blood®
Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Itacitinib for prevention of graft-versus-host disease and cytokine release syndrome in haploidentical transplantation

Ramzi Abboud,[1] Mark A. Schroeder,[1] Michael P. Rettig,[1] Reyka G. Jayasinghe,[1] Feng Gao,[2] Jeremy Eisele,[1] Leah Gehrs,[1] Julie Ritchey,[1] Jaebok Choi,[1] Camille N. Abboud,[1] Iskra Pusic,[1] Meagan Jacoby,[1] Peter Westervelt,[1] Matthew Christopher,[1] Amanda Cashen,[1] Armin Ghobadi,[1] Keith Stockerl-Goldstein,[1] Geoffrey L. Uy,[1] and John F. DiPersio[1]

[1]Division of Oncology, Department of Medicine, and [2]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, St. Louis, MO

KEY POINTS

- **Itacitinib with haploidentical transplantation, when added to standard GVHD prophylaxis, was well tolerated without impairing engraftment.**
- **Itacitinib resulted in low rates of CRS, acute and chronic GVHD, and encouraging GVHD-free relapse-free survival and OS after haplo-HCT.**

Haploidentical hematopoietic cell transplantation (haplo-HCT) is an increasingly used treatment for hematologic malignancies. Although posttransplant cyclophosphamide (PtCy) has improved graft-versus-host disease (GVHD) prophylaxis in haplo-HCT, patients continue to experience life-threatening complications. Interferon gamma and interleukin-6 are central in the pathophysiology of GVHD and cytokine release syndrome (CRS), and both cytokines signal through Janus kinase 1 (JAK-1). We tested the effect of adding the JAK-1 selective inhibitor, itacitinib, to PtCy–haplo-HCT to mitigate these complications and improve overall survival (OS). This open-label, single-arm study evaluated the safety and efficacy of itacitinib combined with standard GVHD prophylaxis after haplo-HCT. A total of 42 patients were treated with itacitinib 200 mg daily from day −3 through +100 or +180, followed by a taper. Itacitinib resulted in low CRS grades, all patients had grade 0 (22%) or grade 1 (78%) CRS and there were no cases of grade 2 to 5 CRS. There were no cases of primary graft failure. No patients developed grade 3 to 4 acute GVHD (aGVHD) through day +180. The cumulative incidence of grade 2 aGVHD at day +100 was 21.9%. The 1-year cumulative incidence of moderate or severe chronic GVHD was 5%. The cumulative incidence of relapse at 2 years was 14%. OS at 1 year was 80%. The cumulative incidence of nonrelapse mortality (NRM) at day 180 was 8%. Itacitinib, when added to standard GVHD prophylaxis, was well tolerated and resulted in low rates of CRS, acute and chronic GVHD, and NRM, and encouraging rates of GVHD-free relapse-free survival and OS after haplo-HCT. This trial was registered at www.ClinicalTrials.gov as #NCT03755414.

Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a cornerstone of therapy for hematologic malignancies. Many patients lack an available HLA-matched donor, but most have a readily available haploidentical donor.[1-5] T-cell replete, peripheral blood (PB) grafts are widely used in haploidentical HCT (haplo-HCT), but graft-versus-host disease (GVHD) and cytokine release syndrome (CRS) remain challenges.[6-9]

Acute GVHD (aGVHD) after PB haplo-HCT affects 40% to 50% of patients and 10% to 15% experience life-threatening aGVHD.[6,7,10] The interferon gamma and interleukin-6 (IL-6) cytokine pathways, which signal through Janus kinase 1 and 2 (JAK1/2), mediate aGVHD.[11-13] Severe (grade 3-5) CRS after haplo-HCT is associated with poor outcomes, with long-term survival of ~25%, driven by excessive nonrelapse mortality (NRM).[8,9,14-17] Conversely, minor (grade 1-2) CRS is associated with improved overall survival (OS), driven by lower relapse rates.[9] As with aGVHD, IFN gamma and IL-6 play a central role in the pathophysiology of CRS, and anti–IL-6 and anti–IL-6 receptor therapies such as tocilizumab and siltuximab have been used to treat severe CRS.[8,18,19]

Preclinical models suggest that the JAK/STAT pathway is central in both aGVHD and CRS, and that JAK inhibition can prevent and treat aGVHD.[18,20-22] Clinically, ruxolitinib, a JAK1/2 inhibitor, is effective in the treatment of aGVHD and chronic GVHD (cGVHD).[23,24] Early studies currently underway using JAK inhibitors for prevention of GVHD have encouraging preliminary results.[25-28]

We hypothesized that itacitinib, a JAK1-specific inhibitor, would be effective in preventing both aGVHD and CRS, without impairing engraftment after PB haplo-HCT. In this study, patients undergoing haplo-HCT for hematologic malignancy received itacitinib combined with standard tacrolimus (Tac), mycophenolate mofetil (MMF), and posttransplant cyclophosphamide (PtCy) as GVHD prophylaxis.

Methods

Trial design

This open-label, single-arm, pilot and expansion study was conducted at the Siteman Cancer Center at Washington University School of Medicine in St Louis (ClinicalTrials.gov identifier: NCT03755414; https://clinicaltrials.gov/study/NCT03755414). The protocol was approved by the institutional review board of Washington University in St. Louis and written informed consent was obtained from all patients before screening.

Patients

Eligible patients were aged ≥18 years and diagnosed with hematologic malignancies. Patients with acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) in morphologic complete remission, myelodysplastic syndrome with ≤10% blasts, and non-Hodgkin lymphoma or Hodgkin lymphoma in complete or partial remission were eligible. Both myeloablative (MA) and reduced intensity conditioning (RIC) were allowed. Exclusion criteria included prior allo-HCT and the presence of donor-specific antibodies with mean fluorescence intensity of ≥2000.

Treatment

In the pilot study, patients received itacitinib 200 mg daily from day −3 through +100, followed by a taper (Figure 1A). In the expansion study, patients received itacitinib 200 mg daily from day −3 through +180, followed by a taper. The taper period consisted of itacitinib 100 mg daily for 1 month then 100 mg every other day for 1 month. If patients developed new GVHD or worsening of existing GVHD during the itacitinib taper period, itacitinib could be increased by 1 dose level. Dose modifications and guidance for growth factor support are described in supplemental Material, available on the *Blood* website. Patients received MA or RIC, followed by infusion of filgrastim-mobilized PB stem and progenitor cells on day 0. All patients received standard GVHD prophylaxis with Tac (beginning on day +5), MMF (beginning on day +5), and PtCy (50 mg/kg on days +3 and +4). In the absence of GVHD or toxicity, Tac was tapered at day +100 and MMF was tapered by day +35.

End points

The primary end points were the incidence of primary graft failure, and the incidence of grade 3 to 4 aGVHD. Secondary

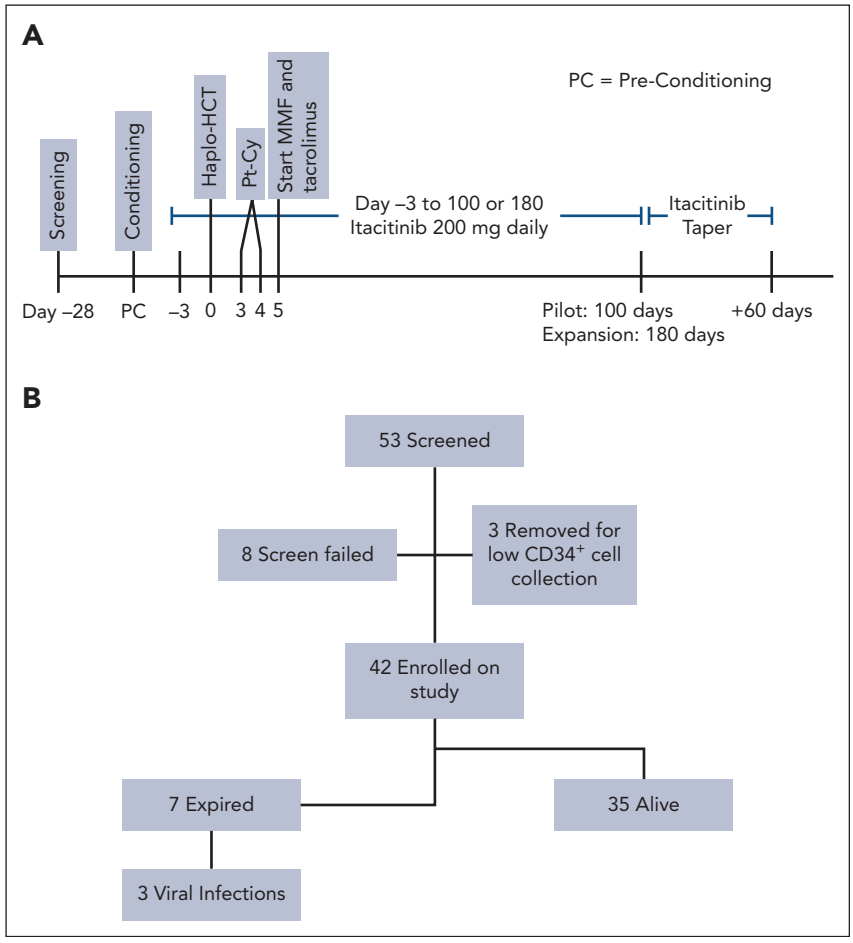

Figure 1. Study design. (A) Study schema. (B) CONSORT diagram.

outcomes were incidence and severity of CRS, and NRM by day +180. Exploratory outcomes included cGVHD, OS, and first event among grade 3 to 4 aGVHD, severe cGVHD, relapse, and death (GVHD-free relapse-free survival [GRFS]).[29] CRS was graded using Lee criteria,[30] aGVHD using the Mount Sinai Acute GVHD International Consortium criteria,[31] and cGVHD using the National Institutes of Health consensus criteria.[32]

Correlative studies

PB and serum samples were collected from patients on study and banked at multiple time points. Control samples were collected from patients undergoing standard-of-care PB haplo-HCT during the same time period; these patients all received the same Tac/MMF/PtCy GVHD prophylaxis except did not receive itacitinib (supplemental Table 4). Correlative studies include flow cytometry (flow) with 5 28-color panels for cellular subsets (supplemental Table 2) and single-cell RNA sequencing (scRNAseq). Additional methods are included in supplemental Materials.

Statistical analysis

As a pilot study, the sample sizes in both pilot phase and expansion cohort were determined primarily based on clinical considerations rather than statistical power. Based on extensive simulation studies, however, Piantadosi recommended that a sample size of 15 to 20 participants would estimate the preliminary information with reasonable precision.[33] For example, there would be 88% chance to observe at least 1 event out of 20 patients if the "true" incidence of primary graft failure was ≥10%. Exact binomial method was used to calculate the incidences of primary graft failure, CRS, and their 95% confidence intervals (CIs). The Gray subdistribution method was used to estimate the cumulative incidences of grade 3 to 4 aGVHD, cGVHD, platelet and neutrophil engraftment, and relapse, while treating death as a competing event. Kaplan-Meier product limit estimator was used to describe the distribution of the GRFS and OS. The statistical analysis was performed using SAS 9.2 (SAS Institute, Cary, NC).

Results

Patient characteristics

Forty-two patients (20 in pilot phase, 22 in extension phase) received HCT on study between November 2019 and December 2022 (Figure 1B). Baseline characteristics of the patients are shown in Table 1. Median follow-up was 453 days (range, 142-1259), and median age was 60 years (range, 20-73), with most patients having AML (60%), ALL (21%) or myelodysplastic syndrome (10%). All patients with AML and ALL were in a complete morphologic remission, although 45% had disease by either cytogenetics, next-generation sequencing, or measurable residual disease assay (flow cytometry for AML; next-generation sequencing for ALL). Most patients received MA conditioning (79%). Forty-seven percent of patients had an HCT-specific comorbidity index score of ≥3.

Engraftment There were no cases of primary graft failure (95% CI, 0-6.9). The median time to neutrophil engraftment was 15 days (range, 12-35; Figure 2A), and the median time to platelet engraftment was 22 days (range, 13-54). All patients had complete donor chimerism by short tandem repeats on bone marrow at day +30, +100, and +180. PB counts at days +28, +60, and +100 after haplo-HCT revealed similar white blood cell count, absolute neutrophil count (ANC), hemoglobin, and platelet count for patients on itacitinib and control patients (Figure 3A-D). Itacitinib was well tolerated, and 28 patients (67%) remained on study drug at the time of planned taper. Dose modifications for neutropenia or thrombocytopenia occurred in 23 (55%) patients, most cases were transient, and itacitinib was restarted in 15 of 23 patients (65%). The median duration of hold in these patients was 10 days. Other reasons for discontinuing study drug included patient choice (n = 1), elevated aspartate aminotransferase/alanine aminotransferase (n = 2), and relapsed disease (n = 3). $CD34^+$

Table 1. Patient characteristics

Characteristic	Value
Female, n (%)	18 (43)
Age at transplantation, median (range), y	59 (19-72)
HCT-CI score, n (%)	
0-1	11 (26)
2	11 (26)
3	6 (14)
4	3 (7)
≥5	11 (26)
Diagnosis, n (%)	
ALL	9 (21)
AML	25 (60)
MDS	4 (10)
Myelofibrosis	2 (5)
Diffuse large B-cell lymphoma	1 (2)
Mycosis fungoides	1 (2)
Measurable disease at transplantation (AML/MDS/ALL only), n (%)	
None	21 (55)
Cytogenetics only positive	4 (11)
NGS only positive	6 (16)
DAT mutation	1 (3)
Non-DAT mutation	5 (13)
MRD only positive	3 (8)
Cytogenetics and NGS (non-DAT) positive	3 (8)
NGS (non-DAT) and flow MRD positive	1 (3)
MA conditioning, n (%)	33 (79)
Conditioning regimen, n (%)	
Flu-Cy-SD TBI	2 (5)
Flu-Bu2	7 (17)
Flu-Bu4	20 (48)
Flu-Fx TBI	13 (31)

Flu-Cy-SD TBI had 200 cGy of TBI; Flu-Fx TBI had 1200 cGY of TBI.

Bu, busulfan; Cy, cyclophosphamide; DAT, DNMT3A, ASXL1, and TET2; Flu, fludarabine; Fx, fractionated; HCT-CI, HCT-specific comorbidity index; MDS, myelodysplastic syndrome; MRD, measurable residual disease by fluorescence-activated cell sorting (AML, MDS) or NGS (ALL); NGS, next-generation sequencing; SD, single dose; TBI, total-body irradiation.

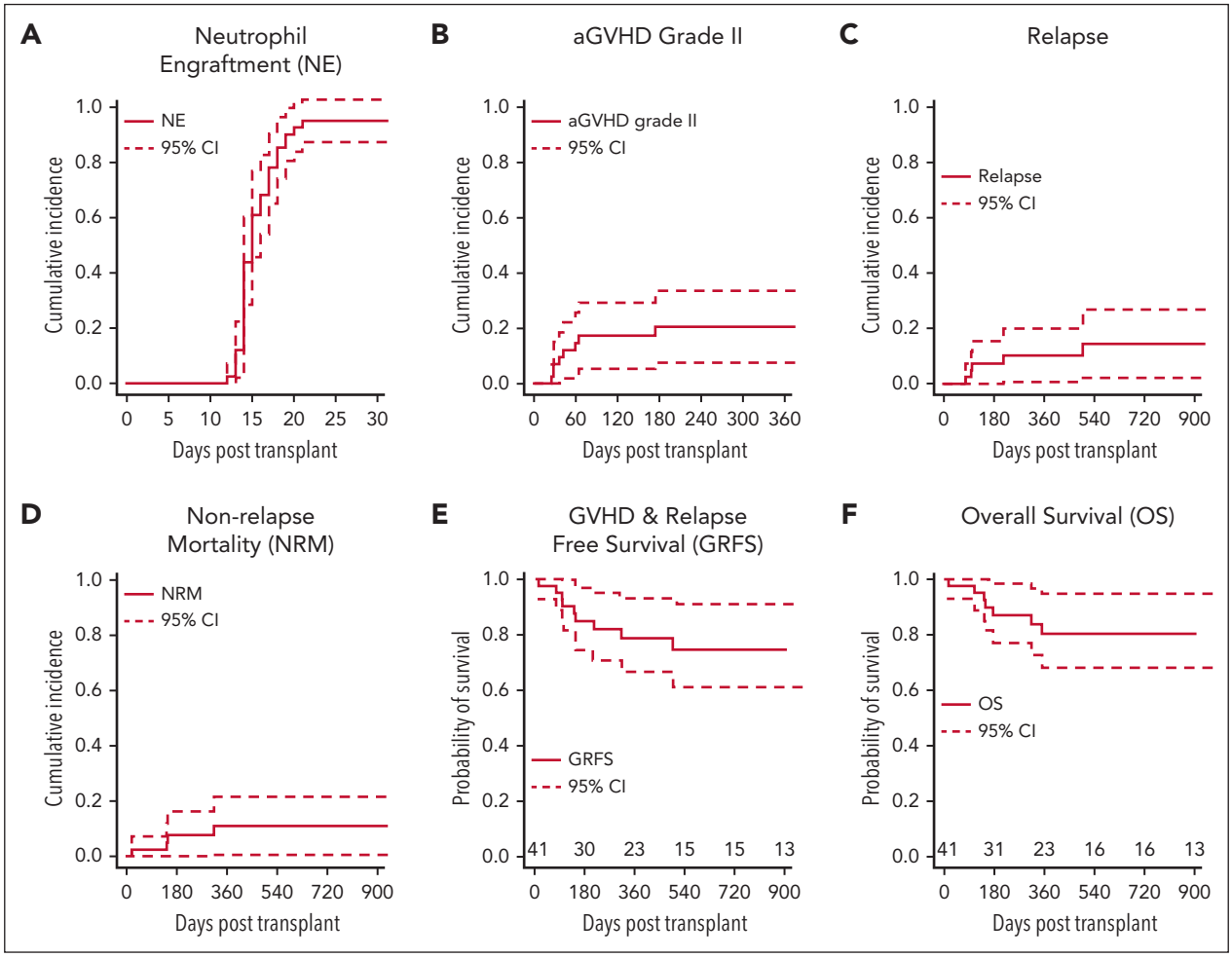

Figure 2. Kaplan-Meier plots of outcomes. (A) Neutrophil engraftment. (B) Acute grade 2 GVHD. (C) Relapse. (D) NRM. (E) GRFS. (F) OS.

selected cell boosts were given at the discretion of treating physicians and 4 patients (10%) received boosts. All 4 patients were subsequently transfusion independent.

aGVHD and cGVHD No patients developed grade 3 to 4 aGVHD through day +100 or day +180. The cumulative incidence of grade 2 aGVHD at day +100 was 21.9% (95% CI, 10.7-35.7; Figure 2B). Two cases of grade 2 skin aGVHD developed during the pilot when itacitinib and Tac were both tapered at day +100. Both patients responded completely to reinstating the previous dose of itacitinib and topical steroids, and neither restarted Tac. In the expansion phase, the duration of itacitinib was extended to day +180 and we observed no increased toxicity and no cases of aGVHD during the taper period. Incidence of aGVHD was similar in all analyzed subgroups, including disease type, conditioning intensity, donor relationship, donor age, and cell dose. In patients who survived without relapse to day +100, the 1-year cumulative incidence of moderate or severe cGVHD was 5% (95% CI, 1-17). At day +365, 36 patients (89%) had no cGVHD, 3 patients had mild cGVHD (7%), and 2 had moderate or severe cGVHD (5%). At day +365, treatments for cGVHD included ruxolitinib alone (n = 2), Tac alone (n = 2), and ruxolitinib with Tac (n = 2).

Relapse, GRFS, and OS Five patients had relapse at a median of 101 days (range, 78-498). The cumulative incidence of relapse at 1 year was 10% (95% CI, 3-22) and at 2 years was 14% (95% CI, 5-29; Figure 2C). No relapses occurred in patients with grade 0 CRS. The GRFS at day +180 was 85% (95% CI, 69-93) and at day +365 was 79% (95% CI, 62-89; Figure 2E), which exceeded historical rates for PB haplo-HCT of 36% to 43%.[7,34] The high GRFS observed on study was driven by low rates of severe aGVHD, extensive cGVHD, and relapse. OS at 180 days 87% (95% CI, 72-94) and at 1 year was 80% (95% CI, 63-90; Figure 2F).

NRM, toxicity, and infectious complications The cumulative incidence of NRM at day 180 was 8% (95% CI, 2-19) and at day 365 was 11% (95% CI, 3-24; Figure 2D). Adverse events were as expected for patients undergoing haplo-HCT (Table 2). There were 3 deaths on trial attributable to infection and all were viral pneumonias; 2 were COVID-19 and 1 was rhinovirus/enterovirus. There were no cases Epstein-Barr virus reactivation or posttransplant lymphoproliferative disorder. Regarding cytomegalovirus (CMV), 34 patients never had detectable CMV in the blood and had no CMV disease. Another 7 patients had detectable CMV levels in the blood as early as

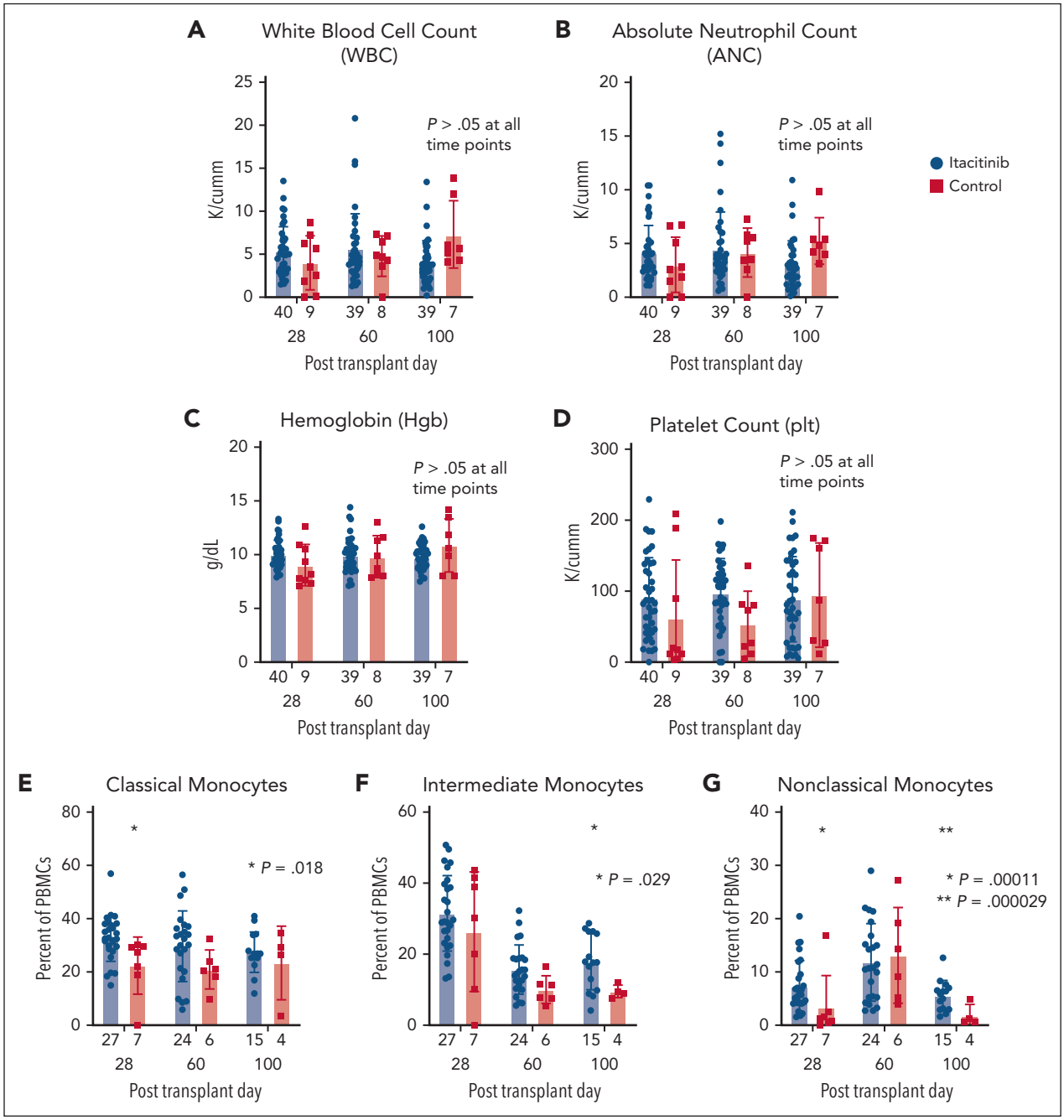

Figure 3. Peripheral blood cell counts at days 28, 60, and 100 after haplo-HCT. (A-D) PB cell counts in study patients (blue) and controls (red) collected on days 28, 60, and 100. White blood cell count (WBC; A), ANC (B), hemoglobin (Hgb; C), and platelet (Plt; D) count were similar at all time points ($P > .05$). (E-G) Percent of peripheral blood mononuclear cells (PBMCs) that are monocyte subsets in itacitinib-treated patients (blue) vs controls (red) at days 28, 60, and 100. There are significantly higher percentages of classical monocytes ($CD14^+CD16^-CD33^+$; E) at day 28 ($P = .018$), intermediate monocytes ($CD14^{+/dim}CD16^{+/dim}CD33^+$; F) at day 100 ($P = .029$), and nonclassical monocytes ($CD14^-CD16^+CD33^+$; G) at day 28 ($P = .00011$) and 100 ($P = .000029$) in itacitinib-treated patients compared with controls.

day 130 and serum DNA levels as high as 6833 IU/mL, but none developed CMV disease. In all cases the CMV viremia resolved with no intervention or restarting letermovir. In the pilot study, 1 patient developed CMV pneumonia at day 145 and serum CMV levels peaked at 72 286 IU/mL on day +157. This patient was treated with ganciclovir, valganciclovir, and CYTOGAM. CMV DNA became undetectable in the blood at day +168 and remained undetectable and the pneumonia resolved. One patient was diagnosed with COVID-19 pneumonia on day +6 after transplant and died of respiratory failure on day +16. One patient had itacitinib held on day +35 per protocol because of thrombocytopenia and experienced prolonged secondary poor graft function evidenced by persistent thrombocytopenia. On day +86 the patient developed COVID-19 and persistent thrombocytopenia and lymphopenia with a normal ANC. On day +133 the patient developed acute hypoxemic respiratory failure and died of progressive respiratory failure on day +143. One patient was taken off itacitinib on day +75 because of drop

Table 2. Grade 3 to 5 adverse events for pilot and expansions phases combined

Adverse event	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Acute kidney injury	3 (7)	1 (2)	0 (0)
Alanine aminotransferase increased	3 (7)	2 (5)	0 (0)
Alkaline phosphatase increased	2 (5)	0 (0)	0 (0)
Anorexia	1 (2)	0 (0)	0 (0)
Aspartate aminotransferase increased	2 (5)	1 (2)	0 (0)
Colitis	1 (2)	0 (0)	0 (0)
Creatinine increased	1 (2)	0 (0)	0 (0)
Cystitis noninfective	2 (5)	0 (0)	0 (0)
Diarrhea	3 (7)	0 (0	0 (0)
Dysphagia	1 (2)	0 (0)	0 (0)
Dyspnea	1 (2)	0 (0)	0 (0)
Electrocardiogram QT corrected interval prolonged	2 (5)	0 (0)	0 (0)
Enterocolitis infectious	6 (14)	0 (0)	0 (0)
Esophagitis	1 (2)	0 (0)	0 (0)
Febrile neutropenia	35 (83)	0 (0)	0 (0)
Flank pain	1 (2)	0 (0)	0 (0)
Generalized muscle weakness	1 (2)	0 (0)	0 (0)
Headache	2 (5)	0 (0)	0 (0)
Hematuria	1 (2)	0 (0)	0 (0)
Hyperglycemia	1 (2)	0 (0)	0 (0)
Hyperkalemia	2 (5)	0 (0)	0 (0)
Hypernatremia	0 (0)	1 (2)	0 (0)
Hyperphosphatemia	1 (2)	0 (0)	0 (0)
Hypertension	1 (2)	0 (0)	0 (0)
Hypertriglyceridemia	6 (14)	0 (0)	0 (0)
Hypocalcemia	1 (2)	0 (0)	0 (0)
Hypokalemia	2 (5)	0 (0)	0 (0)
Hypomagnesemia	2 (5)	0 (0)	0 (0)
Hyponatremia	1 (2)	0 (0)	0 (0)
Hypotension	2 (5)	0 (0)	0 (0)
Hypoxia	2 (5)	0 (0)	1 (2)
Infections and infestations-other, specify	9 (21)	0 (0)	0 (0)
Insomnia	1 (2)	0 (0)	0 (0)
Lung infection	7 (17)	3 (7)	0 (0)
Mucositis oral	12 (29)	0 (0)	0 (0)

Table 2 (continued)

Adverse event	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Nausea	4 (10)	0 (0)	0 (0)
Paronychia	1 (2)	0 (0)	0 (0)
Rectal pain	1 (2)	0 (0)	0 (0)
Renal calculi	1 (2)	0 (0)	0 (0)
Renal colic	1 (2)	0 (0)	0 (0)
Respiratory failure	0 (0)	1 (2)	0 (0)
Reversible posterior leukoencephalopathy syndrome	0 (0)	1 (2)	0 (0)
Sepsis	3 (7)	2 (5)	1 (2)
Skin infection	2 (5)	0 (0)	0 (0)
Supraventricular tachycardia	1 (2)	0 (0)	0 (0)
Urinary tract infection	1 (2)	0 (0)	0 (0)
Weight loss	1 (2)	0 (0)	0 (0)
Wound infection	1 (2)	0 (0)	0 (0)

in platelet count under $100\times10^3/\mu L$ in the context of 100% donor chimerism and preserved hemoglobin and ANC. The patient developed rhinovirus/enterovirus pneumonia on day +86, which progressed to acute hypoxemic respiratory failure resulting in death on day +147. One developed prerenal azotemia because of poor oral intake, resulting in end stage renal disease requiring hemodialysis on day +221. Three months after initiation of hemodialysis, the patient chose to stop renal replacement therapy and died in remission 313 days after transplant. One patient developed severe *Clostridium difficile* colitis on day +221, 61 days after completion of study drug period. The patient underwent emergent colectomy, recovered well and remains alive, in remission, and free of GVHD 1262 days after transplantation.

CRS Itacitinib therapy resulted in low incidence and grade of CRS compared with published haplo-HCT rates[9,14-16] (supplemental Table 1). There were no cases of severe (grade 3-5) CRS, compared with an expected 17% incidence in PB haplo-HCT. There were no cases of grade 2 CRS, compared with expected rate of 28%.[9] All patients on itacitinib had either grade 1 CRS (78% on trial vs 45% expected) or no CRS (22% on trial vs 10% expected).[9] No patients received steroids or anti–IL-6 therapy such as tocilizumab or siltuximab. The median day of onset of CRS was day +2 (range, 1-5).

Correlative study of immune reconstitution

Itacitinib is associated with increased monocyte activation and numbers after haplo-HCT We performed hematopoietic cell identification by flow, identifying 16 distinct populations of cells from the PB (Figure 4A-D, supplemental Methods). Itacitinib patients had a significantly higher proportion of classical monocytes, intermediate monocytes, nonclassical monocytes, basophils, myeloid cells, myeloid dendritic cells (DCs), and plasmacytoid DCs at day +28 (Figures 3E-G and 4E-F; supplemental Figure 1A-D). Although these subsets were not significantly different at day +60 (supplemental Figure 1A-B), we observed greater proportions of circulating intermediate monocytes, nonclassical monocytes, myeloid DCs and plasmacytoid DCs at day +100 (supplemental Figure 1C-D). We further examined monocyte subsets and phenotype by flow and found greater expression of HLA-DR, major histocompatibility complex I, CD38, CD40, CD80, and CD86, all antigens associated with monocyte activation, in bulk monocytes and monocyte subsets from itacitinib-treated patients than control patients (Figure 5A-B). Likewise, scRNAseq revealed increased RNA expression of genes associated with activation in monocytes (Figure 5C).

Itacitinib treatment was associated with decreased numbers of naïve T cells and T cells with reduced activation and exhaustion markers compared with control haplo-HCT We examined T-cell subsets and activation/exhaustion marker expression at days +28, +60, and +100 after haplo-HCT by flow. T cells were identified as regulatory T cells, γδ T-cell receptor T cells, or $CD4^+$ or $CD8^+$ central memory T cells, effector memory T cells, effector T cells, or naïve T cells (supplemental Figure 5). Overall, numbers of circulating T-cell subtypes were similar in patients on itacitinib and controls at days +28, +60, and +100 (supplemental Figure 5). However, there were significantly fewer naïve $CD4^+$ and $CD8^+$ T cells in itacitinib-treated patients at multiple time points (Figure 6B-C), and there were more $CD4^+$ central and effector memory T cells (supplemental Figure 5A-C) and a trend toward increased $CD8^+$ effector memory T cells (supplemental Figure 5D) in the itacitinib-treated patients. Both flow and scRNAseq revealed that itacitinib-treated patients had lower expression of T-cell activation and exhaustion markers *LAG3*, *TIM-3*, *KLRG1*, *PDCD1*, *CTLA4*, *HAVCR2*, and *CD69* at multiple time points (supplemental Figure 2). There were no differences

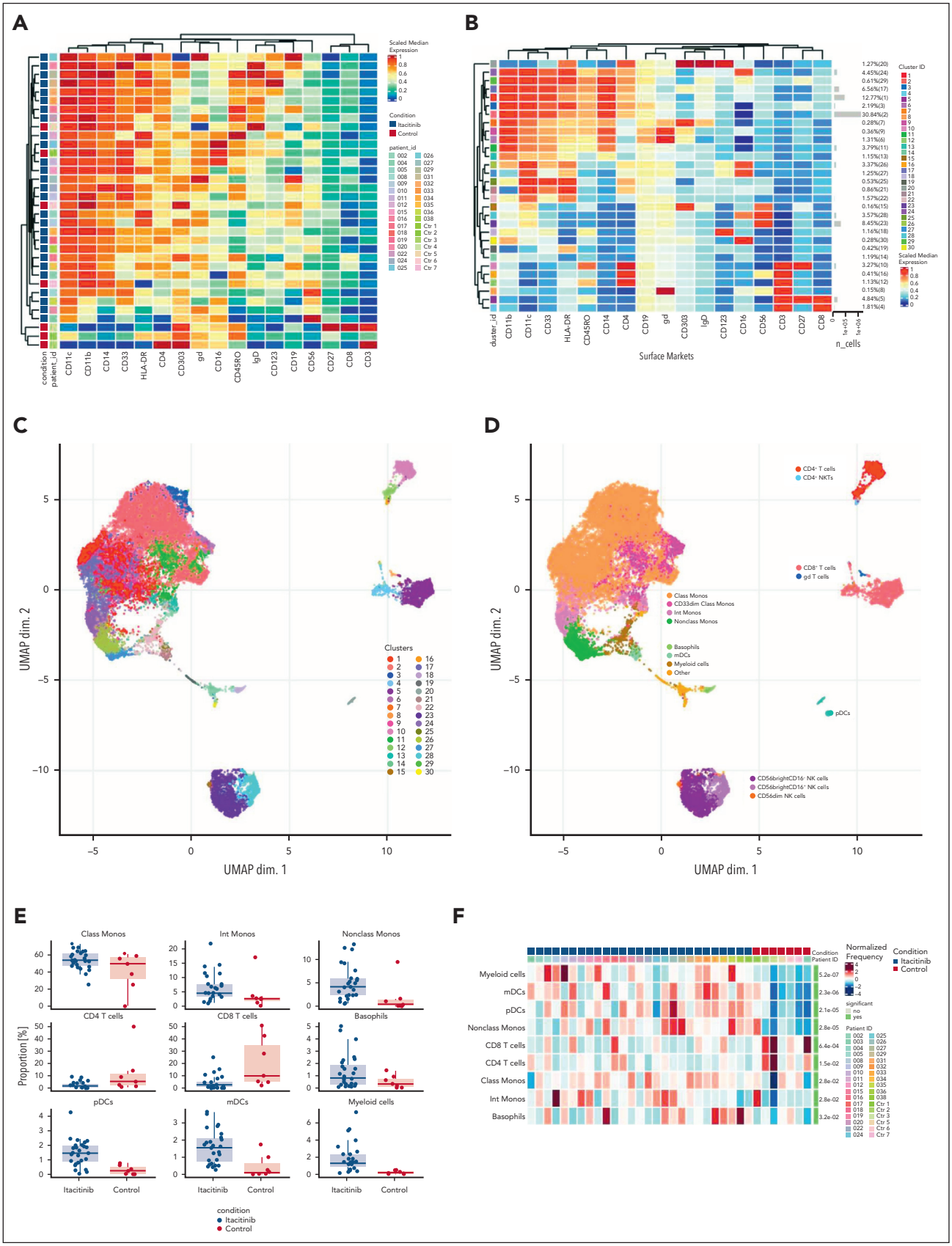

Figure 4. Hematopoietic cell identification by flow panel 1 performed on thawed PBMC samples collected on posttransplant day 28. Detailed methods included in supplemental Materials. Itacitinib samples (n = 27) were from patients on the clinical trial. Control samples (n = 7) were from patients undergoing PB haplo-HCT as standard of care, enrolled during the same time period as the clinical trial. (A) Heat map of median surface marker intensities of 17 lineage markers. Patient and control samples were well

in granzyme A, granzyme B, perforin, or Ki67 expression (data not shown).

Patients on itacitinib and control patients had similar numbers of regulatory T cells and γδ T-cell receptor T cells (supplemental Figure 5G-H). We examined natural killer (NK) cells overall and NK cell subsets (Figure 4D, bottom island in the uniform manifold approximation and projection plot), and found no differences in NK cell numbers between itacitinib-treated patients and controls and any time points (data not shown). We further examined NK cell phenotype by flow and found no differences in CD16, granzyme A, granzyme B, perforin, or Ki67 expression (data not shown).

Discussion

In this trial we assessed the safety of adding itacitinib to the PB haplo-HCT Tac/MMF/PtCy platform in patients with hematologic malignancies, while assessing its effect on severe aGVHD and CRS. The rationale for targeting the JAK/STAT pathway stemmed from preclinical models suggesting its central role in both aGVHD and CRS and the potential of JAK inhibitors in preventing these complications. Itacitinib was safe, with no adverse effect on engraftment. Furthermore, itacitinib was associated with extremely low rates of severe aGVHD, cGVHD, relapse, NRM and CRS, resulting in high GRFS and OS.

Engraftment was not impaired or delayed with itacitinib treatment beginning before allo-HCT, and we observed no cases of primary graft failure. Per protocol design, itacitinib was not held for cytopenias before day +35 and all patients engrafted while on itacitinib. All patients had full donor chimerism at days +30, +100, and +180. Lineage-specific PB chimerism was done at treating physician discretion (supplemental Table 5). This is in keeping with preliminary data using baricitinib prophylaxis in the matched-donor setting.[25] Ruxolitinib for GVHD prophylaxis has been tested in the myelofibrosis setting by multiple groups, with mixed results on engraftment.[27,35] Our study demonstrates that itacitinib can be started before allo-HCT and continued in the posttransplant period without interfering with engraftment in this haplo-HCT platform.

Itacitinib was effective at eliminating grade 2 to 4 CRS. Although the absence of CRS in haplo-HCT has been associated with increased incidence of relapse,[9,17] this was not seen in the context of itacitinib in this trial.

We did not observe an increase in severe aGVHD or in moderate/extensive cGVHD, suggesting itacitinib does not impair the protective effect of PtCy. No patient developed grade 3 or 4 aGVHD. This is encouraging given that with the PB haplo-HCT–PtCy platform, especially with MA conditioning, the expected rate of grade 3 to 4 aGVHD is >15%.[6,7] The incidence of grade 2 to 4 was also low, 16.7% at day +100, compared with historical rates of 38% to 44%.[6,7] After 2 cases of grade 2 skin aGVHD developed in the pilot study during the concurrent itacitinib and Tac taper at day +100, the protocol was amended to extend itacitinib dosing to day +180 before taper. The rationale for extending the duration of itacitinib in the expansion cohort was twofold. First, both patients responded to itacitinib resumption and topical steroids and Tac was not restarted. Second, itacitinib was well tolerated in the pilot, and we wanted to avoid tapering both Tac and itacitinib concurrently at day +100. One of our goals for using JAK inhibitors for prevention of GVHD is to reduce patient exposure to calcineurin inhibitors and systemic steroids, which are associated with toxicities and may be associated with higher relapse rates.[36] In the expansion phase, we saw no increased toxicity related to longer dosing and we saw no cases of aGVHD or cGVHD during the itacitinib taper period in the expansion phase. This suggests that separating the tapering of calcineurin inhibitor and itacitinib is the best approach, with the relatively favorable toxicity profile of itacitinib favoring extending its dosing. Rates of cGVHD were likewise low, with 89% of patients being free of cGVHD at 1 year.

Any approach at reducing GVHD must be balanced with potential increased relapse. Likewise, it has been previously demonstrated that grade 0 CRS after PB haplo-HCT is associated with a higher rate of relapse.[37] In this study, which enrolled a high disease risk population, relapse rate was low, 10% at 1 year, and 14% at 2 years. This is consistent with preclinical models, in which JAK inhibition is not associated with impaired graft-versus-leukemia effect.[38] None of the patients who relapsed had grade 0 CRS, suggesting that grade 0 CRS in the context of itacitinib prophylaxis may not be associated with increased risk of relapse. Low rates of GVHD, NRM, and relapse resulted in a GRFS rate of 85% at day +180 and 79% at 1-year, exceeding historical rates for PB haplo-HCT of 36% to 43%.[7,34]

We examined the effect of itacitinib therapy on immune reconstitution after haplo-HCT and itacitinib was not associated with major suppression of any immune cell subset after haplo-HCT.

We were surprised to find that itacitinib therapy is associated with an increased number of circulating monocytes, with higher expression of several activation markers. The low rates of aGVHD in this study, despite higher monocyte expression of CD80 and CD86, suggest that itacitinib prevents or reduces

Figure 4 (continued) distributed, with clustering of patient samples toward the top of the hierarchy. (B) Heat map of median surface marker intensities of 17 lineage markers across all patient samples. (C) Uniform manifold approximation and projection (UMAP) for dimension reduction plot based on clustering of the 17 lineage markers. Cells are colored the same as in panel B according to the 30 metaclusters. UMAP visualization revealed 5 distinct islands of cells, with minimal cluster blending between or within islands. (D) UMAP after manual cluster merging into specific hematopoietic cell lineages. We identified 16 distinct populations of cells. The upper left island is the largest including classical monocytes (ClassMonos; $CD14^{+}CD16^{-}CD33^{+}$), CD33dim classical monocytes ($CD33^{dim}$ClassMonos; $CD14^{+}CD16^{-}CD33^{dim}$), intermediate monocytes (IntMonos; $CD14^{+/dim}CD16^{+/dim}CD33^{+}$), nonclassical monocytes (NonclassMonos; $CD14^{-}CD16^{+}CD33^{+}$), basophils ($CD123^{+}CD11b^{+}$HLA-DR^{-}), and myeloid DCs (mDCs; $CD33^{hi}$HLA-DR^{hi}). Plasmacytoid DCs (pDCs; $CD123^{+}CD303^{+}$) cluster separately. The $CD4^{+}$ T cells ($CD3^{+}CD4^{+}$), $CD4^{+}$ NK T cells ($CD3^{+}CD4^{+}CD56^{+}$), $CD8^{+}$ T cells ($CD3^{+}CD8^{+}$), and γδ T-cell receptor T cells (γδ T cells; $CD3^{+}\gamma\delta^{+}$) form distinct clusters. The lower middle island includes $CD56^{bright}CD16^{-}$ NK cells ($CD56^{bright}CD16^{-}$), $CD56^{bright}CD16^{+}$ NK cells ($CD56^{bright}CD16^{+}$), and $CD56^{dim}$ NK cells ($CD56^{dim}$).[50,51] (E) Relative abundance of selected hematopoietic cell subtypes, by group (itacitinib in black, control in red), represented by box plots. (F) Differential analysis test results and normalized proportions for hematopoietic cell subtypes. Bar and numbers at the right indicate significant differentially abundant clusters (green) and adjusted *P* values. Control patients have a significantly higher proportion of $CD4^{+}$ T cells and $CD8^{+}$ T cells. Conversely, itacitinib patients have a significantly higher proportion of myeloid cells, mDCs, pDCs, nonclassical monocytes, classical monocytes, intermediate monocytes, and basophils.

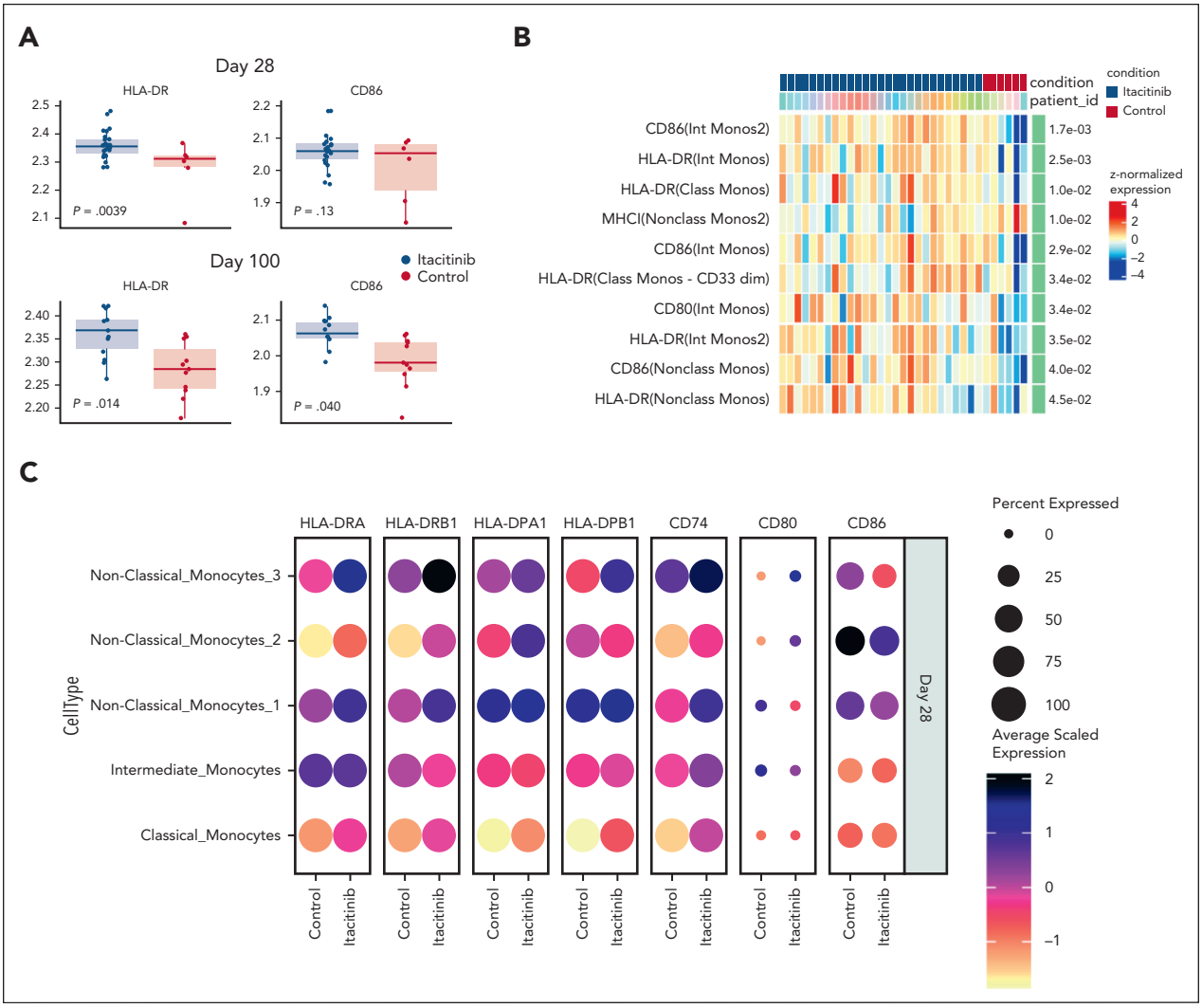

Figure 5. Flow cytometric (A-B) and scRNAseq (C) evaluation of PB monocytes. (A) HLA-DR expression was higher at day 28 ($P = .0039$) and day 100 ($P = .014$), and CD86 expression was higher at day 100 ($P = .04$) in the bulk monocyte population of itacitinib-treated patients. (B) HLA-DR, CD80, and CD86 expression were higher in itacitinib-treated patients at day 28 in monocyte subsets, including classical monocytes ($CD14^{+}CD16^{-}CD33^{+}$), intermediate monocytes ($CD14^{+/dim}CD16^{+/dim}CD33^{+}$), and nonclassical monocytes ($CD14^{-}CD16^{+}CD33^{+}$) by flow. (C) Higher expression of HLA genes, CD74, CD80, and CD86 by scRNAseq at day 28 in monocyte subsets in itacitinib-treated patients compared with control patients.

alloreactive T-cell responses enhanced by these costimulatory molecules. It is also possible that the monocyte activation state in the PB compartment, which we sampled in this study, is different from that in the tissues and in GVHD target organs.

We found no global impairment in T-cell or NK cell reconstitution in patients on itacitinib compared with controls. Overall circulating numbers of T-cell subsets were similar between itacitinib-treated and control patients, with lower numbers of naïve T cells and increased $CD4^{+}$ central and effector memory T cells were observed in itacitinib-treated patients. Preclinical studies support the role of naïve T cells in promoting more severe GVHD compared with central memory or effector memory T cells.[39-41] Naïve T-cell–depleted PB stem cell grafts were administered in a single-arm clinical trial resulting in low incidence of cGVHD.[42] Consistent with reduced alloreactivity, T-cell expression of activation and exhaustion markers was lower in the itacitinib group, including LAG3, TIM-3, KLRG1, and CD69, this was more pronounced at early time points. Although there is a paucity of data on the effect of itacitinib on NK cells, other JAK inhibitors have been shown to reduce the numbers and functional activity of NK cells.[43] Our observation that itacitinib treatment had no effect on NK cell numbers or phenotype is encouraging, because NK cells provide important antiviral and graft-versus-leukemia effects after allo-HCT.[44,45]

Several novel non-JAK inhibitor immunosuppressives have recently been tested for GVHD prevention, all in matched-donor, non-PtCy platforms. Abatacept, a CTLA4 immunoglobulin, was recently approved for prevention of aGVHD in the matched and mismatched unrelated donor, non-PtCy setting, with a grade 3 to 4 aGVHD rate of 6.8%.[46] Sitagliptin, a dipeptidyl peptidase 4 inhibitor was associated with a grade 3 to 4 aGVHD rate of 3%.[47] CD24Fc, which targets damage-associated molecular pattern–mediated signaling,

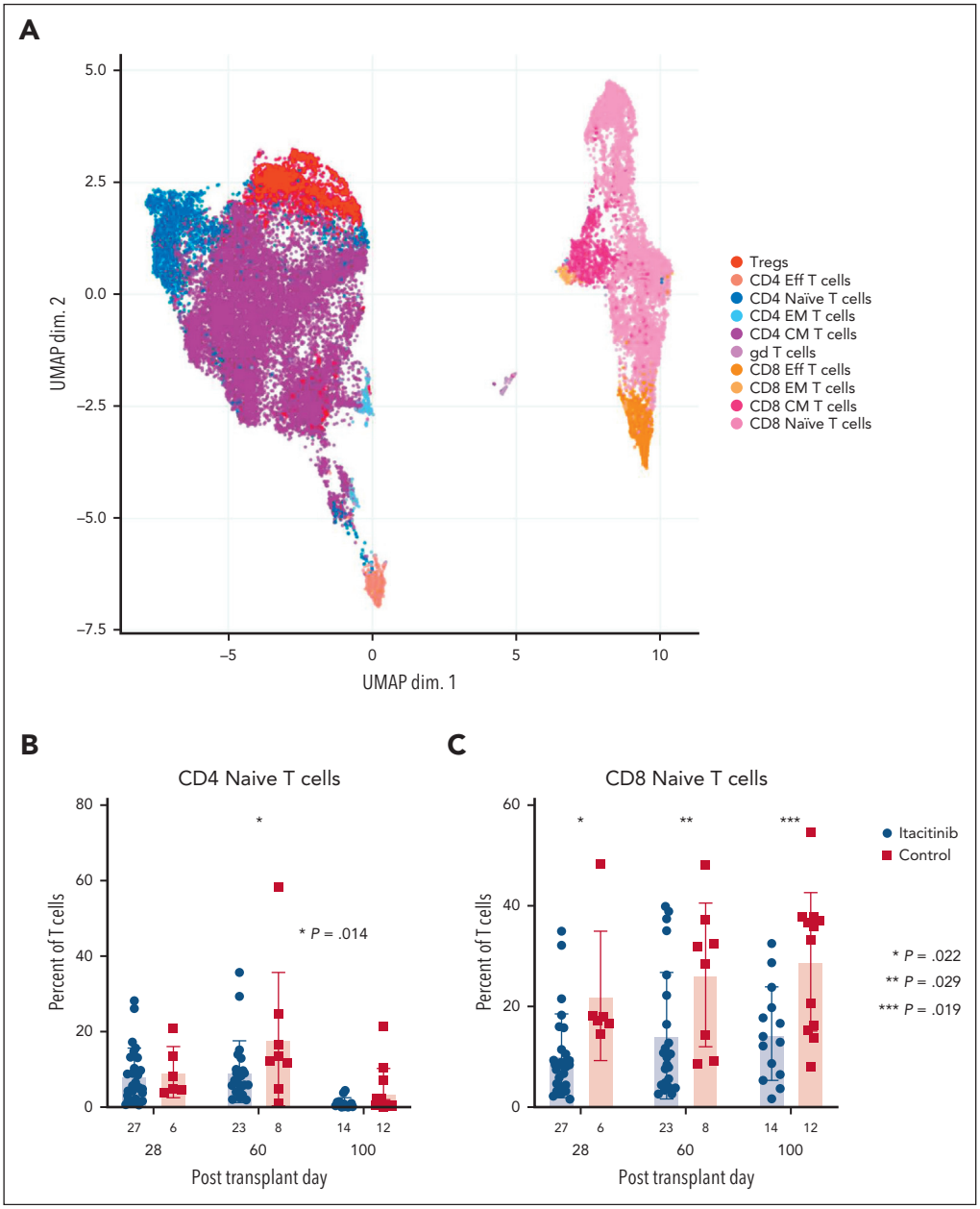

Figure 6. Flow cytometric evaluation of PB T cells. (A) UMAP plot from fluorescence-activated cell sorting (FACS) panel 3 of 1000 randomly selected cells per sample after manual merging of the 30 metaclusters into specific T-cell subtypes. We identified 11 distinct populations of T cells. The left island contains $CD4^+$ T cells, and the right island contains $CD8^+$ T cells. These are characterized as regulatory T cells (Tregs; $CD3^+CD4^+FoxP3^+CD25^{bright}CD127^{dim}$), γδ T cells ($CD3^+\gamma\delta^+$), or $CD4^+$ or $CD8^+$ central memory ($CD3^+CD45RA^-CD197^+$), effector memory ($CD3^+CD45RA^-CD197^-$), effector ($CD3^+CD45RA^+CD197^-$), or naïve ($CD3^+CD45RA^+CD197^+$) T cells. (B-C) Percent naïve CD4 and CD8 T cells of circulating T cells at days 28, 60, and 100. In itacitinib-treated patients there were fewer circulating $CD4^+$ naïve T cells at day 60 ($P = .014$; B) and $CD8^+$ naïve T cells at days 28 ($P = .22$), 60 ($P = .029$), and 100 ($P = .019$) (C).

was associated with a similarly low rate of grade 3 to 4 aGVHD of 3.8%. However, unlike itacitinib, none were associated with significant improvements in cGVHD, with 1-year cGVHD rates of 62%, 37%, and 48.9%, respectively. This may be in part related to the shorter duration of therapy in those studies or the inclusion of PtCy in our study. In a more similar platform to our study, the CAST regimen combined abatacept with shorter course Tac in the PB haplo-HCT–PtCy setting and showed low rates of grade 3 to 4 aGVHD (4.4%) and 1-year moderate-to-severe cGVHD (15.9%).[48]

This study has limitations. It was a single-arm pilot study without an active control group. This study included PB haplo-HCT with Tac/MMF/PtCy GVHD prophylaxis, and the results may not extend to other donor types and transplant platforms. Most patients received MA conditioning, with few patients receiving RIC, because of the overall high-risk disease characteristics of patients enrolled on this study. Although no patient suffered from CMV or Epstein-Barr virus reactivations, there were 3 deaths from viral pneumonia. In rheumatologic diseases, in which JAK inhibitors are given for long treatment periods,

treatment is associated with increased risk of opportunistic and viral infections.[49] It is unclear whether this risk translates to the allo-HCT setting and must be carefully monitored in future studies, especially as the duration of JAK inhibition is extended 6 or 12 months after allo-HCT.

In conclusion, the addition of itacitinib to the PB haplo-HCT Tac/MMF/PtCy platform was safe, without impairment of engraftment. Itacitinib was extremely effective at preventing grade 2 to 4 CRS and grade 3/4 aGVHD, with no clear increase in relapse rate. Itacitinib was well tolerated to day +180, which may allow effective prevention of cGVHD. These data warrant a larger placebo-controlled study of itacitinib in PB haplo-HCT.

Acknowledgments

The authors thank the participants and their families, as well as the nursing and research staff involved in this study.

The authors acknowledge the support of the Alvin J. Siteman Cancer Center, Biostatistics Core, Clinical Trials Core, Bursky Center for Human Immunology and Immunotherapy, Immunomonitoring Laboratory, and Tumor Procurement Core funded via the National Institutes of Health (NIH), National Cancer Institute (NCI) Comprehensive Cancer Center support grant (P30CA091842 [J.F.D]) and the Washington University Institute of Clinical and Translational Sciences grant (UL1TR002345) from the NIH, National Center for Advancing Translational Sciences. Incyte Corporation provided itacitinib and funding to perform this investigator-initiated study. This study was supported by grants from the American Society of Hematology Clinical Research Training Institute, the American Society of Hematology (ASH) research training award for fellows [R.A.], and the NIH, NCI (award number K12 CA167540 [R.A.]); NIH, NCI Outstanding Investigator Award [R35 CA210084 (J.F.D.)]; NIH, NCI Leukemia Specialized Programs of Research Excellence [P50 CA171963 (J.F.D.)]; and NIH, NCI Research Specialist Award [R50 CA211782 (M.P.R.)]).

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or ASH. Incyte Corporation had no influence on trial design, conducting the study, or manuscript creation.

Authorship

Contribution: R.A., R.G.J., F.G., M.A.S., and J.F.D. designed and performed the clinical trial, designed and performed research, analyzed data, and wrote the first version of the manuscript; M.P.R., J.E., L.G., J.R., J.C., C.N.A., I.P., P.W., A.C., M.C., A.G., M.J., G.L.U., and K.S.-G. performed research and contributed to the manuscript; and R.G.J. and F.G. analyzed data.

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

ORCID profiles: R.A., 0000-0001-7786-4155; R.G.J., 0000-0003-2368-4890; J.C., 0000-0002-5590-087X; C.N.A., 0000-0002-6653-3518; I.P., 0000-0003-1725-5184; A.G., 0000-0001-8848-8474; G.L.U., 0000-0002-7809-0996; J.F.D., 0000-0002-0429-3133.

Correspondence: John F. DiPersio, Director of Center for Gene and Cellular Immunotherapy, Washington University School of Medicine, 4940 Parkview, St Louis, MO, 63110; email: jdipersi@wustl.edu.

Footnotes

Submitted 8 August 2024; accepted 5 November 2024; prepublished online on *Blood* First Edition 22 November 2024. https://doi.org/10.1182/blood.2024026497.

Presented in abstract form at the 63rd annual meeting of the American Society of Hematology, Atlanta, GA, 11 December 2021; and the 65th annual meeting of the American Society of Hematology, San Diego, CA, 10 December 2023.

Data can be found at the Gene Expression Omnibus (available at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE282021; accession number GSE282021).

The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

REFERENCES

1. Bashey A, Zhang X, Sizemore C a, et al. T-cell-replete HLA-haploidentical hematopoietic transplantation for hematologic malignancies using post-transplantation cyclophosphamide results in outcomes equivalent to those of contemporaneous HLA-matched related and unrelated donor transplantation. *J Clin Oncol*. 2013;31(10):1310-1316.
2. Luznik L, O'Donnell P V, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14(6):641-650.
3. Kanakry J a, Kasamon YL, Gocke CD, et al. Outcomes of related donor HLA-identical or HLA-haploidentical allogeneic blood or marrow transplantation for peripheral T cell lymphoma. *Biol Blood Marrow Transplant*. 2013;19(4):602-606.
4. Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. Registry. *N Engl J Med*. 2014;371(4):339-348.
5. Raiola AM, Dominietto A, Ghiso A, et al. Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning. *Biol Blood Marrow Transplant*. 2013;19(1):117-122.
6. Ruggeri A, Labopin M, Bacigalupo A, et al. Bone marrow versus mobilized peripheral blood stem cells in haploidentical transplants using posttransplantation cyclophosphamide. *Cancer*. 2018;124(7):1428-1437.
7. Bashey A, Zhang MJ, McCurdy SR, et al. Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell–replete haploidentical donor transplantation using post-transplant cyclophosphamide. *J Clin Oncol*. 2017;35(26):3002-3009.
8. Abboud R, Keller J, Slade M, et al. Severe cytokine-release syndrome after T cell–replete peripheral blood haploidentical donor transplantation is associated with poor survival and anti–IL-6 therapy is safe and well tolerated. *Biol Blood Marrow Transplant*. 2016;22(10):1851-1860.
9. Abboud R, Wan F, Mariotti J, et al. Cytokine release syndrome after haploidentical hematopoietic cell transplantation: an international multicenter analysis. *Bone Marrow Transplant*. 2021;56(11):2763-2770.
10. Weisdorf D, Zhang MJ, Arora M, Horowitz MM, Rizzo JD, Eapen M. Graft-versus-host disease induced graft-versus-leukemia effect: greater impact on relapse and disease-free survival after reduced intensity conditioning. *Biol Blood Marrow Transplant*. 2012;18(11):1727-1733.
11. Choi J, Ziga ED, Ritchey J, et al. IFNγR signaling mediates alloreactive T-cell trafficking and GVHD. *Blood*. 2012;120(19):4093-4103.
12. Abboud R, Choi J, Ruminski P, et al. Insights into the role of the JAK/STAT signaling pathway in graft- versus -host disease. *Ther Adv Hematol*. 2020;11:2040620720914489.
13. Schroeder MA, Choi J, Staser K, DiPersio JF. The role of Janus kinase signaling in graft-versus-host disease and graft versus leukemia. *Biol Blood Marrow Transplant*. 2018;24(6):1125-1134.

14. Raj RV, Hamadani M, Szabo A, et al. Peripheral blood grafts for T cell–replete haploidentical transplantation increase the incidence and severity of cytokine release syndrome. *Biol Blood Marrow Transplant*. 2018;24(8):1664-1670.

15. Mariotti J, Taurino D, Bramanti S, et al. Severe cytokine release syndrome is a fatal complication after PBSC, but not after BM haploidentical transplantation with post-transplant cyclophosphamide [abstract]. *Blood*. 2017;130(suppl 1):665.

16. Imus PH, Blackford AL, Bettinotti M, et al. Severe cytokine release syndrome after haploidentical peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant*. 2019;25(12):2431-2437.

17. Shapiro RM, Kim HT, Ansuinelli M, et al. Cytokine release syndrome in haploidentical stem cell transplant may impact T-cell recovery and relapse. *Blood Adv*. 2023;7(15):4080-4088.

18. Singh N, Hofmann TJ, Gershenson Z, et al. Monocyte lineage–derived IL-6 does not affect chimeric antigen receptor T-cell function. *Cytotherapy*. 2017;19(7):867-880.

19. De Togni E, Wan F, Slade M, Plach K, Abboud R. The impact of tocilizumab treatment for cytokine release syndrome on the incidence of early blood stream infections after peripheral blood haploidentical hematopoietic cell transplantation. *Leuk Lymphoma*. 2022;63(12):2975-2981.

20. Choi J, Cooper ML, Staser K, et al. Baricitinib-induced blockade of interferon gamma receptor and interleukin-6 receptor for the prevention and treatment of graft-versus-host disease. *Leukemia*. 2018;32(11):2483-2494.

21. Kim S, Ashami K, Lim S, et al. Baricitinib prevents GvHD by increasing Tregs via JAK3 and treats established GvHD by promoting intestinal tissue repair via EGFR. *Leukemia*. 2022;36(1):292-295.

22. Ma HH, Ziegler J, Li C, et al. Sequential activation of inflammatory signaling pathways during graft-versus-host disease (GVHD): early role for STAT1 and STAT3. *Cell Immunol*. 2011;268(1):37-46.

23. Zeiser R, von Bubnoff N, Butler J, et al. Ruxolitinib for glucocorticoid-refractory acute graft-versus-host disease. *N Engl J Med*. 2020;382(19):1800-1810.

24. Zeiser R, Polverelli N, Ram R, et al. Ruxolitinib for glucocorticoid-refractory chronic graft-versus-host disease. *N Engl J Med*. 2021;385(3):228-238.

25. Schroeder MA, Choi J, Atluri H, et al. Phase I study of baricitinib GVHD prophylaxis in HLA-matched, peripheral blood allogeneic hematopoietic cell transplant [abstract]. *Blood*. 2022;140(suppl 1):1875-1876.

26. Morozova EV, Barabanshikova MV, Moiseev IS, et al. A prospective pilot study of graft-versus-host disease prophylaxis with post-transplantation cyclophosphamide and ruxolitinib in patients with myelofibrosis. *Acta Haematol*. 2021;144(2):158-165.

27. Ali H, Tsai NC, Synold T, et al. Peritransplantation ruxolitinib administration is safe and effective in patients with myelofibrosis: a pilot open-label study. *Blood Adv*. 2022;6(5):1444-1453.

28. Defilipp Z, Kim HT, Knight L, et al. Maintenance ruxolitinib is associated with low rates of chronic GVHD requiring systemic treatment in older adults with AML/MDS undergoing allogeneic HCT: results of a phase II multicenter trial [abstract]. *Blood*. 2023;142(suppl 1):653.

29. Ruggeri A, Labopin M, Ciceri F, Mohty M, Nagler A. Definition of GvHD-free, relapse-free survival for registry-based studies: an ALWP–EBMT analysis on patients with AML in remission. *Bone Marrow Transplant*. 2016;51(4):610-611.

30. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood*. 2014;124(2):188-195.

31. Harris AC, Young R, Devine S, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai Acute GVHD International Consortium. *Biol Blood Marrow Transplant*. 2016;22(1):4-10.

32. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 Diagnosis and Staging Working Group Report. *Biol Blood Marrow Transplant*. 2015;21(3):389-401.e1.

33. Piantadosi S. Translational clinical trials: an entropy-based approach to sample size. *Clin Trials*. 2005;2(2):182-192.

34. Mehta RS, Saliba RM, Alsfeld LC, et al. Bone marrow versus peripheral blood grafts for haploidentical hematopoietic cell transplantation with post-transplantation cyclophosphamide. *Transplant Cell Ther*. 2021;27(12):1003.e1-1003.e13.

35. Morozova E V, Moiseev IS, Barabanshikova M V, et al. Graft-versus-host disease prophylaxis with posttransplantation cyclophosphamide and ruxolitinib in patients with myelofibrosis [abstract]. *Blood*. 2017;130(suppl 1):4492.

36. Morsink LM, Hazenberg CLE, Choi G, et al. Improved outcome of allogeneic transplantation in older patients treated for myeloid malignancies using post-transplantation cyclophosphamide and reduced duration of immune suppression. *Transplant Cell Ther*. 2024;30(8):794-807.

37. Abboud R, Wan F, Mariotti J, et al. Cytokine release syndrome after haploidentical hematopoietic cell transplantation: an international multi-center collaboration. *Biol Blood Marrow Transplant*. 2020;26(3):S55-S56.

38. Choi J, Cooper ML, Alahmari B, et al. Pharmacologic blockade of JAK1/JAK2 reduces GvHD and preserves the graft-versus-leukemia effect. *PLoS One*. 2014;9(10):e109799, 7.

39. Jiang H, Fu D, Bidgoli A, Paczesny S. T cell subsets in graft versus host disease and graft versus tumor. *Front Immunol*. 2021;12.

40. Anderson BE, McNiff J, Yan J, et al. Memory CD4+ T cells do not induce graft-versus-host disease. *J Clin Invest*. 2003;112(1):101-108.

41. Chen BJ, Cui X, Sempowski GD, Liu C, Chao NJ. Transfer of allogeneic CD62L-memory T cells without graft-versus-host disease. *Blood*. 2004;103(4):1534-1541.

42. Bleakley M, Heimfeld S, Loeb KR, et al. Outcomes of acute leukemia patients transplanted with naive T cell-depleted stem cell grafts. *J Clin Invest*. 2015;125(7):2677-2689.

43. Vian L, Le MT, Gazaniga N, et al. JAK inhibition differentially affects NK cell and ILC1 homeostasis. *Front Immunol*. 2019;10:2972.

44. Mushtaq MU, Shahzad M, Shah AY, et al. Impact of natural killer cells on outcomes after allogeneic hematopoietic stem cell transplantation: a systematic review and meta-analysis. *Front Immunol*. 2022;13:1005031.

45. Gill S, Olson JA, Negrin RS. Natural killer cells in allogeneic transplantation: effect on engraftment, graft- versus-tumor, and graft-versus-host responses. *Biol Blood Marrow Transplant*. 2009;15(7):765-776.

46. Watkins B, Qayed M, McCracken C, et al. Phase II trial of costimulation blockade with abatacept for prevention of acute GVHD. *J Clin Oncol*. 2021;39(17):1865-1877.

47. Farag SS, Abu Zaid M, Schwartz JE, et al. Dipeptidyl peptidase 4 inhibition for prophylaxis of acute graft-versus-host disease. *N Engl J Med*. 2021;384(1):11-19.

48. Al-Homsi AS, Cirrone F, Wo S, et al. PTCy, abatacept, and a short course of tacrolimus for GVHD prevention after haploidentical transplantation. *Blood Adv*. 2023;7(14):3604-3611.

49. Winthrop KL. The emerging safety profile of JAK inhibitors in rheumatic disease. *Nat Rev Rheumatol*. 2017;13(5):320, 243.

50. Koch S, Larbi A, Derhovanessian E, Ozcelik D, Naumova E, Pawelec G. Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. *Immun Ageing*. 2008;5:6.

51. Ong SM, Teng K, Newell E, et al. A novel, five-marker alternative to CD16-CD14 gating to identify the three human monocyte subsets. *Front Immunol*. 2019;10:1761.