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CD34+-selected stem cell boost for poor graft function after allogeneic hematopoietic stem cell transplantation: a large cohort study focusing on haploidentical transplantation

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Authors' contributions

Yunqian Sun and Aiming Pang designed the study. The data analysis and manuscript development were led by Yun He and Linwei Shi. All authors contributed to providing clinical data and approved the final version for submission.

Data-sharing statement

The data involving in this study are available from the corresponding author upon reasonable request.

Disclosures

No conflicts of interest to disclose.

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Declaration of AI Use

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Abstract

Poor graft function (PGF) is a life-threatening complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). $CD34^+$-selected stem cell boost has shown promise; however, data in the haploidentical setting and after failure of thrombopoietin receptor agonists (TPO-RAs) are limited. Patients who received purified $CD34^+$ boost for refractory PGF after HSCT at two centers were retrospectively analyzed. Outcomes included overall response rate (ORR), graft-versus-host disease (GVHD) and overall survival (OS). Among the 116 evaluable patients, 86.79% (103/116) received haploidentical transplants and 113 (97.4%) had received at least one platelet-stimulating agent, including TPO-RA and/or recombinant human thrombopoietin (rhTPO). The ORR was 67.24% (CR 62.93%, PR 4.31%). The 180-day cumulative recovery rates of neutrophils, hemoglobin, and platelets were 56.7%, 57.1%, and 65.7%, respectively. Active infection at the time of $CD34^+$-selected stem cell boost was the sole independent predictor of lower ORR (OR = 3.786, 95% CI: 1.584–9.048, P = 0.003). The incidence of grade III–IV acute GVHD was 9.48%, and that of moderate-to-severe chronic GVHD was 11.21%. The OS was 64.66%. Responders had a significantly better OS than non-responders (81.65% vs. 11.67%, P < 0.001). In conclusion, $CD34^+$-selected stem cell boost is safe and highly effective for refractory PGF after allo-HSCT, including in haploidentical recipients and those who have failed TPO-RAs. Active infection at infusion predicts a poor treatment response, and non-responders have a dismal prognosis, underscoring the need for alternative strategies.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for various hematological disorders. Poor graft function (PGF) is defined as persistent cytopenia affecting one, two, or three hematopoietic lineages after allo-HSCT, with a reported incidence of 5%–27%,[1-7] which increases the risks of infection and bleeding, and drives high non-relapse mortality; therefore, it is one of the most challenging post-transplant complications to manage.[5, 8-10]

Current treatment strategies for PGF include supportive care (transfusions and growth factors), thrombopoietin receptor agonists (TPO-RAs), $CD34^+$-selected stem cell boost, mesenchymal stem cell infusions, and second transplantation.[3, 5, 11-14] However, supportive care only provides temporary relief, and although TPO-RAs have shown some efficacy, a considerable proportion of patients fail to achieve durable trilineage recovery. A second transplantation provides a new source of stem cells but carries a high non-relapse mortality rate and is suitable only for a minority of medically fit patients.[15]

$CD34^+$ boost delivers a large number of purified donor hematopoietic stem/progenitor cells without additional conditioning. Their efficacy in promoting hematopoietic recovery and low risk of GVHD was first demonstrated by Larrocca et al.[25] Subsequently, multiple studies have reported overall response rates (ORRs) of 68%–100% and complete response (CR) rates of 32%–92% for $CD34^+$ boost in PGF. [16-28] Nevertheless, these studies have notable limitations: most previous reports on $CD34^+$ boost included only small cohorts (10–62 patients). [16-29] Moreover, most previous studies did not systematically document prior TPO-RA exposure. Consequently, whether $CD34^+$ boost retains its efficacy in patients who have failed TPO-RA therapy remains unresolved. Furthermore, the proportion of haploidentical donors in these studies ranged from 5% to 50%. Given the increasing use of haploidentical transplantation, with its inherent immunologic barriers and higher risk of PGF, data on the efficacy of $CD34^+$ boost in this setting are urgently needed.

We have previously reported 27 cases of refractory PGF following haplo-HSCT, with an ORR of 55.6%.[30] However, the sample size was small and no analysis was performed on the factors associated with GVHD, treatment response, or other outcomes after $CD34^+$-cell

reinfusion. In this study, we conducted the largest (to date) two-center retrospective study of 116 patients with PGF after allo-HSCT, of whom 86.79% received haploidentical transplants and 97.4% had prior exposure to rhTPO/TPO-RA. We aimed to evaluate the safety and efficacy of $CD34^+$ boost and identify factors associated with ORR and overall survival (OS).

Methods

Study Design and Patients

A total of 122 patients diagnosed with PGF after allo-HSCT at the two centers between January 2019 and March 2026 received a purified $CD34^+$-selected stem cell boost. Three patients were lost to follow-up, and three did not meet the diagnostic criteria for PGF. Finally, 116 patients were included in the final analysis (Figure 1). This study was approved by the Ethics Committee of Peking University People's Hospital.

$CD34^+$ Cell Selection and Infusion

$CD34^+$ cells were selected using a CliniMACS system (Miltenyi Biotec). The final product was infused intravenously within 24 h of selection, without conditioning or additional GVHD prophylaxis.

Definitions and Outcomes

Neutrophil engraftment was defined as the first of three consecutive days with absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L without granulocyte colony-stimulating factor (G-CSF). Platelet engraftment was defined as the first of seven consecutive days with a platelet count $\geq 20 \times 10^9$/L without transfusion.[6]

PGF was defined as dependence on blood and/or platelet transfusions and/or growth factor support in the absence of other explanations, such as disease relapse, drugs, or infections.[6] All patients maintained complete donor chimerism (>95% in both lineages) throughout follow-up before $CD34^+$ boost, no mixed or absent chimerism was detected.

CR was defined as achievement of ANC > 0.5×10^9/L (no G-CSF support), platelet count > 20×10^9/L, and hemoglobin > 80 g/L (no transfusions) after $CD34^+$ boost. Partial response (PR)

was defined as improvement in two lineages but not meeting the CR criteria. PGF non-response represented failure to achieve a response in at least two lineages. ORR was the sum of CR and PR.

GVHD

Acute GVHD was diagnosed and graded according to the Glucksberg criteria[31] (stages I–IV for organ involvement, overall grade I–IV), and chronic GVHD was classified as mild, moderate, or severe according to the NIH consensus criteria.[32]

Statistical Analysis

Categorical variables were compared using the chi-square or Fisher's exact test, and continuous variables were compared using the Mann–Whitney U test. Multivariate logistic regression was used to identify the independent predictors of ORR, OS was estimated using the Kaplan–Meier method, and multivariate Cox regression was performed for survival analysis. A two-sided $P < 0.05$ was considered statistically significant.

Results

Patient Characteristics

A total of 116 patients (64 men; 55.17%) were included in the final analysis. The median age was 35.5 years (range: 5–75 years). Underlying diseases included aplastic anemia (AA, n = 29), myelofibrosis (n = 7), acute leukemia (n = 46), myelodysplastic syndrome (n = 28), lymphoma (n = 4), and chronic myeloid leukemia in blast crisis (n = 2). Eleven patients (11/116, 9.48%) were DSA-positive at the time of HSCT. Regarding donor type, 103 patients (88.79%) underwent haplo-HSCT, 13 underwent matched sibling donor (MSD) transplantation, and one underwent matched unrelated donor (MUD) transplantation. A busulfan-based conditioning regimen was used in 88.79% of patients, while a total body irradiation-based regimen was used in 9.48%. The median infused mononuclear cell dose was 10.075×10^8/kg (range, 5.98–23.29 $\times 10^8$/kg), and the median $CD34^+$-cell dose was 2.75×10^6/kg (range, 0.41–9.57 $\times 10^6$/kg). The median time to neutrophil engraftment was 13 days (range: 10–27 days) and the median time to engraftment was 17 days (range: 7–55 days). Notably, 43 (37.07%) patients experienced

primary platelet engraftment failure (Supplemental table 1).

Regarding post-transplantation complications, cytomegalovirus (CMV) infection occurred in 56 patients (48.28%), of whom three were diagnosed with CMV disease. Letermovir was used as CMV prophylaxis in 54 patients. No significant association was observed between letermovir use and the ORR(p=0.503). Epstein–Barr virus (EBV) infection was observed in 24 patients (20.69%), 22 received rituximab as preemptive or therapeutic intervention, and 11 developed EBV-related post-transplantation lymphoproliferative disease or EBV-associated hemophagocytic lymphohistiocytosis. Grades III–IV acute GVHD (aGVHD) occurred in 23 patients (19.83%), and moderate-to-severe chronic GVHD (cGVDH) occurred in 8 patients (6.90%) (Supplemental Table 1).

Prior treatment for PGF before $CD34^+$ infusion

Before CD34+ boost, 113 of 116 patients (97.4%) had received at least one platelet-stimulating agent, including TPO-RA and/or recombinant human thrombopoietin (rhTPO). n total, 45 patients (38.8%) were treated with a single agent, most commonly hetrombopag (17 patients, 2.5–7.5 mg/day), eltrombopag (15 patients, 50–100 mg/day), rhTPO (7 patients), avatrombopag (4 patients, 20–40 mg/day), or romiplostim (2 patients). The remaining 68 patients (58.6%) received two or more agents concurrently or sequentially: 51 (44.0%) received two agents, 16 (13.8%) received three, and 1 (0.9%) received four. The median rhTPO/TPO-RA treatment time was 96 days (IQR, 61-153; range, 13–463).

Among the 116 evaluable patients, 48 (41.4%) had received Mesenchymal Stem Cells **(**MSCs) infusion before CD34+ boost, and 68 (58.6%) had not; all MSCs were from third-party umbilical cord sources. The ORRs were 66.7% (32/48) and 67.6% (46/68) in the MSCs-exposed and MSCs-naïve groups, respectively, with no significant difference between the two groups ($\chi^2 = 0.013$, P = 0.910; Supplementary Table 2). Two patients in this study underwent re-transplantation for PGF after the initial transplantation and subsequently developed secondary PGF, after which they received a CD34+ boost (Supplementary Table 3).

PGF and $CD34^+$ Infusion Details

Among the 116 patients, 16 (13.79%) had primary PGF, and 100 (86.21%) had secondary PGF (Table 1). The median time from transplantation to PGF diagnosis was 90.5 days (range: 20–1,217 days), the median time from transplantation to $CD34^+$ boost was 196 days (range: 58–1,255 days), and the median interval from PGF diagnosis to $CD34^+$ boost was 74 days (range: 2–829 days).

The CD34+ boost product was infused at a media dose of 3.37×10^6/kg (range, 0.55–16.8 $\times 10^6$/kg), with a median purity of 94% (range: 61.95%–99.64%) (Supplemental Table 1). In this retrospective study, $CD3^+$ T-cell dose data were available for only 42 patients (median, 3.89×10^3 cells/kg; range, 0.47–330 $\times 10^3$ cells/kg). At the time of $CD34^+$ infusion, 2 patients had uncontrolled aGVHD, 12 had active cGVHD, 27 (36.45%) had uncontrolled infection, and 7 had uncontrolled thrombotic microangiopathy (TMA). At the time of $CD34^+$ cell infusion, 96.55% of patients were still receiving immunosuppressive agents.

Efficacy Assessment

At 180 days post-infusion, 73 patients (62.93%) achieved CR and 5 (4.45%) achieved PR, representing an ORR of 67.24% (Table 1). The median time to neutrophil recovery was 15 days (range, 3–150 days), corresponding to a 180-day cumulative recovery rate of 56.7% (95% CI, 47.2%–66.2%) . For hemoglobin, recovery occurred at a median of 14 days (range, 3–78 days), with a 180-day cumulative recovery rate of 57.1% (95% CI 47.7%–66.6%). For platelets, the median recovery time was 17 days (range, 3–94 days), and the associated 180-day cumulative recovery rate was 65.7% (95% CI 56.6%–74.8%) (Figure 2).

Factors Influencing ORR

In the univariate analysis, none of the factors examined showed a statistically significant association with the ORR across the age groups (P = 0.177). Similarly, no difference was found between patients with bone marrow failure disorders (AA) and those with hematological malignancies (P = 0.936). Donor type (haploidentical *vs.* MSD/MUD, (P = 0.391), donor source

(maternal/collateral relatives *vs.* other donors, P = 0.955), and donor age (P = 0.721) did not significantly affect the ORR. The type of PGF (primary PGF *vs.* secondary PGF) was not associated with the ORR (P = 0.063). Active infection at the time of $CD34^+$ boost infusion was a significant predictor of poor outcome (P< 0.001). Concurrent TMA at the time of infusion was significantly associated with treatment failure of $CD34^+$-BOOST (Fisher's exact test, P< 0.001).

Univariate logistic regression showed that concurrent infection at the time of $CD34^+$ infusion was significantly associated with ORR (OR = 3.786, 95% CI: 1.584–9.048, P = 0.003).

Incidence of GVHD after Purified $CD34^+$ boost

After CD34 boost, 17 of 116 patients (14.7%) developed aGVHD, including 3 with grade I, 3 with grade II, and 11 with grade III–IV. Nine patients (52.9%) were steroid-dependent or refractory. Similarly, 17 patients (14.7%) developed cGVHD, including 4 with mild, 9 with moderate, and 4 with severe disease. Eleven patients (64.7%) were steroid-dependent.

Factors associated with the occurrence of aGVHD after infusion were analyzed. No significant associations with donor type (HID *vs*. MSD/MUD, P = 1.000), donor source (maternal/collateral donors *vs*. others, P = 0.07), prior aGVHD before infusion (P = 0.649), or prior cGVHD (P = 0.437) were observed. Prior aGVHD was significantly associated with the development of post-infusion cGVHD (P = 0.040).

Survival Outcomes

A total of 41 deaths were recorded. The mortality rates was 12.8% (10/78) and 81.58% (31/38) in the ORR (CR + PR) and no response (NR) groups (P < 0.001). Infection was the leading cause of death in both groups, whereas transplant-associated thrombotic microangiopathy occurred exclusively in the NR group.

With a median follow-up of 218 days (range, 2–2,733 days) the OS rate was 87.2% among responders and only 18.42% among non-responders (Figure 3). Survival analysis demonstrated significantly superior OS in the ORR group versus the NR group, with a median survival of 48.5 days in the NR group (OR = 0.033, 95% CI: 0.012–0.095, P < 0.001). Active infection at

the time of $CD34^+$ boost was significantly associated with an increased risk of death (OR = 3.192, 95% CI 1.446–7.045; P= 0.004). The presence of concurrent TMA at the time of infusion was associated with OS (Fisher's exact test, P < 0.001). The interval from PGF diagnosis to $CD34^+$-boost was not significantly associated with mortality (Mann–Whitney U = 1147.500, Z=-2.070, P = 0.038). Donor type (haploidentical donor vs. MSD/MUD, p = 0.961) and underlying disease (AA *vs*. others, P = 0.391) were not significantly associated with OS (Figure 3).

Multivariate Cox regression analysis identified response status (NR *vs*. ORR) as an independent risk factor for death (HR = 11.615, 95% CI 5.203–25.929, P < 0.001). Concurrent TMA at $CD34^+$ boost was significantly associated with poorer survival (OR = 0.337, 95% CI: 0.139–0.820, P = 0.017). However, the time from diagnosis to $CD34^+$ infusion (P = 0.323) and concurrent infection (P = 0.364) were not independent predictors.

Discussion

We demonstrated that a $CD34^+$-selected stem cell boost is a safe and highly effective treatment strategy for PGF after allo-HSCT, with an ORR of 67.24%. Our cohort is unique in several aspects: (i) it is the largest reported series of $CD34^+$ boost to date; (ii) it demonstrated that $CD34^+$ boost is as effective in haplo-SCT setting and the proportion of haploidentical donors was exceptionally high (88.78%), whereas haploidentical donors accounted for only 5% in a meta-analysis[23]; and (iii) the majority of our patients had previously received TPO-RA therapy (97.4%), and 41.4% had been treated with a combination of TPO-RA and MSCs. Thus, our study provides critical real-world evidence of refractory PGF in the modern era of haploidentical transplantation.

Our ORR of 67.24% is consistent with previously reported values,[18, 20-26, 28, 30] despite inter-study variability in the definitions of CR and PR. Despite the stronger human leukocyte antigen barrier and higher immunologic risks associated with haploidentical transplantation, donor type was not significantly associated with ORR in the univariate analysis (P = 0.333), consistent with the reports by Al-Ramahi et al.[25] and Cuadrado et al.[22] With adequate T-cell depletion (median $CD3^+$ cell dose 3.89×10^3/kg), $CD34^+$ boost from haploidentical donors is

equally safe and effective as from matched donors. The major hurdles in haploidentical transplantation, graft rejection and primary graft failure, are primarily confined to the initial engraftment phase, where bidirectional alloreactivity is most intense. In contrast, $CD34^+$ boost serves as a cell-repletion strategy aimed at correcting established hematopoietic insufficiency rather than re-establishing immune tolerance. The impact of donor type on boost outcomes is substantially attenuated, provided adequate T-cell depletion is achieved and no active graft rejection occurs.

Interestingly, a substantial proportion of patients in whom TPO-RA therapy had failed responded to $CD34^+$ boost. Elevated reactive oxygen species, endothelial and mesenchymal dysfunction, and aberrant immune responses further exhaust residual CD34+ cells,[4, 33, 34] making TPO-RA alone insufficient. Under these conditions, even high-dose TPO-RA may fail to rescue the defective endogenous $CD34^+$ cells. Purified donor $CD34^+$ cell infusion may restore multilineage hematopoiesis by supplying a pool of functionally intact progenitors. Clinical series[17, 18, 20-28, 30] reporting durable trilineage recovery in 68–100% of patients after $CD34^+$ boost are consistent with this mechanism. These observations argue for the use of $CD34^+$-selected boosts in haploidentical transplantation and after TPO-RA failure, underscoring the importance of replenishing the stem cell compartment rather than relying solely on pharmacological stimulation.

We confirmed that infection is the most important predictor of treatment response. Univariate analysis identified active infection at the time of infusion as a significant negative predictor of ORR ($P < 0.001$), and multivariate logistic regression confirmed it as an independent predictor (OR = 3.786, 95% CI: 1.584–9.048, P = 0.003). This finding aligns closely with previous studies.[22, 25, 28] Mechanistically, the pro-inflammatory milieu induced by infection (e.g., elevated IFN-γ and TNF-α) impairs hematopoietic stem cell self-renewal, promotes apoptosis, and damages the bone marrow niche.[35, 36] Therefore, active infections should be rigorously controlled before treating $CD34^+$ boost.

A key contribution of our study is comprehensive data on GVHD. The incidence of grade III-IV acute GVHD was 9.48%, and that of moderate-to-severe chronic GVHD was 11.21%, which is relatively low compared with most published series (Shahzad et al. meta-analysis:

any-grade aGVHD 17%, cGVHD 18% [23]; Al-Ramahi et al.: grade II-IV aGVHD 35%, cGVHD 45% [25]; Metheny et al.: grade III-IV aGVHD 7% [26]; Shapiro et al.: grade III-IV aGVHD 3.4% and moderate-severe cGVHD 12%[28]). The relatively low $CD3^+$ cell dose observed in our cohort may have contributed to this favorable safety profile, although this interpretation is limited by the small number of patients with available $CD3^+$ data (n=42) and should be taken as hypothesis-generating. Notably, a prior history of aGVHD was significantly associated with post-infusion cGVHD (P = 0.040); therefore, that caution is warranted in patients with pre-existing aGVHD.

We found that patients who failed to respond to $CD34^+$ boost had a poor prognosis. In our cohort, responders achieved an OS rate of 81.65%, whereas non-responders achieved an OS rate of only 15.35% (P < 0.001). Survival analysis demonstrated significantly superior overall survival in the ORR group versus the NR group, with a median survival of 92 days in the NR group (HR = 37.73, 95% CI 14.96–95.17, P < 0.001). Similar survival differences have been reported by others, such as Al-Ramahi et al. (1-year OS: 51% for CR vs. 7% for PR/NR)[25]. These findings highlight the need for early identification of patients who are unlikely to respond and for more effective salvage strategies. For this ultra-high-risk population, secondary transplantation, bone marrow niche-targeted therapies, and novel immunomodulatory drugs warrant further investigation.

This study had some limitations. First, the retrospective design introduced potential selection and information biases. The cohort was heterogeneous regarding underlying diseases, disease status at transplantation, and type of PGF, which reflects real-world practice but complicates the subgroup analysis. Second, this was a single-arm retrospective analysis without a control group; therefore, direct comparisons with other treatments (such as second transplantation and immunosuppressive therapy) cannot be made.

In conclusion, this large, two-center, real-world study confirmed that $CD34^+$-stem cell infusion is a safe and highly effective treatment for PGF after allo-HSCT, including in patients undergoing haploidentical transplantation and in those who have failed prior TPO-RA therapy. Active infection at infusion is a strong predictor of a poor response and should be controlled beforehand. Non-responders have extremely poor prognosis, highlighting the urgent need for

novel therapeutic strategies. Prospective studies are warranted to define the optimal timing, patient selection, and comparative effectiveness of $CD34^+$ boost versus other salvage options.

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Table 1. The outcomes of $CD34^+$ Boost

Characteristics	ORR		NR (n = 38)	P value
	CR (n = 73)	PR (n = 5)		
PGF type, n				
primary PGF	12	2	2	0.063
secondary PGF	61	3	36	
Time to PGF since HSCT, median days (range)	95 (28–1217)	90 (50–110)	63 (22–543)	0.246
Time to PGF to boost, median days (range)	67 (2–829)	23 (2–93)	84 (5–793)	0.534
HSCT-related complications during boost (n)				
Active aGVHD	1(grade III)	-	1(grade IV)	0.450
Total Active cGVHD	9	-	3	0.725
Mild	4	-	1	
moderate	4	-	2	
severe	1	-	-	
Active Infection	20	3	24	0.014
TA-TMA	-	-	7	0.018
I-II aGVHD after boost, n	5	-	2	0.225
III-IV aGVHD after boost, n	7	2	1	
Total cGVHD	15	-	2	0.147
M-S cGVHD after boost, n	12	-	1	
Mortality, n	8	2	31	< 0.001
Cause of death, n				
Infection	6	1	22	
TA-TMA	0	-	5	
Hemorrhagic/thrombotic events	1 (ischemic stroke)	1 (ICH)	1(ICH)	
Relapse	0	0	2	
Others	1(breast cancer)		1 (demyelination)	-

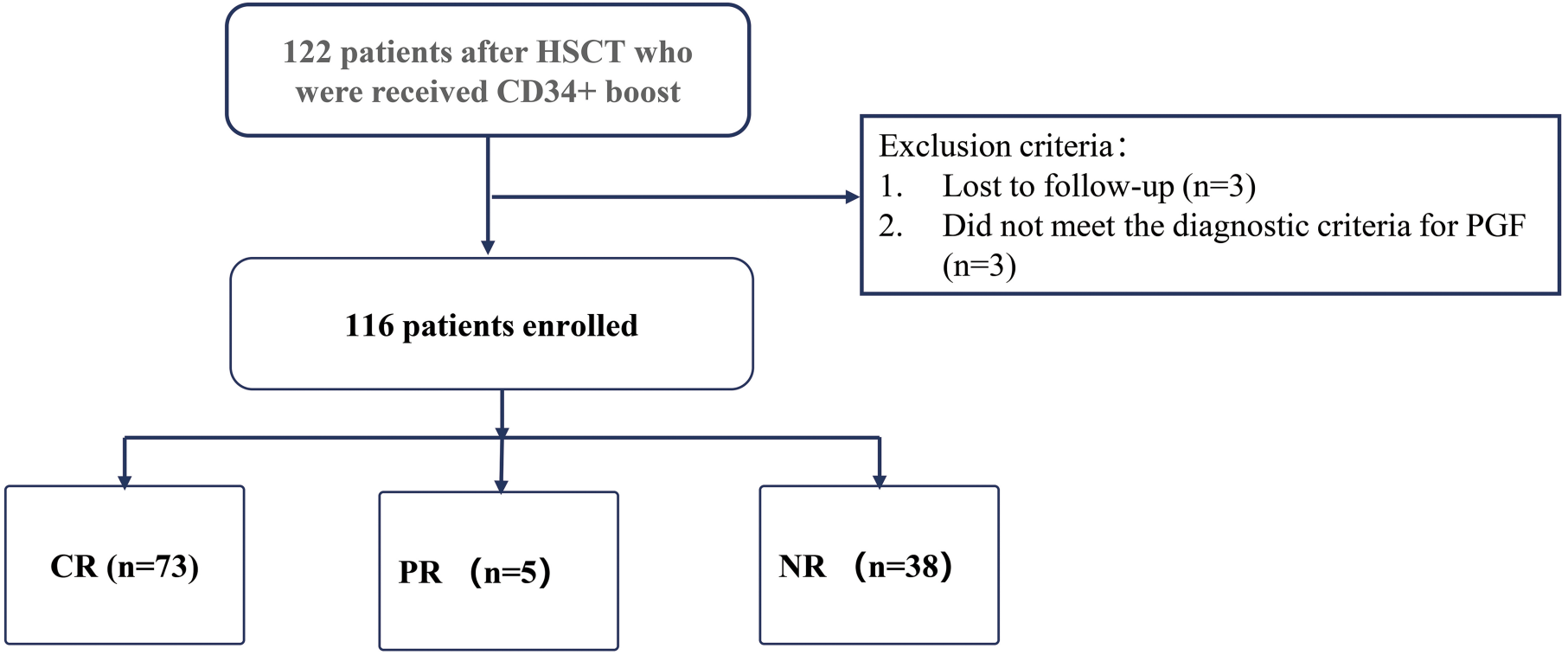
Abbreviations: a/c GVHD, acute/chronic graft-versus-host disease; CR, complete remission; ICH, intracerebral hemorrhage; M-S, moderate-to-severe; PGF, poor graft function; TA-TMA, transplant-associated thrombotic microangiopathy

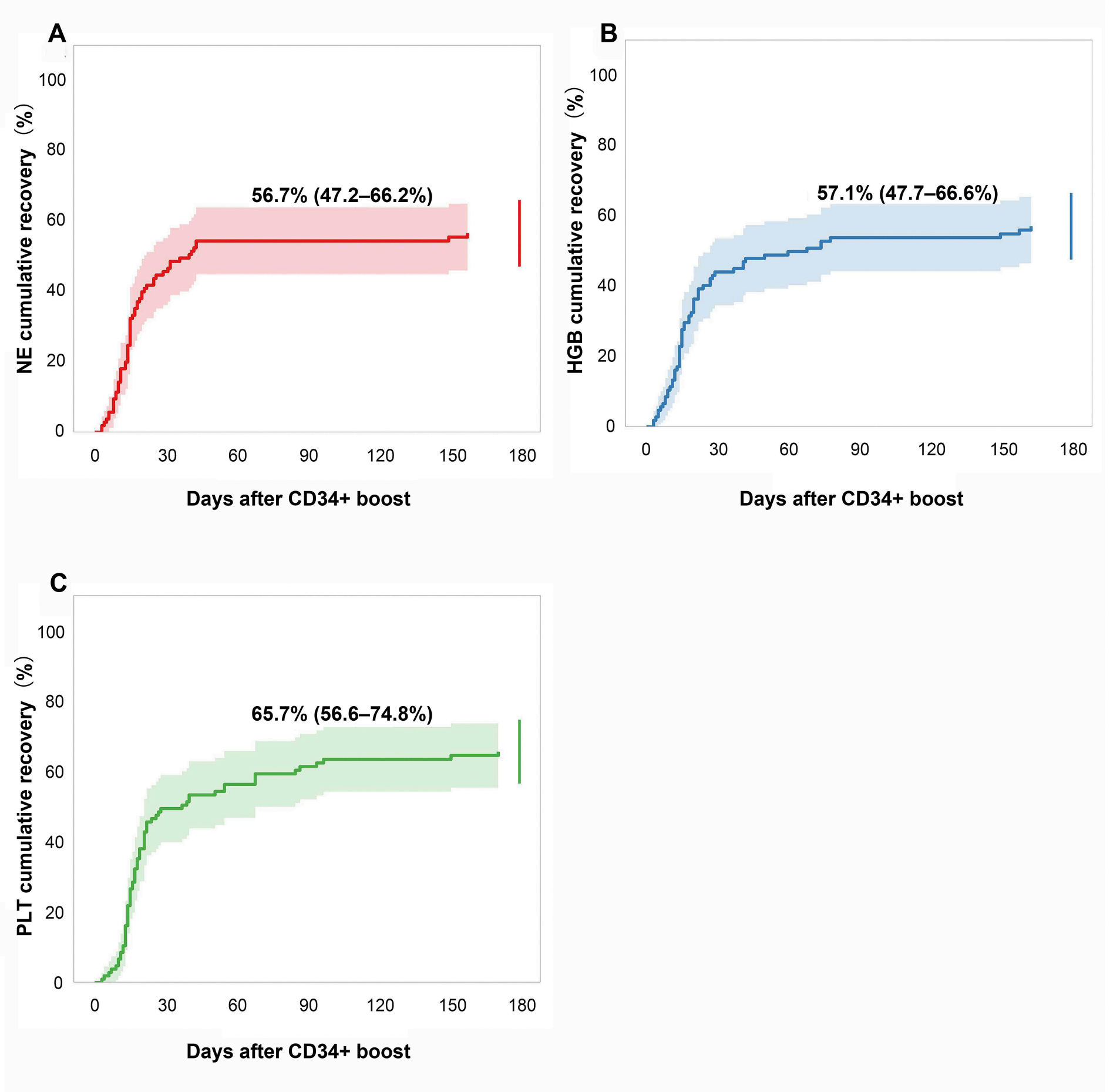
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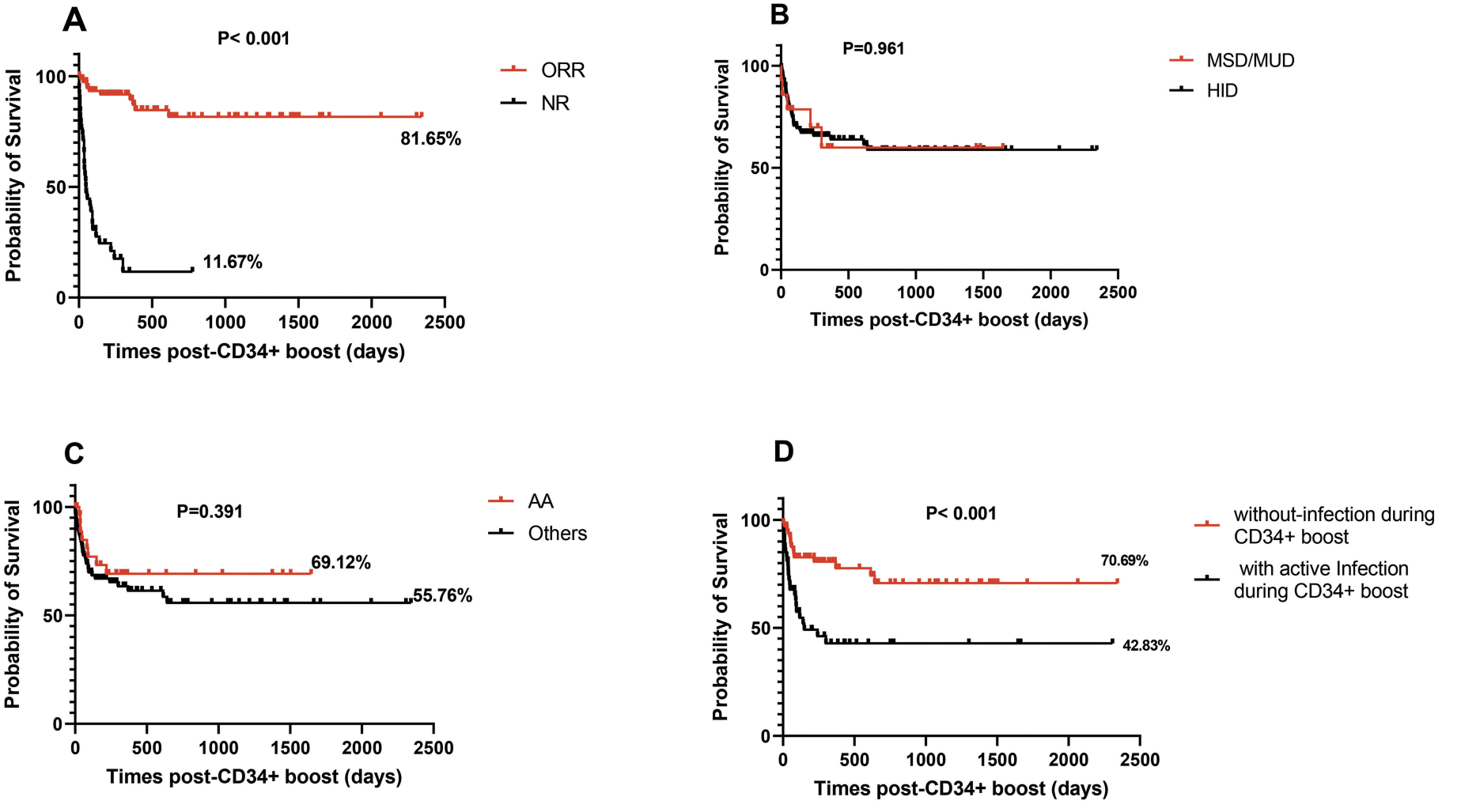
Figure 1. Flow chart of screening

Figure 2. Cumulative hematologic recovery rate at 180 days after CD34+ boost. (A) Cumulative NE recovery rate over time. The 180-day cumulative NE recovery rate was 56.7% (95% CI: 47.2–66.2%). (B) Cumulative HGB recovery rate over time. The 180-day cumulative HGB recovery rate was 57.1% (95% CI: 47.7–66.6%). (C) Cumulative PLT recovery rate over time. The 180-day cumulative PLT recovery rate was 65.7% (95% CI: 56.6–74.8%). Shaded areas represent 95% confidence intervals. Abbreviations: NE, neutrophil; HGB, hemoglobin; PLT, platelet; CI, confidence interval.

Figure 3. Kaplan-Meier estimates of survival probability among different subgroups after CD34+ boost. (A) Comparison of survival probabilities between patients achieving ORR and those with NR. The ORR group demonstrated a significantly higher survival probability (81.65%) compared to the NR group (11.67%; $P < 0.001$). (B) Comparison of survival probabilities between patients receiving MSD/MUD transplants and those receiving HID transplants. No significant difference was observed between the two groups ($P = 0.961$). (C) Comparison of survival probabilities between patients with AA and those with other underlying diseases. No significant difference was observed between the two groups ($P = 0.391$). (D) Comparison of survival probabilities between patients without infection and those with active infection during CD34+ boost. Patients without infection had a significantly higher survival probability (70.69%) compared to those with active infection (42.83%; $P < 0.001$). Abbreviations: ORR, overall response rate; NR, no response; MSD/MUD, matched sibling donor/matched unrelated donor; HID, haploidentical donor; AA, aplastic anemia.


122 patients after HSCT who were received CD34+ boost
Exclusion criteria：
1. Lost to follow-up (n=3)
2. Did not meet the diagnostic criteria for PGF (n=3)
116 patients enrolled
CR (n=73)
PR (n=5)
NR (n=38)


A
NE cumulative recovery (%)
100
80
60
40
20
0
56.7% (47.2–66.2%)
0
30
60
90
120
150
180
Days after CD34+ boost
B
HGB cumulative recovery (%)
100
80
60
40
20
0
57.1% (47.7–66.6%)
0
30
60
90
120
150
180
Days after CD34+ boost
C
PLT cumulative recovery (%)
100
80
60
40
20
0
65.7% (56.6–74.8%)
0
30
60
90
120
150
180
Days after CD34+ boost


A
P< 0.001
Probability of Survival
100
50
0
81.65%
11.67%
0
500
1000
1500
2000
2500
Times post-CD34+ boost (days)
ORR
NR
B
P=0.961
Probability of Survival
100
50
0
0
500
1000
1500
2000
2500
Times post-CD34+ boost (days)
MSD/MUD
HID
C
P=0.391
Probability of Survival
100
50
0
69.12%
55.76%
0
500
1000
1500
2000
2500
Times post-CD34+ boost (days)
AA
Others
D
P< 0.001
Probability of Survival
100
50
0
70.69%
42.83%
0
500
1000
1500
2000
2500
Times post-CD34+ boost (days)
without-infection during CD34+ boost
with active Infection during CD34+ boost

Supplemental Table 1. General characteristics of patients with PHR

Characteristics	ORR		NR (n = 38)	P value
	CR (n = 73)	PR (n = 5)		
Age, median years (range)	35 (5–71)	42 (19–51)	34 (11–75)	
≤18, n	13	0	12	
18 < age < 60, n	54	5	23	0.177
≥60, n	6	0	3	
Sex, n				
Male	42	4	18	0.238
Female	31	1	20	
Hematologic diagnosis, n				
AA	18	1	10	
ALL/AML	31	0	15	
MDS	17	1	10	0.409
MF	5	2	0	
other	2 (lymphoma)	1 (CML)	3 (2 lymphoma,1CML)	
Time from diagnosis to HSCT (months), (range)	6.49 (1.1–246)	12.76 (0.99–96)	7.6 (0.89–264)	0.070
Donor age, median years (range)	37.5 (11–71)	48 (19–64)	43 (7–61)	0.721
Donor Type (n)				
MSD	8		4	
HID	65	5	33	0.391
MUD	0	0	1	
Donor source				
Mother/collateral donors	10	2	6	0.955
Other donors	63	3	32	
DSA positive	4	-	7	0.748

ABO compatibility, n				
Matched	44	3	18	0.380
Minor mismatched	10	1	10	
Major mismatched	16	1	9	
Bidirectional mismatch	3	0	1	
Conditioning regimen, n				
BU-based regimen	64	4	35	0.564
TBI-based regimen	8	1	2	
Others	1	0	1	
Stem cell source				
BM+PB	12	1	8	0.565
PB	61	4	30	
MNC ($\times 10^8$/kg) (range)	10.22 (6.71–23.29)	9.73(7.63–11.26)	10.05 (5.98–13.56)	0.289
$CD34^+$ ($\times 10^6$/kg) (range)	2.255 (0.41–7.31)	6.38 (1.89–8.41)	3.46(1.04–9.57)	0.095
Engraftment time, days (range)				
Neutrophils	13 (10–27)	12 (11–20)	17.5 (10–24)	0.681
Platelets	24 (7–43)	25 (9–55)	15 (10–43)	0.136
Prior CMV viremia, n	36	1	19	0.895
CMV prophylaxis with letermovir	38	-	16	0.503
Prior CMV diseases	1	-	2	0.253
Prior EBV viremia, n	14	1	9	0.578
Prior EBV-PTLD or HLH	8	-	4	1.000
Prior HC, n	15	1	10	0.482
Prior TA-TMA	2	0	6	0.024
Prior aGVHD, n				0.053
Grade I-II	19	2	10	

Grade III-IV	12	-	11	
Prior cGVHD				
Total cGVHD	11	-	3	0.809
M-S cGVHD	5	-	3	0.767
Prior therapy of PHR				
TPO/TPO-RA	72	4	38	1.000
MSCs	29	3	16	0.910
2^{nd} HSCT	1	0	1	1.000
Time to SCB since HSCT, median days (range)	208 (89–1255)	133 (58–183)	191 (84–853)	0.947
Number of $CD34^+$ cells ($\times 10^6$/kg)	3.37 (0.73–14)	7.43 (2.66–11.07)	2.60(0.55–16.81)	0.156
Purity of $CD34^+$ stem cells	94.47% (61.95%–99.64%)	94.24% (86.5%–98.72%)	92.33% (83.2%–98.3%)	0.476
Number of $CD3^+$ cells ($\times 10^3$/kg)	3.7 (0.47–330)	7.17(4.61–13.7)	3.92 (1.69–10.1)	0.874
Immunosuppressive therapy at time of SCB, n	66	5	35	0.843

Abbreviations: AA: aplastic anemia, AML: acute myeloid leukemia, ALL: acute lymphocytic leukemia, BM: bone marrow blood stem cell, BU: busulfan, CML: chronic myeloid leukemia, CMV: cytomegalovirus, CR: complete response, EBV: Epstein–Barr virus, GVHD: graft-versus-host disease, HC: hemorrhagic cystitis, HID: haploidentical donor, HLH: hemophagocytic lymphohistiocytosis, HSCT: hematopoietic stem cell transplantation, MDS: myelodysplastic syndrome, MF: myelofibrosis, MNC: mononuclear cell count, MSD: matched sibling donor, MUD: matched unrelated donor, NR: no response, ORR: overall response rate, PB: peripheral stem cell, PR: partial response, PTLD: post-transplantation lymphoproliferative disease, TA-TMA: transplant-associated thrombotic microangiopathy, TBI: total body irradiation.

Supplementary Table 2. Previous exposure to MSC

Group	CR	PR	NR	ORR	P value
Prior MSCs exposure (n=48)	29	3	16	66.7%	0.910
No prior MSCs exposure (n=68)	44	2	22	67.6%	

Abbreviations: MSCs: Mesenchymal Stem Cells

Supplementary Table 3. Clinical characteristics of patients undergoing re-transplantation for PGF

No	Age	Sex	disease	donor	DSA before 2nd HSCT	Type of PGF	Indications for 2nd HSCT	Time from 2nd HSCT to PGF diagnosis (days)	Purified $CD34^+$ cell dose ($\times 10^6$/kg)	Time from 2nd HSCT to $CD34^+$ boost infusion (days)	Time from PHR diagnosis to $CD34^+$ boost infusion (days)	Time to neutrophil recovery after $CD34^+$ boost (days)	Time to hemoglobin recovery after $CD34^+$ boost (days)	Time to platelet recovery after $CD34^+$ boost (days)	Comorbidities at $CD34^+$ boost	Efficacy of $CD34^+$ boost
1	14	Female	SAA	1st: father to daughter; 2nd: mother to daughter	negative	After 1st HSCT：Secondary PGF After 2nd HSCT：Secondary PGF	62	62	1.91	125	63	—	—	—	Multi-serous effusion, cardiac dysfunction, COVID-19 positive	NR
2	57	man	MF	1st: HLA-matched sibling; 2nd: haploidentical son	negative	After 1st HSCT: Primary PGF; After 2nd HSCT: Secondary PGF	60	60	8.10	106	46	13	15	15	无	CR