Primary Graft Failure after Myeloablative Allogeneic Hematopoietic Cell Transplantation for Hematologic Malignancies

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Conflict of Interest
The authors do not have any conflicts of interest to disclose.

Supplementary information is available on Leukemia’s website.
Abstract

Clinical outcomes after primary graft failure (PGF) remain poor. Here we present a large retrospective analysis (n=23,272) which investigates means to prevent PGF and early detection of patients at high risk. In patients with hematologic malignancies, who underwent their first myeloablative allogeneic hematopoietic cell transplantation, PGF was reported in 1,278 (5.5%), and there was a marked difference in PGFs using peripheral blood stem cell compared to bone marrow grafts (2.5 vs. 7.3%; P<0.001). A 4-fold increase of PGF was observed in myeloproliferative disorders compared to acute leukemia (P<0.001). Other risk factors for PGF included recipient age below 30, HLA-mismatch, male recipients of female donor grafts, ABO-incompatibility, busulfan/cyclophosphamide conditioning, and cryopreservation. In bone marrow transplants, total nucleated cell doses ≤2.4 × 10^8/kg were associated with PGF (OR 1.39; P<0.001). The use of tacrolimus-based immunosuppression and granulocyte colony-stimulating factor were associated with decreased PGF risk. These data, allow clinicians to do more informed choices with respect to graft source, donor selection, conditioning and immunosuppressive regimens to reduce the risk of PGF. Moreover, a novel risk score determined on day 21 post-transplant may provide the rationale for an early request for additional hematopoietic stem cells.

Keywords

Allogeneic hematopoietic cell transplantation; primary graft failure; myeloablative; leukemia; myelodysplastic syndrome; myeloproliferative disorders

Introduction

Graft failure was one of the major causes of treatment failure in the early era of allogeneic hematopoietic cell transplantation (allo-HCT)\(^1\). Today, allo-HCT is standard of care for many hematological diseases; however, graft failure remains a significant complication\(^3\)–\(^5\). Primary graft failure (PGF) is characterized by the absence of initial donor cell engraftment; the patient never recovers from the neutropenia [absolute neutrophil count (ANC) <0.5×10^9/l] induced by the conditioning regimen. PGF is particularly devastating after myeloablative HCT because autologous hematopoietic recovery is rare, and death from infection and/or other complications of prolonged pancytopenia is likely in the absence of a second HCT. In contrast, secondary graft failure is defined as loss of donor cells after initial engraftment. The latter is more common after reduced intensity conditioning\(^5\), and the need for a second transplant is usually less urgent since autologous hematopoietic recovery is more likely to occur. Historically, a number of factors including cell dose, cellular and humoral rejection, viral infections, and defective bone marrow stroma have been associated...
with PGF$^{6-20}$. Most previous reports on PGF have included patients with aplastic anemia, and a large analysis of risk factors for PGF in the modern era of allo-HCT for hematological malignancies has not yet been conducted. The aim of the present study was therefore to investigate risk factors for PGF after myeloablative allo-HCT in more than 20,000 patients with hematologic malignancies, and to develop a novel predictive risk score for PGF in patients that have not yet engrafted two-three weeks post-transplant to acquire more lead-time to improve the clinical outcome in patients that do not engraft.

**Patients and Methods**

**Data Source**

This retrospective study used the Center for International Blood and Marrow Transplant Research (CIBMTR) database, a voluntary research affiliation of more than 450 transplantation centers worldwide that contribute detailed data on all completed autologous- and allo-HCT to a Statistical Center at the Medical College of Wisconsin in Milwaukee. The CIBMTR maintains an extensive database including detailed transplant-related information. Participating centers are required to report all transplants consecutively; patients are followed longitudinally with yearly follow-ups and compliance is monitored by computerized checks for discrepancies, physicians’ review of submitted data and on-site audits. Observational studies conducted by the CIBMTR are performed in compliance with the Privacy Rule (HIPAA) as a Public Health Authority, as well as all applicable federal regulations pertaining to the protection of human research participants.

**Patient Selection**

We retrieved 23,272 patients from the CIBMTR database with acute leukemia, chronic leukemia, myelodysplastic syndrome (MDS) or myeloproliferative disorder [primary myelofibrosis, polycythemia vera, essential thrombocytemia (MPD)], who were reported for their first myeloablative allo-HCT using unrelated or HLA identical sibling donors and either bone marrow (BM) or peripheral blood stem cells (PB) between 1995 and 2008. The CIBMTR working definition of regimen intensity was utilized in this study to select transplants that only included myeloablative conditioning regimens.$^{21}$ The myeloablative properties of all conditioning regimens were confirmed by reported doses of the myeloablative agents (≥5 Gy single dose or ≥8 Gy fractionated TBI, ≥9 mg/kg busulfan, or ≥150 mg/m$^2$ melphalan). We did not discriminate between oral and intravenous busulfan.

All classification on HLA match in the present study cohort was based on Weisdorf et al.$^{22}$, which classifies HLA matching in three major groups: well matched (no known disparity at HLA A,B,C,DRB1), partially matched (one locus known or likely disparity), and mismatched (≥2 locus disparity). HLA typing methodology has evolved over time, the determination of matching in the earlier years might be considered not fully matched by today's standard, but this classification enables analyses that span over a long period of time as the present study.

**Study Endpoint**

PGF was defined as: 1) alive on day 28 with ANC <0.5×10$^9$/l, or 2) ANC <0.5×10$^9$/l and donor cell infusion (donor lymphocyte infusion, boost, re-transplantation) within 28 days.
post-transplant. Death (n=717) and progressive disease (n=100) within 28 days were competing risks. Chimerism data was not available for most patients in this study.

Statistical Analysis

To summarize the characteristics of the dataset, descriptive tables of patient-, disease- and transplant-related variables were performed for all patients in the cohort as well as for patients surviving without engraftment on day 14 and day 21 following HCT. For discrete factors, the number of cases and their respective percentages were calculated. For continuous factors, the median and ranges were calculated. To determine risk factors for PGF, a forward stepwise logistic regression was used to model the odds of PGF as a function of pre-transplant prognostic factors (patient and donor age, gender match, Karnofsky/Lansky score, disease, disease status, conditioning regimen, unrelated donor age, donor pregnancies and transfusions, HLA match, ABO incompatibility, graft type, cell dose, cryopreservation, growth factor, immunosuppression, anti-thymocyte globulin (ATG), alemtuzumab, CMV match, major fungal infections pre-transplant, and year of transplant). All two-way interactions were checked, and P-values <0.05 were considered significant. After a preliminary model building stage, the model was adjusted to compensate for any center effects using generalized estimating equations with a sandwich variance estimate, and 17 cases with unknown center were excluded. Secondary objectives included evaluation and modeling of the risk of PGF in children, and in the subgroup of patients alive but without engraftment or progressive disease day 14 after HCT. Similar techniques were used for this analysis as for the analysis in the larger cohort. Patients at risk for PGF on day 21 post-transplant were randomly divided in training (2/3) and validation (1/3) datasets. By means of multivariate modelling, a risk score was created based on approximate coefficients from the logistic models built on the training dataset. A cutpoint for high vs. low risk was determined based on sensitivity and specificity in the training dataset, and the predictive capability was confirmed in the validation dataset. SAS Version 9.1 (SAS Institute, Cary, NC) were used for all analyses.

Results

PGF was reported in 1,278 out of 23,272 (5.5%) patients. In 1,253 patients the ANC was <0.5×10^9/l on day 28, and 25 patients with ANC <0.5×10^9/l received a second donor cell infusion before day 28 post-transplant. The main patient characteristics are described in table 1, and detailed univariate analyses are depicted in supplemental data 1. The results of the multivariate model for risk factors at transplant (table 2) are described below. Interactions were formally tested for all variables that entered the final model and none were significant besides the built-in interactions for donor/HLA and spleen status by disease.

Patient-related factors

Age ≥30 years was associated with decreased risk of PGF (OR=0.75, P<0.001), whereas female to male gender mismatch (OR=1.28, P=0.001), and Karnofsky/Lansky score <90% (OR=1.18, P=0.042) were associated with increased risk.
**Disease-related factors**

Compared to AML, an increased risk of PGF was observed in chronic lymphocytic leukemia (CLL) (OR=1.57, P=0.003) and chronic myelogenous leukemia (CML) (OR=1.88, P<0.001). In patients with MPD or MDS, the effect of disease was dependent on spleen status, where presence of splenomegaly was associated with even greater risk of PGF (MPD: OR=3.92, P=0.001; MDS: OR=2.34, P=0.002). After splenectomy, MPD and MDS patients had similar risk of PGF as corresponding patients with normal spleen (MPD OR=1.68, P=0.341; MDS OR=1.68, P=0.193). Due to a high percentage (~5%) of missing data the impact of spleen status was not investigated in acute and chronic leukemia. In AML, ALL, and CML higher PGF rates were observed in advanced disease (OR=1.54, P<0.001). In MDS, disease status was not associated with PGF, and disease status was not evaluated in CLL and MPD.

**Transplant-related factors**

Busulfan/Cyclophosphamide (Bu/Cy) was associated with increased risk of PGF when compared to TBI/Cy (OR=1.35, P=0.002). The dose of TBI (range 5–20 Gy) or Bu (range 9–30 mg/kg) in combination with Cy did not influence PGF rates (data not shown). Donor age was not investigated in HLA identical siblings due to the close relationship to recipient age. Unrelated donor age, donor transfusions, or pregnancies did not affect PGF rates (data not shown). Well matched unrelated donors were associated with PGF when compared to HLA identical siblings (OR=1.38, P<0.001), and the highest risk of PGF was seen in mismatched donors (OR=1.79, P<0.001). Mismatched grafts had significantly higher PGF compared to both well matched (OR=1.30; P=0.019) and partially matched (OR=1.38; P=0.015) grafts, whereas partially matched grafts had similar PGF risk when compared to well matched grafts (OR=0.94; P=0.540). Minor ABO mismatch did not affect PGF, whereas in major ABO mismatch a higher risk was noted (OR=1.24, P=0.012).

The incidence of PGF was markedly lower in PB compared to BM grafts (2.5 vs. 7.3%; P<0.001). In BM, a total nucleated cell (TNC) dose ≤2.4×10^8/kg was associated with PGF (OR=1.39, P<0.001). However, low CD34 cell doses (0.1–2 × 10^6/kg) had no impact on PGF in 533 patients who received PB. Irrespective of graft source cryopreservation (OR=1.43, P=0.013) was associated with PGF, whereas G-CSF was observed to reduce the risk (OR=0.36, P<0.001). Compared to standard immunosuppression with cyclosporine (CSA) and methotrexate (MTX), tacrolimus and MTX (OR=0.61, P<0.001) was associated with lower PGF risk. Notably, neither ex vivo T-cell depletion (OR=1.13, P=0.383) nor in vivo T-cell depletion with ATG or alemtuzumab were associated with PGF. Pre-HCT CMV match or prior major fungal infections were not associated with PGF. Impact of viral infections was not investigated due to a high number of missing data. Transplantation after 2003 was associated with PGF (OR=1.28, P=0.011). During 1995–2002 death within 28 days post-transplant without engraftment was increased (4.17% vs. 2.13%, P<0.001), whereas disease progression within 28 days was similar (0.43% vs. 0.46%, P=0.742). Donor cell infusions within 28 days in non-engrafted subjects were more common in the early era (0.14% vs. 0.05%, P=0.028).
Children

In 5,975 children and adolescents (<21 years old) there were 406 (6.8%) PGFs. Age, disease, spleen status, HLA match, cryopreservation, and year of transplant were no longer significant when the analysis was restricted to recipients younger than 21 years (table 3). Other risk factors were similar to those reported for the whole population.

**Landmark analysis on day 14 or day 21 post-transplant, and PGF risk score**

Two weeks post-transplant 16,653 subjects were at risk for PGF, and the incidence of PGF was 7.7%. Most risk factors were similar to those reported at transplant (supplemental data 2). Of note, ex vivo T-cell depletion became impactful on PGF (OR=1.36, P=0.022). The incidence of PGF in patients at risk two weeks post-transplant was too low to develop a useful risk score. Three weeks post-transplant, 6,050 subjects were at risk for PGF, and the incidence of PGF was 21.0%. In the training cohort, nine variables affected the risk of PGF (table 4). In contrast to our previous analyses, PB grafts were associated with increased risk of PGF (OR=1.41, P=0.010), and ex vivo T-cell depletion remained an important risk factor (OR=2.30, P<0.001). A risk score was developed (table 5) and a cut-off at risk score 7 was noted (table 6). In the training dataset the sensitivity was 20% and the specificity 90%, whereas in the validation dataset the sensitivity was 16% and the specificity 89%. In the training and validation datasets the positive predictive values for risk score ≥7 were 36% and 28%, respectively. In both cohorts, the negative predictive values for risk score <7 were 81%.

**Overall Survival and Causes of death**

One-year probabilities of overall survival among patients who are alive at day 28 post transplant were 45% (95% Confidence Interval [CI],42–48 %) and 61% (95% CI, 60–62%, p<0.001) if they developed or not PGF (Figure 1). Irrespective of prior PGF, primary disease was the cause of death in approximately 30% of all deceased subjects. Infection was the second most common cause of death with similar percentages in both groups (~15%), whereas graft failure as cause of death appeared more common among patients with prior PGF (11% vs. <1%). All causes of death are depicted in supplemental data 3.

**Discussion**

Recent data show that one year overall survival after re-transplantation due to PGF without relapse is as low as 11%4. Thus, the rationale for the present study was to conduct a large CIBMTR registry study to establish parameters to predict and avoid PGF in the future. Risk factors for PGF may be related to characteristics of the graft, the patient, the donor, or the transplantation procedure. In this study, graft type was the strongest risk factor in the multivariate model for PGF, with a 3 times higher risk in BM compared to PB grafts. Many factors, such as cell dose and the 10-fold higher number of CD3 cells in PB likely facilitate engraftment23, and contribute to the lower incidence of PGF using PB. That cell dose is important for the establishment of proper graft function is also supported by previous studies showing associations between cell dose and infection, GVHD, overall treatment failure, and survival24–29. In the present study, BM grafts with low cell dose (TNC doses ≤2.4×10^8/kg) resulted in a 40% rise in PGF, which is similar to previously reported data28. Previous
studies report a weak association between CD34 cell dose of PB grafts and days to engraftment\(^{30, 31}\). However, the current analysis did not reveal any impact of PB CD34 cell dose on PGF, and even in recipients of cell doses below \(2\times10^6/\text{kg}\) we were not able to define any threshold associated with increased risk of PGF. This suggests that PB products \textit{per se} are associated with cell doses above the threshold that would affect PGF, or other cell subtypes such as T-cells may be equally or more important for engraftment. Nevertheless, while other factors seem more important for PGF CD34 cell dose is probably important for subsequent secondary graft failure\(^{32}\).

Patient-related risk factors for PGF included age below 30 years, Karnofsky/Lansky score \(<90\%\), and primary diseases such as chronic leukemia, MDS, and MPD. In reduced intensity allo-HCT, CLL has been reported to be associated with graft failure\(^{33}\), and it is likely that primary disease may affect the probability of PGF indirectly due to intensity differences in pre-transplant chemotherapeutic protocols. Indeed, the disorders with the highest risk of PGF in our cohort were diseases which usually require chemotherapy of low to moderate intensity pre-transplant. Furthermore, the association between PGF and MPD has been well recognized\(^{34, 35}\), and is most likely dependent on multiple factors, including the impact of a defective bone marrow stroma, splenic consumption of infused stem cells\(^{36}\), and increased risk of allo-immunization following multiple transfusions\(^{37}\). Splenomegaly is a factor contributing to PGF in MPDs according to the present data. The availability of JAK2 inhibitors provides the opportunity to test whether treatment before transplantation can decrease the spleen size and offset the risk of PGF after transplantation for MPD\(^{38}\). Any further evaluations of the impact of spleen size by examination and/or imaging studies were unfortunately not possible since these data were not available in the database. The main purpose of the conditioning regimen is to suppress the recipient’s hematopoietic system to prevent an immunological rejection as well as to provide space for the infused donor cells to engraft\(^{39}\). In view of this, the higher risk of PGF in patients below 30 years may reflect that children immunity rather than hematopoiesis is more resistant to conditioning. Moreover, advanced disease and Karnofsky/Lansky score \(<90\%\) were associated with increased PGF risk, which may imply that the donor cells are implanted into an impaired hematopoietic microenvironment. Further studies are needed to reveal the true mechanisms behind these findings.

HLA-compatibility between donor and recipient is of major importance for predicting graft failure\(^{40}\). As expected, HLA match was associated with the risk of PGF. Well matched unrelated donors are mismatched at DPB1 in more than 80\% of the cases, and DPB1 can drive T-cells and antibody responses associated with graft failure\(^{41, 42}\). Thus, the observed increased PGF risk with well matched unrelated transplants should not be surprising, since DPB1 disparity may well be responsible for the increased risk of PGF compared to HLA identical sibling grafts. Furthermore, the risk of PGF was similar in well matched and partially mismatched unrelated grafts. This was; however, rather surprising since prior studies have observed that HLA class I and HLA-C mismatches are important determinants for graft failure\(^{43, 44}\). However, the higher risk of PGF in mismatched compared to both well and partially matched unrelated grafts still suggests that immunological T cell mediated responses towards HLA contributes to PGF.
Interestingly, in the sub-analysis in children there was no HLA effect detected, whereas the increase of PGF in major ABO-incompatibility remained. In contrast to adults, almost 90% of children received BM and a slightly higher TNC dose of \( \geq 3.0 \times 10^8 \) /kg was needed to reduce PGFs, which may reflect a higher median TNC dose in children due to their lower weight. Major ABO mismatch during unrelated donor allo-HCT has been associated with graft failures. Our data show that this is true also for HLA identical sibling donors, and regardless of the stem cell source is BM or PB. Erythrocyte and/or plasma depletion from ABO incompatible BM or PB products may compromise stem cell and T-cell viability as well as reduce graft cell numbers. Unfortunately, the CIBMTR database did not include data on erythrocyte/plasma depletion. However, previous single center reports, where red cell depletion and plasma reduction always are performed before cell counting, have reported similar ABO-effect. Major ABO-incompatibility was associated with PGF even when cell dose was adjusted for, but since hematopoietic stem cells and granulocytes do not express AB antigens the mechanism for this finding requires further investigations. The impact of donor gender and PGF remains unclear. However, in line with the present data male donor has previously been reported to be associated with decreased risk of PGF. To date, the present study is probably the largest analysis on PGF and donor/recipient gender match. We report that female to male gender mismatch increases the risk of PGF, whereas all other gender combinations have similar risk of PGF. Thus, when considering the risk for PGF male donor is preferred to a male patient, whereas donor sex does not matter for female recipients. In association to GVHD, immunological responses against minor antigens associated with the recipients Y chromosome are well known; it is, however, surprising that this gender mismatch also increases PGFs. Any underlying immunological mechanism has probably no association with the Y chromosome, although the true mechanism, which may not be immunological, seems elusive. Moreover, there are previous studies that report that female recipients reject male donor grafts most likely due to an allo-immunity towards antigens associated with the Y chromosome. In general these reports refer to rejection/secondary graft failure in H-Y immunized subjects, which may explain the discrepancy with the present analysis.

There is a well-documented association between ex vivo T-cell depletion and graft failure, but we did not observe such an effect in the overall population. Notably, the present study only included myeloablative conditioning regimens and increased conditioning intensity has previously been reported to decrease graft failures using T-cell depleted grafts. Moreover, our model adjusts for cell dose suggesting that a higher graft cell dose may reduce the risk of graft failure. However, in the present analysis T-cell depleted products were still associated with PGF among patients that had not engrafted two-three weeks post-transplant. In contrast to previous reports, in vivo T-cell depletion using ATG or alemtuzumab, was not associated with graft failures. That T-cell depletion, in the overall population, does not increase the risk of PGF may seem counterintuitive, and we have not been able to fully explain this finding. However, since the vast majority (87%) of all T-cell depleted grafts was BM; the strong graft source effect may conceal the impact of T-cell depletion at transplant. Moreover, the association between cryopreservation of the stem cell product prior to infusion and increased PGF risk is likely attributable to T-cell impairment as well as lower cell dose after thawing.
With respect to factors associated with the HCT procedure, TBI/Cy conditioning and tacrolimus-based immunosuppression both were associated with decreased PGF risk. All patients receiving TBI-based myeloablative conditioning regimens received ≥5 Gy single or 8 Gy fractionated dose, and higher doses had no effect on PGF. As previously reported, G-CSF initiated within one week post-transplant diminished the PGF risk. Surprisingly, in the multivariate model there was 28% higher risk of PGF in patients transplanted 2003–2008 compared to 1995–2002. This was not explained by any differences associated with the present PGF definition including deaths, progressive disease or donor cell infusions within 28 days post-transplant. Notably, the absolute number of PGFs was lower in the latter era, mainly due to increased use of PB, suggesting that even fewer PGFs should be expected in recent years. Conditioning regimens and intensity have also changed over the years, but this did not explain the increased PGF risk in the latter era. The reason for this remains to be determined.

Lastly, we developed a risk score for PGF in non-engrafted patients on day 21 post-transplant, and the predictive capability of the risk score was confirmed in a validation cohort. The time to engraftment is markedly different for BM and PB, but when we first modeled BM and PB separately we found similar results except for cell dose. We therefore chose to model them together to improve the statistical power of the analysis. The sensitivity and specificity were approximately 20% and 90%, respectively, and in the validation cohort PGF was predicted in 28% of high risk patients and no PGF was predicted in 81% of low risk patients. Since the positive predictive value of this model is low too much uncertainty exist to modify practice, besides ordering appropriate diagnostic testing, bone marrow biopsy and blood T cell chimerism, and develop contingency plans in the case of PGF.

All retrospective registry studies are dependent on the quality of data input. Indeed the CIBMTR routinely monitor data reported from participating centers to ensure data quality. There are; however, limitations associated with the retrospective nature of this study, but it is likely the only feasible strategy to study a rare event such as PGF. The power of the present risk score to predict PGF is limited, but due to the high specificity we believe that it contributes to the future management of patients who have not yet engrafted on day 21 post-transplant. In high risk patients, we suggest that G-CSF treatment is started, although the present analysis only show that pre-planned G-CSF starting within one week post-transplant decrease the risk of PGF, and the transplant physician may initiate early planning for a rescue transplant, whereas in low risk patients there is no reason for an early planning since a significant number of these patients will engraft.

In conclusion, PGF remains an important clinical problem in myeloablative allo-HCT. In this largest PGF analysis ever undertaken, we have identified many important risk factors, which should prove helpful in assessing the risk of PGF pre-transplant, and allow clinicians to make more informed choices for their patients with respect to BM versus PB, donor selection, immunosuppressive regimens, and when to plan for a rescue transplantation.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.
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Overall Survival for Primary Graft Failure on Day 28 Landmark

Figure 1.
Landmark analysis for overall survival on day 28 post-transplant in subjects who have engrafted successfully versus those with PGF.
Table 1
Characteristics of patients at risk for PGF after first myeloablative allo-HCT.

| Variable                          | N    | PGF (%) | P-valuea |
|----------------------------------|------|---------|----------|
| Number of patients               | 23272| 1278 (5) |          |
| Number of centers                | 372  | 249     |          |
| Year of transplant               |      |         | <0.001   |
| 1995–1996                        | 4449 | 333 (7) |          |
| 1997–1998                        | 3933 | 227 (6) |          |
| 1999–2000                        | 3336 | 200 (6) |          |
| 2001–2002                        | 2887 | 135 (5) |          |
| 2003–2004                        | 3151 | 152 (5) |          |
| 2005–2006                        | 3374 | 144 (4) |          |
| 2007–2008                        | 2142 | 87 (4)  |          |
| Recipient age (years), median (range) | 35 (<1–75) | 31 (<1 – 66) | <0.001 |
| 0–10                             | 2558 | 172 (7) |          |
| 11–20                            | 3417 | 234 (7) |          |
| 21–30                            | 3876 | 233 (6) |          |
| 31–40                            | 4758 | 253 (5) |          |
| 41–50                            | 5146 | 249 (5) |          |
| 51–60                            | 3084 | 122 (4) |          |
| >60                              | 433  | 15 (3)  |          |
| Donor/recipient gender match     |      |         | <0.001   |
| Male/Male                        | 8347 | 447 (5) |          |
| Female/Male                      | 4979 | 338 (7) |          |
| Male/Female                      | 5375 | 254 (5) |          |
| Female/Female                    | 4458 | 231 (5) |          |
| Unknown                          | 113  | 8 (7)   |          |
| Karnofsky/Lansky score (%)       |      |         | <0.001   |
| ≥90                              | 16439| 892 (5) |          |
| <90                              | 5643 | 343 (6) |          |
| Unknown                          | 1190 | 43 (4)  |          |
| Disease                          |      |         | <0.001   |
| AML                              | 8300 | 337 (4) |          |
| ALL                              | 5762 | 280 (5) |          |
| CLL                              | 844  | 44 (5)  |          |
| CML                              | 5776 | 440 (8) |          |
| MDS                              | 2128 | 127 (6) |          |
| MPPb                             | 462  | 50 (11) |          |
| Conditioning regimen             |      |         | <0.001   |
| Variable                      | N     | PGF (%) | P-value<sup>a</sup> |
|-------------------------------|-------|---------|---------------------|
| TBI Cy and other             | 11921 | 636 (5) |                     |
| Bu Cy and other              | 7779  | 473 (6) |                     |
| TBI and other                | 1418  | 79 (6)  |                     |
| Bu and other                 | 1400  | 48 (3)  |                     |
| Melphalan and other          | 166   | 6 (4)   |                     |
| Unknown dosage<sup>c</sup>   | 588   | 36 (6)  |                     |

**HLA match status<sup>d</sup>**  

| Related                      | N     | PGF (%) | P-value<sup>a</sup> |
|------------------------------|-------|---------|---------------------|
| - HLA identical sibling     | 10059 | 477 (5) |                     |

**Unrelated**  

| - Well matched               | 7445  | 421 (6) |                     |
| - Partially matched          | 3695  | 203 (5) |                     |
| - Mismatched                 | 1831  | 159 (9) |                     |
| - Unknown                    | 242   | 18 (7)  |                     |

**Graft type**  

| BM                           | 14272 | 1051 (7) | P-value<sup>a</sup> |
|------------------------------|-------|----------|---------------------|
| PB                           | 8906  | 219 (2)  |                     |
| BM+PB                        | 94    | 8 (9)    |                     |

**GVHD prophylaxis**  

| CSA + MTX +/- other          | 13349 | 837 (6)  | P-value<sup>a</sup> |
| CSA + MMF +/- other          | 256   | 8 (3)    |                     |
| CSA +/- other                | 1764  | 83 (5)   |                     |
| Tacrolimus + MTX +/- other   | 4336  | 140 (3)  |                     |
| Tacrolimus + MMF +/- other   | 539   | 13 (2)   |                     |
| Tacrolimus +/- other         | 601   | 12 (2)   |                     |
| Ex vivo T-cell depletion     | 1817  | 128 (7)  |                     |
| MTX +/- other                | 171   | 17 (10)  |                     |
| Other or none                | 439   | 45 (10)  |                     |

**Median follow-up of survivors Range (months)**  

| BM                           | 62 (<1–179) | 70 (1 – 171) | P-value<sup>a</sup> |

<sup>a</sup> Chi-Square.

<sup>b</sup> Myeloproliferative disorder (MPD) including primary myelofibrosis, polycythemia vera, and essential thrombocytopenia.

<sup>c</sup> Reported as myeloablative, but unknown dose of the myeloablative agent (TBI, busulfan, or melphalan).

<sup>d</sup> HLA match status: Well matched was defined as no known disparity at HLA A,B,C,DRB1, partially matched as one locus known or likely disparity with their donors and mismatched as ≥2 locus disparity.
Multivariate risk model for PGF in all patients at risk at transplant.

| Variable                                      | N  | OR  | Lower | Upper | P-value |
|-----------------------------------------------|----|-----|-------|-------|---------|
| Recipient age (years)                         |    |     |       |       | <0.001  |
| <30 years                                     | 9440| 1.00|       |       |         |
| ≥30 years                                     | 13815| 0.75| 0.65  | 0.86  | <0.001  |
| Donor/recipient gender match                  |    |     |       |       | <0.001  |
| Other                                         | 18166| 1.00|       |       |         |
| Female/Male                                   | 4976| 1.28| 1.10  | 1.49  | 0.001   |
| Unknown                                       | 113 | 1.39| 0.64  | 2.99  | 0.406   |
| Karnofsky/Lansky score (%)                    |    |     |       |       | 0.005   |
| <90                                           | 16431| 1.00|       |       |         |
| <90                                           | 5634| 1.18| 1.01  | 1.38  | 0.042   |
| Unknown                                       | 1190| 0.69| 0.49  | 0.96  | 0.030   |
| Disease                                       |    |     |       |       | <0.001  |
| AML<sup>a</sup>                               | 8296| 1.00|       |       |         |
| ALL<sup>a</sup>                               | 5758| 1.12| 0.90  | 1.39  | 0.299   |
| CLL                                           | 843 | 1.57| 1.17  | 2.10  | 0.003   |
| CML<sup>a</sup>                               | 5771| 1.88| 1.57  | 2.25  | <0.001  |
| MDS<sup>b</sup>                               | 2126| 1.38| 1.07  | 1.79  | 0.013   |
| MPD<sup>b,c</sup>                             | 461 | 1.81| 0.97  | 3.39  | 0.062   |
| Disease status - AML/ALL/CML<sup>d</sup>      |    |     |       |       | <0.001  |
| Early                                         | 10203| 1.00|       |       |         |
| Intermediate                                  | 5534| 0.98| 0.84  | 1.15  | 0.816   |
| Advanced                                      | 3746| 1.54| 1.25  | 1.89  | <0.001  |
| Unknown                                       | 342 | 0.85| 0.52  | 1.40  | 0.533   |
| Spleen status - MPD                           |    |     |       |       | <0.001  |
| Normal                                        | 181 | 1.00|       |       |         |
| Splenectomy                                   | 91 | 1.68| 0.58  | 4.87  | 0.341   |

<sup>a</sup> Indicates previously defined models.
<sup>b</sup> Indicates previously defined models.
<sup>c</sup> Indicates previously defined models.
<sup>d</sup> Indicates previously defined models.
| Variable                      | N  | OR (Lower, Upper) | P-value |
|-------------------------------|----|-------------------|---------|
| Splenomegaly                  |    |                   |         |
| Splenomegaly - MDS normal     | 161| 3.92 (1.79, 8.55) | 0.001   |
| Splenomegaly - MDS unknown    | 28 | 0.82 (0.10, 6.76) | 0.854   |
| Splenomegaly - MDS unknown    | 174| 2.34 (1.36, 4.03) | 0.002   |
| Splenomegaly - MDS unknown    | 192| 1.59 (0.84, 2.66) | 0.167   |
| Conditioning regimen          |    |                   |         |
| Conditioning regimen - TBI    | 1905| 1.00 (1.11, 1.65) | 0.002   |
| Conditioning regimen - Bu     | 1400| 1.19 (1.09, 1.27) | 0.0285  |
| Conditioning regimen - Bu     | 1481| 1.30 (1.00, 1.73) | 0.046   |
| Conditioning regimen - TBI    | 588 | 1.60 (0.98, 2.61) | 0.001   |
| Conditioning regimen - TBI    | 24 | 1.79 (0.98, 2.94) | 0.021   |
| Conditioning regimen - TBI    | 1418| 1.30 (1.09, 1.73) | 0.069   |
| Conditioning regimen - TBI    | 252 | 1.35 (1.07, 1.71) | 0.013   |
| Conditioning regimen - TBI    | 166 | 1.27 (0.60, 2.66) | 0.533   |
| Conditioning regimen - TBI    | 10059| 1.00 (1.11, 1.65) | 0.002   |
| Conditioning regimen - TBI    | 7439| 1.38 (1.15, 1.64) | <0.001  |
| Conditioning regimen - TBI    | 3686| 1.29 (1.05, 1.60) | 0.018   |
| Conditioning regimen - TBI    | 1829| 1.79 (1.41, 2.27) | <0.001  |
| Conditioning regimen - TBI    | 24 | 1.79 (0.98, 2.94) | 0.021   |
| Conditioning regimen - TBI    | 1418| 1.30 (1.09, 1.73) | 0.069   |
| Conditioning regimen - TBI    | 252 | 1.35 (1.07, 1.71) | 0.013   |
| Conditioning regimen - TBI    | 166 | 1.27 (0.60, 2.66) | 0.533   |
| Conditioning regimen - TBI    | 10059| 1.00 (1.11, 1.65) | 0.002   |
| Conditioning regimen - TBI    | 7439| 1.38 (1.15, 1.64) | <0.001  |
| Conditioning regimen - TBI    | 3686| 1.29 (1.05, 1.60) | 0.018   |
| Conditioning regimen - TBI    | 1829| 1.79 (1.41, 2.27) | <0.001  |
| Conditioning regimen - TBI    | 24 | 1.79 (0.98, 2.94) | 0.021   |
| Conditioning regimen - TBI    | 1418| 1.30 (1.09, 1.73) | 0.069   |
| Conditioning regimen - TBI    | 252 | 1.35 (1.07, 1.71) | 0.013   |

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| Variable                                                                 | N    | OR  | Lower | Upper | P-value |
|-------------------------------------------------------------------------|------|-----|-------|-------|---------|
| BM<sup>h</sup>                                                          | 14255| 1.00|       |       |         |
| PB                                                                     | 8906 | 0.29| 0.23  | 0.37  | <0.001  |
| BM+PB                                                                  | 94   | 0.97| 0.49  | 1.91  | 0.921   |
| BM-total nucleated cell dose (10<sup>8</sup>/kg)                       |       |     |       |       | <0.001  |
| ≤2.4                                                                   | 5773 | 1.00|       |       |         |
| >2.4                                                                   | 7369 | 0.72| 0.61  | 0.84  | <0.001  |
| Unknown                                                                | 1113 | 1.00| 0.76  | 1.30  | 0.980   |
| Cryopreservation                                                        |       |     |       |       | 0.026   |
| No                                                                     | 13299| 1.00|       |       |         |
| Yes                                                                    | 2112 | 1.43| 1.08  | 1.89  | 0.013   |
| Unknown                                                                | 7844 | 0.94| 0.78  | 1.14  | 0.530   |
| Growth factor<sup>i</sup>                                               |       |     |       |       | <0.001  |
| No                                                                     | 14390| 1.00|       |       |         |
| G-CSF                                                                  | 7213 | 0.36| 0.30  | 0.44  | <0.001  |
| GM-CSF                                                                 | 505  | 0.70| 0.43  | 1.15  | 0.155   |
| Both G-CSF and GM-CSF                                                  | 69   | 0.57| 0.16  | 2.02  | 0.381   |
| Unknown                                                                | 1078 | 0.63| 0.45  | 0.87  | 0.005   |
| GVHD prophylaxis                                                        |       |     |       |       | <0.001  |
| CSA+MTX +/- other                                                       | 13348| 1.00|       |       |         |
| CSA +/- other                                                           | 2020 | 0.73| 0.58  | 0.92  | 0.007   |
| Tacrolimus + MTX +/- other                                             | 4336 | 0.61| 0.48  | 0.78  | <0.001  |
| Tacrolimus +/- other                                                    | 1140 | 0.50| 0.30  | 0.82  | 0.007   |
| Ex vivo T-cell depletion                                                | 1817 | 1.13| 0.86  | 1.49  | 0.383   |
| Other or unknown                                                       | 594  | 1.87| 1.20  | 2.93  | 0.006   |
| Year of Transplant                                                      |       |     |       |       | 0.011   |
| 1995–2002                                                              | 14588| 1.00|       |       |         |
| 2002–2008                                                              | 8667 | 1.28| 1.06  | 1.56  | 0.011   |

<sup>a</sup> Early disease status.

<sup>b</sup> Normal spleen.
Myeloproliferative disorder (MPD) including primary myelofibrosis, polycythemia vera, and essential thrombocythemia.

Disease status pre-transplant defined as: Early=AML or ALL in first CR; CML in first chronic phase. Intermediate=AML or ALL in second CR, third CR or fourth CR; CML in accelerated phase or greater than or equal to second chronic phase. Advanced=primary induction failure and relapse for AML and ALL; CML in blastic phase.

Splenomegaly data on size of the spleen by examination and/or imaging studies were not available in the database.

Reported as myeloablative, but unknown dose of the myeloablative agent (TBI, busulfan, or melphalan).

HLA match status: Well matched was defined as no known disparity at HLA A,B,C,DRB1, partially matched as one locus known or likely disparity with their donors and mismatched as ≥2 locus disparity.

BM TNC \( \leq 2.4 \times 10^8 \) /kg.

Growth factor, G-CSF, GM-CSF or both were delivered to promote engraftment. This was pre-planned and initiated between day -1 and day 7.
Table 3

Multivariate risk model for PGF in children (<21 years old) at risk at transplant.

| Variable                        | N  | OR  | Lower | Upper | P-value |
|---------------------------------|----|-----|-------|-------|---------|
| Donor/recipient gender match    |    |     |       |       | <0.001  |
| Other                           | 4517| 1.00|       |       |         |
| Female/Male                     | 1432| 1.54| 1.20  | 1.96  | <0.001  |
| Unknown                         | 26 | 2.92| 1.00  | 8.56  | 0.050   |
| Karnofsky/Lansky score (%)      |    |     |       |       | <0.001  |
| ≥90%                            | 4725| 1.00|       |       |         |
| <90%                            | 994 | 1.57| 1.22  | 2.03  | <0.001  |
| Unknown                         | 256 | 0.68| 0.34  | 1.39  | 0.291   |
| Disease                         |    |     |       |       | <0.001  |
| AML                             | 1806| 1.00|       |       |         |
| ALL                             | 2796| 1.16| 0.86  | 1.55  | 0.329   |
| CLL                             | 155 | 1.80| 1.03  | 3.13  | 0.039   |
| CML                             | 764 | 1.97| 1.36  | 2.85  | <0.001  |
| MDS                             | 402 | 1.10| 0.65  | 1.85  | 0.734   |
| MPD<sup>a</sup>                 | 52  | 0.93| 0.28  | 3.02  | 0.898   |
| Conditioning regimen            |    |     |       |       | 0.034   |
| TBI Cy and other                | 3597| 1.00|       |       |         |
| Bu Cy and other                 | 1646| 1.46| 1.10  | 1.93  | 0.008   |
| Bu and other                    | 164 | 1.08| 0.43  | 2.71  | 0.868   |
| Melphalan and other             | 10  | 3.67| 0.60  | 22.31 | 0.158   |
| TBI and other                   | 465 | 1.49| 1.01  | 2.20  | 0.046   |
| Unknown dosage<sup>b</sup>      | 93  | 2.22| 1.12  | 4.40  | 0.022   |
| ABO incompatibilities           |    |     |       |       | 0.018   |
| Matched                         | 2772| 1.00|       |       |         |
| Major                           | 1489| 1.50| 1.14  | 1.97  | 0.004   |
| Minor                           | 1112| 1.48| 1.07  | 2.06  | 0.019   |
| Variable                              | N   | OR  | Lower | Upper | P-value |
|--------------------------------------|-----|-----|-------|-------|---------|
| Unknown                              | 602 | 1.58| 1.06  | 2.35  | 0.024   |
| Graft type                           |     |     |       |       | 0.001   |
| BM                                  | 4792| 1.00|       |       |         |
| PB                                  | 1171| 0.61| 0.40  | 0.92  | 0.018   |
| BM+PB                               | 12  | 3.00| 1.02  | 8.82  | 0.046   |
| BM-total nucleated cell dose (10^8/kg) |     |     |       |       | 0.050   |
| ≥3.0                                | 2182| 1.00|       |       |         |
| <3.0                                | 2174| 1.37| 1.06  | 1.77  | 0.015   |
| Unknown                             | 436 | 1.15| 0.76  | 1.75  | 0.503   |
| Growth factor^d                     |     |     |       |       | <0.001  |
| No                                  | 3830| 1.00|       |       |         |
| G-CSF                               | 1753| 0.37| 0.27  | 0.50  | <0.001  |
| GM-CSF                              | 110 | 1.05| 0.48  | 2.32  | 0.897   |
| Unknown                             | 282 | 0.80| 0.47  | 1.36  | 0.409   |
| GVHD prophylaxis                    |     |     |       |       | 0.014   |
| CSA+MTX +/- other                   | 3702| 1.00|       |       |         |
| CSA +/- other                       | 759 | 0.71| 0.51  | 1.00  | 0.047   |
| Tacrolimus + MTX +/- other          | 573 | 0.66| 0.38  | 1.14  | 0.139   |
| Tacrolimus +/- other                | 131 | 0.25| 0.06  | 0.97  | 0.045   |
| Ex vivo T-cell depletion            | 602 | 1.30| 0.87  | 1.95  | 0.196   |
| Other or unknown                    | 208 | 1.31| 0.87  | 1.99  | 0.195   |

^a Myeloproliferative disorder (MPD) including primary myelofibrosis, polycythemia vera, and essential thrombocythemia.

^b Reported as myeloablative, but unknown dose of the myeloablative agent (TBI, busulfan, or melphalan).

^c BM TNC ≥3.0 × 10^8/kg.

^d Growth factor, G-CSF, GM-CSF or both were delivered to promote engraftment. This was pre-planned and initiated between day -1 and day 7.
Table 4

Multivariate risk model for PGF in patients at risk on day 21 post-transplant (training dataset).

| Variable                          | N     | OR   | Lower | Upper | P-value |
|-----------------------------------|-------|------|-------|-------|---------|
| Recipient age (years)             |       |      |       |       |         |
| <30                               | 1915  | 1.00 |       |       |         |
| ≥30                               | 2144  | 0.79 | 0.66  | 0.94  | 0.010   |
| Karnofsky/Lansky score (%)        |       |      |       |       |         |
| ≥90%                              | 2876  | 1.00 |       |       |         |
| <90%                              | 1016  | 1.23 | 1.01  | 1.51  | 0.043   |
| Unknown                           | 167   | 0.75 | 0.51  | 1.10  | 0.142   |
| Disease                           |       |      |       |       | <0.001  |
| AML<sup>a</sup>                   | 1186  | 1.00 |       |       |         |
| ALL<sup>a</sup>                   | 972   | 1.04 | 0.79  | 1.36  | 0.779   |
| CLL                               | 125   | 1.54 | 1.00  | 2.39  | 0.051   |
| CML<sup>a</sup>                   | 1296  | 1.58 | 1.27  | 1.98  | <0.001  |
| MDS                               | 369   | 1.27 | 0.96  | 1.68  | 0.096   |
| MPD<sup>b</sup>                   | 111   | 1.79 | 1.04  | 3.07  | 0.034   |
| Disease status - AML/ALL/CML<sup>c</sup> |     |      |       |       | 0.020   |
| Early                             | 1773  | 1.00 |       |       |         |
| Intermediate                      | 1011  | 0.89 | 0.71  | 1.11  | 0.304   |
| Advanced                          | 599   | 1.30 | 0.97  | 1.73  | 0.075   |
| Unknown                           | 71    | 0.84 | 0.45  | 1.58  | 0.596   |
| HLA match status<sup>d</sup>      |       |      |       |       | 0.018   |
| Related                           |       |      |       |       |         |
| - HLA identical sibling           | 1522  | 1.00 |       |       |         |
| Unrelated                         |       |      |       |       |         |
| - Well matched                    | 1390  | 1.15 | 0.90  | 1.45  | 0.263   |
| - Partially matched               | 712   | 1.06 | 0.81  | 1.39  | 0.661   |
| - Mismatched                      | 406   | 1.56 | 1.18  | 2.07  | 0.002   |

<sup>a</sup> Leukemia.
| Variable                                      | N   | OR  | Lower | Upper | P-value |
|----------------------------------------------|-----|-----|-------|-------|---------|
| - Unknown                                    | 29  | 0.76| 0.29  | 2.01  | 0.581   |
| Graft type/total nucleated cell dose (10⁸/kg) |     |     |       |       | 0.010   |
| BM >2.4                                      | 1440| 1.00|       |       |         |
| BM ≤2.4                                      | 1702| 1.32| 1.09  | 1.60  | 0.005   |
| PB                                           | 659 | 1.41| 1.09  | 1.83  | 0.010   |
| BM unknown total nucleated cell dose          | 258 | 1.22| 0.88  | 1.71  | 0.238   |
| Conditioning regimen                          |     |     |       |       | <0.001  |
| TBI+/− other                                 | 2518| 1.00|       |       |         |
| Other                                        | 1541| 1.53| 1.24  | 1.89  | <0.001  |
| GVHD prophylaxis                              |     |     |       |       | <0.001  |
| Calcineurin inhibitor<sup>d</sup>+ MTX+/− other | 3410| 1.00|       |       |         |
| Ex vivo T-cell depletion                      | 255 | 2.30| 1.75  | 3.03  | <0.001  |
| Other GVHD prophylaxis                        | 394 | 1.72| 1.33  | 2.22  | <0.001  |

<sup>a</sup> Early disease status.

<sup>b</sup> Myeloproliferative disorder (MPD) including primary myelofibrosis, polycythemia vera, and essential thrombocytopenia.

<sup>c</sup> Disease status pre-transplant defined as: Early=AML or ALL in first CR; CML in first chronic phase. Intermediate=AML or ALL in second CR, third CR or fourth CR; CML in accelerated phase or greater than or equal to second chronic phase. Advanced=primary induction failure and relapse for AML and ALL; CML in blastic phase.

<sup>d</sup> HLA match status: Well matched was defined as no known disparity at HLA A,B,C,DRB1, partially matched as one locus known or likely disparity with their donors and mismatched as ≥2 locus disparity.
Table 5

Weighted risk scores for PGF in all patients who are at risk for graft failure on day 21 post-transplant

| Day 21 weighted PGF risk scores | Score |
|---------------------------------|-------|
| Recipient age                   |       |
| ≥30 years                       | 0     |
| <30 years                       | 1     |
| Karnofsky/Lansky score          |       |
| ≥90%                            | 0     |
| <90%                            | 1     |
| Disease                         |       |
| AML                             | 0     |
| ALL                             | 0     |
| MDS                             | 1     |
| CLL                             | 2     |
| CML                             | 2     |
| MPD\(^a\)                       | 3     |
| Disease status\(^b\)            |       |
| Other                           | 0     |
| Advanced AML/ALL/CML            | 1     |
| HLA match status\(^c\)          |       |
| HLA identical sibling           | 0     |
| Well matched unrelated          | 0     |
| Partially matched unrelated     | 0     |
| Mismatched unrelated            | 2     |
| Graft type/total nucleated cell dose (TNC) |   |
| BM TNC > 2.4 × 10^9/kg          | 0     |
| BM TNC ≤ 2.4 × 10^9/kg          | 1     |
| Peripheral blood                | 2     |
| Conditioning regimen            |       |
| TBI+/- other                    | 0     |
| Other                           | 2     |
| GVHD prophylaxis                |       |
| Calcineurin inhibitor\(^d\) + MTX +/- other | 0 |
| Other GVHD prophylaxis          | 3     |
| T-cell depletion                | 4     |

\(^a\) Myeloproliferative disorder (MPD) including primary myelofibrosis, polycythemia vera, and essential thrombocythemia.

\(^b\) Disease status pre-transplant defined as: Other= AML, ALL, and CML early/intermediate; CLL, MDS, and MPD irrespective of disease status. Advanced=primary induction failure and relapse for AML and ALL; CML in blastic phase.
c HLA match status: Well matched was defined as no known disparity at HLA A,B,C,DRB1, partially matched as one locus known or likely disparity with their donors and mismatched as ≥2 locus disparity.
d Cyclosporine or tacrolimus.
Table 6

Validation of the PGF prediction score determined on day 21 post-transplant

| Score | Training dataset | Validation dataset |
|-------|------------------|--------------------|
|       | N    | No PGF | PGF | N    | No PGF | PGF | P-value |
| <7    | 3192 | 2590 (81%) | 602 (19%) | <0.001 | 1548 | 1248 (91%) | 300 (19%) | 0.003 |
| ≥7    | 437  | 278 (64%) | 159 (36%) | <0.001 | 205  | 147 (72%) | 58 (28%) |
| Unknown | 432  | 352 (81%) | 80 (19%) | 0.003  | 259  | 190 (73%) | 69 (27%) |
| Total | 4061 | 3220 (79%) | 841 (21%) | 0.003  | 2012 | 1585 (79%) | 427 (21%) |