in 81% (22% CR) at a median of 30 d postreinfusion	Younger recipient and donor age (continuous variable)	17%	2-y OS of 45%	40%
Askaa et al, 2014 <sup>95</sup>	Retrospective cohort	18 adult patients with PGF	CD34 <sup>+</sup> selected infusions without conditioning	No comparator arm	Response in 72% at 200 d posttransplant	Not assessed	22%	9-y OS of 40%	Not reported
Stasia et al, 2014 <sup>96</sup>	Retrospective cohort	41 adult patients with PGF	G-CSF–mobilized CD34 <sup>+</sup> selected infusions without conditioning	No comparator arm	Response in 83% of patients at a median follow-up of 1245 d	None found to be significant	15%	3-y OS of 63%	12%
Ghobadi et al, 2017 <sup>38</sup>	Prospective study with retrospective data included	26 adult patients with PGF across 3 treatment arms	CD34–selected product mobilized using the following strategies: (1) fresh mobilized products using G-CSF and plerixafor; (2) fresh mobilized products, using G-CSF only, and (3) cryopreserved cells mobilized by using G-CSF; all without conditioning	No comparator arm	Response in 81% (62% CR) Median of 54 d postinfusion to time to recovery	Not assessed	23%	3-y OS of 40%	Not reported
Mainardi et al, 2018 <sup>40</sup>	Retrospective cohort	50 pediatric patients with PGF	CD34 <sup>+</sup> Selected infusions without conditioning	No comparator arm	Response in 78% <sup>36</sup> of patients 8 wk postreinfusion	Donor age <40 y of age	6%	5-y OS of 38%	24%
Cuadrado et al, 2020 <sup>37</sup>	Retrospective cohort	62 adult patients with PGF	Fresh G-CSF–mobilized CD34–selected cells without conditioning	No comparator arm	Response in 76% of patients	CMV recipient and donor negative/negative No active infection Matched recipient/donor sex	11%	5-y OS of 54%	30%
<b>Stem cell–directed therapy: TPO agonism</b>									
Peiffaut de Latour et al, 2020 <sup>98</sup>	Prospective phase 1/2 multicenter trial	24 patients with thrombocytopenia post-alloSCT	Romiplostim	No comparator arm	75% had a platelet response; 21 patients with concurrent anemia had improvement in Hb; 4 patients with concurrent neutropenia had improvement in neutrophil count	None identified	70%	Not reported	21%

CR, complete response; G-CSF, granulocyte colony-stimulating factor. See Tables 1 and 2 for expansion of other abbreviations.



**Table 3. (Continued)**

Reference	Study type	Group size	Intervention	Comparator	Efficacy	Factors affecting recovery	Acute GVHD	OS	NRM
<b>Bone marrow microenvironment and NHSC-directed therapy</b>									
Meuleman et al, 2009 <sup>98</sup>	Prospective phase 1 study	6 patients with PGF	Donor-derived MSCs	No comparator	2 of 6 responded	Not reported	1 patient developed acute GVH	Not reported	1 patient died of CMV disease after MSC infusion
Liu et al, 2014 <sup>100</sup>	Prospective study	20 patients with PGF	Third-party donor MSCs	No comparator	17 patients.		1 patient developed acute GVH	1-y OS of 45%	45%
Kong et al, 2019 <sup>79</sup>	Prospective study	74 patients pre-alloSCT 35 treated with NAC	NAC infusion as preventative in patients with EC < 0.1%	Comparators in previous observational study and patients with adequate EC function	Reduction in cumulative incidence of PGF from 38.20%-41.80% to 7.63%-9.51% at 2 mo	N/A	Not reported	Not reported	Not reported

CR, complete response; G-CSF, granulocyte colony-stimulating factor. See Tables 1 and 2 for expansion of other abbreviations.

prevention of GVHD.<sup>83-85</sup> Targeted/cellular immune modulators hold promise in the treatment of PGF and may spare the global immune suppression of steroids and ATG in the already immunosuppressed alloSCT population.

### Seed, soil, or climate: which therapy is best?

Clearly, further data derived from prospective studies involving patients with PGF are needed to understand in further detail the mechanisms of disease and appropriate therapy. Based on the evidence presented, there is rationale for either HSC or bone marrow microenvironment/stromal cell-directed therapy, but neither therapy has been compared with each other to measure efficacy. In our observation, validated by recently published experience, PGF appears to be triggered by a significant pre-, intra- or post-alloSCT stimulus. This may indicate that different treatment modalities may be effective for PGF because of preexisting bone marrow fibrosis vs PGF associated with significant early transplant multiorgan dysfunction vs PGF associated with post-alloSCT viremia/viral therapy. To frame a discussion regarding possible therapy, we have provided 3 common alloSCT scenarios that led to the development of PGF.

**PGF due to preexisting bone marrow microenvironment dysfunction:** This occurs due to preexisting myeloproliferative or longstanding myeloid malignancies. Post-alloSCT count recovery can be fragile due to marrow fibrosis and/or inflammatory bone marrow microenvironment with or without splenomegaly. Preventative measures are favored, such as appropriate patient selection for alloSCT and referral early in the disease course. Pretransplant splenectomy as a method of preventing post-alloSCT cytopenias is controversial but can be considered. Post-alloSCT treatments for this type of PGF are limited, and further assessment of persisting immune or microenvironmental disruption because of malignant clone is required. TPO agonism may be useful.

**PGF due to significant intratransplant illness:** This commonly occurs in patients who have multiorgan dysfunction in the setting of profound sepsis or veno-occlusive disease within the first 30 days of alloSCT. Blood counts are chronically low and rarely recover due to significant suppression from systemic inflammation and HSC loss due to physiologic derangement from organ dysfunction. If patients are well enough, HSC-stimulating therapies such as repeat HSC infusion or TPO agonism may be considered.

**PGF due to posttransplant inflammatory stimuli:** This occurs due to consequences of viral infection plus or minus treatment of viral infection or GVHD. Treatment of the underlying inciting cause, removal, or substitution of myelotoxic drugs is required to assess reversibility. Alternate methods of viral treatment such as viral-specific T cells and TPO agonism may be useful. Further assessment of underlying immunobiology is required to assess whether targeted immunologic agents could be used as adjuncts.

Although TPO agonism appears to be the most useful and least morbid treatment option in these 3 scenarios, there are several unanswered questions relevant to its application. Are there enough HSCs to facilitate complete bone marrow recovery? What is the

inflammatory milieu or stromal disruption within the bone marrow microenvironment and what impact does this have on HSC function? Although we wish to be didactic about how to manage PGF-related scenarios, active investigation is needed with the above questions in mind. Only then can we definitively answer whether seed, soil, and climate, or combination therapy, is best.

## Concluding remarks and future directions

PGF is a complex clinical problem. Immune dysregulation in the form of a graft-versus-bone marrow response offers a unifying explanation for the myriad of problems detected in the bone marrow microenvironment. A standard definition is required to identify an at-risk population with poor clinical outcomes from PGF, facilitate early intervention, and hasten recovery from bone marrow cytopenias. There has been some success in stem cell-directed therapy such as CD34-selected HSC reinfusion and TPO agonism. Targeted immune modulation, either alone or in combination with other bone marrow microenvironment-directed therapies, needs to be investigated.

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## Acknowledgments

This work was supported by the Victorian Cancer Agency and Maddie Riewoldt's Vision.

## Authorship

Contribution: A.P. wrote the manuscript; R.K. provided expert review pertaining to the scientific components of the manuscript; and L.C. and D.R. provided expert review pertaining to clinical components of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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