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Graft-versus-Host Disease Prophylaxis with Post-Transplantation Bendamustine in Patients with Refractory Acute Leukemia: A Dose-Ranging Study

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A B S T R A C T

The prognosis of acute leukemia refractory to induction chemotherapy or immunotherapy is dismal. Salvage allogeneic hematopoietic stem cell transplantation (HSCT) is widely used option for these patients, but only 10% to 15% of patients are cured by the procedure. Preclinical studies indicate that substitution of post-transplantation cyclophosphamide with bendamustine (PTB) in a prophylaxis regimen may be associated with an augmented graft-versus-leukemia (GVL) reaction. The aim of this study was to establish the optimal dose of PTB and evaluate the antileukemic effect of HSCT with this type of graft-versus-host disease (GVHD) prophylaxis. In the prospective trial (NCT02799147), PTB was administered in doses of 140, 100, and 70 mg/m^2 on days +3 and +4. Myeloablative conditioning with fludarabine and oral busulfan was provided to all patients. The first 12 patients received single-agent PTB, and subsequent patients received combination therapy with tacrolimus and mycophenolate mofetil (MMF). Inclusion criteria were acute myelogenous leukemia (AML) or acute lymphoblastic leukemia (ALL) refractory to at least one induction course of chemotherapy or target therapy and ≥5% clonal blasts in the bone marrow. The study cohort comprised 22 patients with AML and 5 with ALL. Seven patients were enrolled in the 140 mg/m^2 group (due to a stopping rule), and 10 each were enrolled in the 100 mg/m^2 and 70 mg/m^2 groups. Primary refractory disease was documented in 41% of the patients, and secondary refractory was documented in 59%. The median blast count in the bone marrow at the start of the conditioning was 18% (range, 6% to 97%). Transplantation was performed with a matched sibling donor in 5 patients, a matched or mismatched unrelated donor in 15, and a haploidentical donor in 7. Engraftment was documented in 93% of the patients, including 89% with complete remission and 63% without measurable residual disease. After PTB prophylaxis, we observed an unusual complication, a cytokine release syndrome (CRS), in 70% of the patients, including grade 3 to 5 CRS in 44%. The most frequent clinical symptoms included high fever in 67% of patients, abnormal liver function tests in 67%, pancreatitis in 63%, skin vasculitis in 56%, enterocolitis in 48%, inflammation of oral mucosa in 37%, disseminated intravascular coagulation in 37%, and central nervous system toxicity in 26%. The development of CRS was associated with use of an HLA-mismatched donor (75% versus 20%; P = .0043). Classic acute GVHD was documented in 44% of the patients. Grade II-IV acute GVHD was associated with grade 3 to 5 CRS (67% versus 25%; P = .031). Moderate and severe chronic GVHD in the 100-day survivors were more often observed after single-agent PTB than after the combination immunosuppression (100% versus 18%; P = .002). A relatively low relapse rate was observed for this patient population. Three-year overall survival was 28% (95% confidence interval [CI], 13% to 46%), and event-free survival was 29% (95% CI, 13% to 46%). Nonrelapse mortality was 46% (95% CI, 25% to 64%), and the cumulative incidence of relapse was 26% (95% CI, 11% to 44%). No relapses were documented after day +100. There were no statistically significant differences among the dose groups (P = .3481); however, survival was higher in the 100 mg/kg group. Survival was higher in patients with AML compared with those with ALL (35% versus 0%; P = .0157). PTB

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represents a promising option to augment the GVL effect in refractory AML; however, the high CRS-associated mortality necessitates additional studies to reduce the risk of this complication. Thus, routine clinical application of PTB cannot be currently recommended. Combination immunosuppression with tacrolimus and MMF partially ameliorates these complications, at least in the setting of HLA-matched allografts. Biological mechanisms of CRS and GVL after PTB require further elucidation.

INTRODUCTION

The prognosis of acute leukemia refractory to induction chemotherapy is poor. Patients with acute myelogenous leukemia (AML) with failure of two 7+3 inductions [1] or high-dose cytarabine induction [2] have a long-term survival probability of only 10% to 15%. In adult acute lymphoblastic leukemia (ALL), novel targeted therapies are associated with a high remission rate, but relapse and resistance are not uncommon [3-5]. Allogeneic hematopoietic stem cell transplantation (HSCT) is a widely used option for relapsed and refractory acute leukemia. Up to a one-quarter of HSCTs are performed in an advanced disease stage [6]; however, the efficacy of these transplantations is limited. Although favorable outcomes with sequential conditioning strategies in high-risk AML have been reported [7], these data are not always supported in the multicenter retrospective [8,9] and prospective studies [10]. Generally, even after HSCT, the long-term survival of patients with refractory acute leukemia is 15% to 25%. Novel transplantation approaches are needed to improve the efficacy of HSCT in this unfavorable group.

The preclinical study of Stokes et al. [11] demonstrated that substitution of post-transplantation cyclophosphamide (PTCY) with posttransplantation bendamustine (PTB) does not compromise engraftment and facilitates comparable graft-versus-host disease (GVHD) control and an improved graft-versus-leukemia (GVL) effect in the mouse model of acute leukemia. The same group lead by Katsanis et al. [12] initiated a clinical study in children and young adults in which the last dose of PTCY was partly substituted with PTB. Because even lower doses of PTCY have been reported to be effective [13], no unexpected toxicities were observed. We conducted a prospective dose ranging study of PTB alone and in combination with other immunosuppressive agents in patients with refractory leukemia.

METHODS

The prospective open-label Phase I/II study was conducted during 2016 to 2020 at the RM Gorbacheva Research Institute of Pavlov University. The plan was to enroll 30 patients, 10 patients in each of PTB dose level groups: 140 mg/m^2, 100 mg/m^2, and 70 mg/m^2 infused at days +3 and +4, administered in deescalating order. The study was approved by the Ethical Committee of the Saint Petersburg Pavlov University and conducted according to the principles of the Declaration of Helsinki. All patients signed informed consent for the clinical intervention, collection of clinical data, and biological samples for research purposes. The study was registered at ClinicalTrials.gov (NCT02799147). The inclusion criteria were diagnosis of AML, ALL, or mixed-lineage acute leukemia. primary or secondary refractory to at least one course of induction chemotherapy or immunotherapy; >5% of clonal blasts in the bone marrow (BM) or peripheral blood at the time of inclusion; an available sibling, 8-10/10 HLA-matched unrelated, or haploidentical donor; absence of an active second malignancy; no severe concurrent illness or organ dysfunction; and no uncontrolled infection. Previous allogeneic HSCT was also considered an immunotherapy, and these patients were eligible for enrollment. Patients with a Karnofsky Performance Status (KPS) ≥70% could be enrolled. The stopping rule for each arm was >3 consecutive cases of nonrelapse mortality (NRM). In the event of the stopping conditions, the study advisory board was to evaluate the data and decide whether to continue the study with the other dose levels. Only enrollment in the 140 mg/m^2 group was stopped, with 7 patients included in the analysis. Enrollment in the 100 mg/m^2 and 70 mg/m^2 groups was as planned.

Patient and Disease Characteristics

Twenty-two patients had AML, 2 had T cell ALL, and 3 had B cell ALL (Table 1). The median patient age was 38 years (range, 20 to 56 years). Matched sibling donor HSCT was performed in 5 patients, matched unrelated donor HSCT was performed in 15, and haploidentical donor HSCT was performed in 7. Fifteen donors had at least one HLA-mismatch, and 12 donors were 10/10 HLA-matched. The graft source was peripheral blood stem cells (PBSCs) in 23 patients and BM in 4 patients. All recipients and 17 of 27 donors were cytomegalovirus-positive. Twenty-four patients had a first allograft, 2 patients had a second allograft, and 1 patient had a third allograft. The median KPS was 80% (range, 70% to

Table 1
Characteristics of the Study Group

Parameter	Value
Males:females, n (%)	13 (48):14 (52)
Age, yr, median (range)	38 (20-56)
Study group, n (%)	
PTB 140 mg/kg^2	7 (26)
PTB 100 mg/kg^2	10 (37)
PTB 70 mg/kg^2	10 (37)
GVHD prophylaxis regimen, n (%)	
Single-agent PTB	12 (44)
PTB + tacrolimus + MMF	15 (56)
Donor, n (%)	
Matched related	5 (19)
10/10 matched unrelated	7 (26)
9/10 matched unrelated	8 (29)
Haploidentical	7 (26)
Graft source, n (%)	
BM	4 (15)
PBSCs	23 (85)
CD34$^+$ cells, × 10^6/kg cells, mean ± SD	6.2 ± 2.0
Diagnosis, n (%)	
AML	22 (82)
T cell ALL	2 (7)
B cell ALL	2 (11)
Previous induction therapies, median (range)	2 (1-7)
Karyotype, n (%)	
Complex (≥3 abnormalities)	9 (33)
Other high risk abnormalities	7 (26)
Intermediate risk	11 (41)
Molecular abnormalities, n (%)	
RUNX1/RUNX1T1	3 (11)
FLT3-ITD	2 (7)
EVI	2 (7)
KRAS	1 (4)
MLL	2 (7)
Philadelphia chromosome-positive	2 (7)
Extramedullary disease, n (%)	7 (26)
Primary refractory disease, n (%)	11 (41)
Secondary refractory disease, n (%)	16 (59)

90%). A significant proportion of patients had iron overload at the time of HSCT. The median serum ferritin level was 1200 ng/mL (range, 48 to 3828 ng/mL). One-half of the patients received systemic antibiotics, and 22% received antifungals for the treatment of febrile neutropenia or infection at the time of enrollment. The mean C-reactive protein level was 32 ± 44 μg/L. The patients' medical histories are summarized in Supplementary Table S1.

The median blast count in the BM at the start of conditioning was 18% (range, 6% to 97%). The median number of previously failed induction courses or therapies was 2 (range, 1 to 7). Complex karyotype was documented in 33% of the patients, extramedullary disease in 26%, and secondary AML in 22%.

Transplantation Procedures

All patients received myeloablative conditioning with fludarabine 180 mg/m^2 and oral busulfan 12 to 14 mg/kg (FluBu3). All patients age >40 years or with a KPS of 70% received busulfan 12 mg/kg. Bendamustine was administered as a 1-hour i.v. infusion on days +3 and +4. The first 12 consecutive patients did not received additional immunosuppression, and the subsequent 15 patients received tacrolimus adjusted to a concentration of 5 to 15 ng/mL and MMF 30 mg/kg/day starting on day +5.

According to the protocol, patients did not receive any relapse prophylaxis, including targeted therapies or donor lymphocyte infusions. Preemptive therapy for measurable residual disease was allowed.

Supportive care included hydration at 3 L/m^2 from the start of conditioning until day +5, allopurinol 600 mg/day, omeprazole 20 mg twice daily, trimethoprim/sulfamethoxazole 960 mg/day, acyclovir 600 mg/day, and unfractionated heparin 100 U/kg/day. All patients received either primary prophylaxis with echinocandins or secondary prophylaxis according to the previously diagnosed invasive fungal infection. No primary antibacterial prophylaxis was administered besides treatment of previous episodes of febrile neutropenia or infection.

Laboratory Assays

All patients with suspected skin or gastrointestinal (GI) GVHD underwent biopsy and histological examination of the affected organ. No liver biopsies were performed during the study period. EDTA plasma was collected at days -7, 0, +7, +14, +21, +30, +60, and +100 for cytokine analysis. After collection, plasma was centrifuged at 1000 × *g*for 15 minutes at 4 °C, aliquoted, and stored at -80 °C until the day of the assay. IL-1β, IL-10, IL-6, IL-17, and TNF were measured using commercially available ELISA kits (LLC Cytokine, Saint Petersburg, Russia) according to the manufacturer's instructions. Flow cytometry was performed in selected patients using a FACSCanto II flow cytometer (BD Biosciences, San Jose, CA) at days +30, +60, and +100. The panel of antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany) included CD3, CD4, CD8, CD16, CD56, CD197, CD45, and CD45RA. Myeloid-derived suppressor cells were measured based on the currently existing recommendations and defined as Lin$^-$CD33^{dim}HLA-DR$^-$ [14].

For patients with recurrent cytogenetic abnormalities, measurable residual disease (MRD) was assessed by RT-PCR using commercially available tests (Inogen, Saint Petersburg, Russia). For patients without recurrent cytogenetic abnormalities, the combination of WT1, EVI1, and BAALC1 expression and common protocols for flow cytometry [15,16] was used.

Clinical Definitions

The times to disease relapse, acute GVHD (aGVHD), moderate to severe chronic GVHD (cGVHD), NRM, overall survival (OS), and event-free survival (EFS) were defined as the times from transplantation to the event. The incidence of aGVHD was calculated at 125 days after HSCT, and the time frame for the other outcomes was 3 years. Disease relapse was defined as morphologic or cytogenetic evidence of disease with pretransplantation characteristics or morphologic evidence without pretransplantation characteristics. Toxicity was evaluated using CTCAE version 4.03. Sepsis and severe sepsis were diagnosed based on international guidelines for management of severe sepsis and septic shock [17]. Invasive mycosis was diagnosed in case of probable or proven infection according to European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) guidelines [18]. The threshold for cytomegalovirus reactivation was >500 copies/mL. Cytokine release syndrome (CRS) was graded according to the MD Anderson Center guidelines before 2019 [19] and based on American Society for Transplantation and Cellular Therapy consensus criteria starting in 2019 [20].

RESULTS

Engraftment and CRS

Ten patients each were enrolled in the 100 mg/m^2 and 70 mg/m^{2}groups, whereas enrollment in the 140 mg/m^{2}group was halted at 7 patients owing to 3 consecutive cases of NRM. Starting from the first patient in the protocol, rapid engraftment and peculiar features of CRS were observed. Engraftment was documented in 93% of the patients. The median time to WBC engraftment was 17 days (range, 9 to 40 days), and the median time to platelet engraftment was 14 days (range, 9 to 40 days). The use of PTB as single-agent prophylaxis was a major predictor of faster engraftment (median, 13 days versus 20 days; *P*= .0237), as was the graft source (median, 29 days for BM grafts versus 16 days for PBSC grafts; *P*= .002).

The observed clinical syndrome with PTB prophylaxis was different from previously reported complications after conventional GVHD prophylaxis based on calcineurin inhibitors. The typical presentation included high fever with poor response to antipyretics starting the day after the first infusion of PTB; appearance of skin vasculitis before engraftment (Supplementary Figures S1 and S2); elevated liver function test findings, predominantly transaminases (Supplementary Figure S3) with moderate to no bilirubin elevation; elevated serum amylase and lactate dehydrogenase; inflammation of soft palate or lips (Supplementary Figure S4); and diarrhea with nonspecific

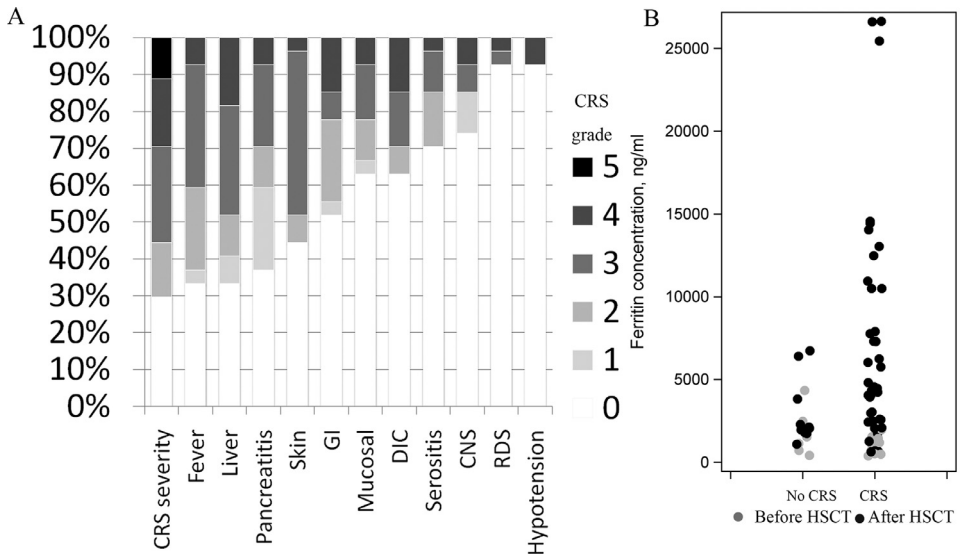

Figure 1. Clinical presentation of CRS (A) and serum ferritin levels in patients without CRS and patients with grade 2-5 CRS (B).

colitis and enteritis (Supplementary Figure S5). The central nervous system toxicity, hypotension, and respiratory distress syndrome observed after chimeric antigen receptor (CAR) T cell therapies also were documented in severe cases of CRS after PTB (Figure 1A).

Overall, CRS was diagnosed in 70% of the patients, including grade 2 in 15%, grade 3 in 26%, grade 4 in 18%, and grade 5 in 11%. The clinical presentation included high fever in 67% of patients, abnormal liver function tests in 67%, pancreatitis in 63%, skin vasculitis in 56%, enterocolitis in 48%, inflammation of the oral mucosa in 37%, disseminated intravascular coagulation in 37%, central nervous system toxicity in 26%, respiratory distress syndrome in 7%, and hypotension in 7%. The median time to manifestation of CRS symptoms, excluding fever, was 9 days (range, 1 to 98 days). The detection of CRS symptoms included extensive laboratory, instrumental, and histological differential diagnosis with sepsis, veno-occlusive disease, and GVHD. An elevated serum ferritin level was observed in the majority of patients with CRS (Figure 1B). The major predictor of CRS was the use of an HLA-mismatched donor (75% versus 20%; P = .0043) and especially of a haploidentical donor (88% versus 42%; P = .0302). The only patient allografted from the haploidentical donor without severe CRS had received a BM graft, whereas the rest of the patients received PBSCs. There was no difference in the incidence of severe CRS (P= .7950), or in the severity of CRS (P= .6416), between patients receiving single-agent PTB and those receiving PTB in combination with tacrolimus and MMF.

Methylprednisone administered at the usual dose of 1 to 2 mg/kg was not effective in controlling CRS. Tocilizumab was administered to 13 patients, 10 of whom had at least a partial response with improvements in clinical manifestations; however, 5 patients required additional anticytokine therapy. Pulsed methylprednisone 300 mg for 2 to 3 days in combination with ruxolitinib 10 to 20 mg/day were effective in controlling CRS manifestations in the majority of patients (Supplementary Figure S6).

Survival, NRM, and Relapse Incidence

One patient died before engraftment, and another patient experienced primary graft failure and disease progression. Complete response was documented in 89% of the patients, and MRD-negative remission was reported in 63%. Three of the 9 patients with MRD cleared it without additional intervention. Although the number of enrolled ALL patients was very small, only 40% of these patients were MRD-negative, compared with 71% of the AML patients.

The 3-year OS was 28% (95% CI, 13% to 46%) and EFS was 29% (95% CI, 13% to 46%). NRM was 46% (95% CI, 25% to 64%), and the cumulative incidence of relapse was 26% (95% CI, 11% to 44%) (Figure 2A, B). There were no statistically significant differences among the dosage groups (P= .3481); however, survival was higher in the 100 mg/kg^2 group. The survival estimates were 14% for the 140 mg/kg^2 group, 40% for the 100 mg/kg^2 group, and 26% for the 70 mg/kg^2 group (Figure 2C). The regimen was not effective in controlling the disease in ALL patients, all but 1 of whom died from disease progression. Survival was 35% in the AML patients versus 0% in the ALL patients (P= .0157) (Figure 2D). In the patients with AML, there was a trend toward worse OS in the presence of grade 3 to 5 CRS (25% versus 44%; P= .092). The relapse incidence was not different between patients with severe CRS and those without severe CRS (P= .9). The cumulative incidence of relapse in the AML patients was 18% (95% CI, 5% to 37%).

Adverse Events and Toxicities

The incidence of early common complications of HSCT was as expected for this patient population. Grade 3-4 mucositis was observed in 30% of the patients, cytomegalovirus reactivation in 48%, invasive breakthrough invasive mycosis in 15%, sepsis in 30%, severe sepsis in 19%, vaso-occlusive disease in 15%, transplant-associated thrombotic microangiopathy in 19%, and grade 3-4 kidney toxicity in 2 patients. Compared with the other transplantation approaches, rates of human herpesvirus (HHV)-6, HHV-1, and HHV-2 reactivation were relatively high, documented by PCR in blood or GI biopsies in 63% of the patients. Two long-term survivors experienced recurrent HHV-1 and -2 infection of the GI tract for 2 years after transplantation until they received a full course of HHV-1 and -2 vaccination.

The cumulative incidence of aGVHD was 44%, including grade I in 2 patients, grade II in 1 patient, grade III in 6 patients, and grade IV in 3 patients. All manifestations of aGVHD were confirmed by biopsy except liver GVHD. Grade II-IV aGVHD was associated with the development of grade 3 to 5 CRS (67% versus 25%; P = .031). The cumulative incidence of moderate and severe cGVHD was 56%. One-hundred-day survivors were more common in the single-agent PTB group compared with the combination immunosuppression group (100% versus 18%; P = .002).

The leading causes of mortality in the study were progressive disease (in 6 patients), CRS-related deaths, including cases of sepsis after immunosuppressive treatment of grade 4 CRS (6 patients), severe cGVHD with concurrent infectious complications (2 patients), and late septic episodes after discharge to local care (2 patients) (Supplementary Table S2). All cases of sepsis-related mortality were observed in patients colonized by carbapenem-resistant gram-negative bacteria.

Cytokine Analysis and Cell Subpopulations

The analysis of cytokines found higher postallograft IL-6 levels in patients with CRS grade 2 to 5 (mean, 60 $\pm$110 pg/mL versus 35 $\pm$ 101 pg/mL; P= .0146) but no associations between CRS and levels of IL-1 (P= .8795), IL-10 (P= .0746), IL-17 (P= .8598), or INF-γ (P = .9011). There also was a significant association between ferritin level and grade 2 to 5 CRS (mean, 7364 $\pm$ 6841 ng/mL versus 2055 $\pm$ 1928 ng/mL; P = .0087) (Supplementary Figures S7 and S8). We observed a rapid decline in both ferritin and lactate dehydrogenase levels after effective anticytokine therapies (Supplementary Figure S6). No association between measured cytokines and GVHD was observed (P= .4490 for INF-γ, P= .6194 for IL-1, P= .2524 for IL-10, P= .7388 for IL-17, and P= .7388 for ferritin), except for higher IL-6 levels in patients with grade III-IV aGVHD (P= .0367).

A population-wide study was conducted to compare immunologic recovery with healthy controls [21]. Owing to the small sample size, high heterogeneity of the group, and extensive use of anticytokine and immunosuppressive therapies in the post-transplantation period, no statistically significant conclusions could be drawn within the group. However, the following observations were made regarding immunologic recovery: (1) in some patients, levels of natural killer (NK) cells and NK T (NKT) cells exceeded those in healthy control both during the early period after engraftment and at long-term follow-up; (2) a close to normal ratio of CD4:CD8 early post-transplantation with high absolute CD4$^+$ counts was observed, with subsequent skewing toward the prevalence of CD8$^+$ cells; (3) overall rapid lymphocyte recovery; and (4) sparing of B cells in some of the patients with their subsequent elimination and recovery starting at 1 year post-HSCT (Supplementary

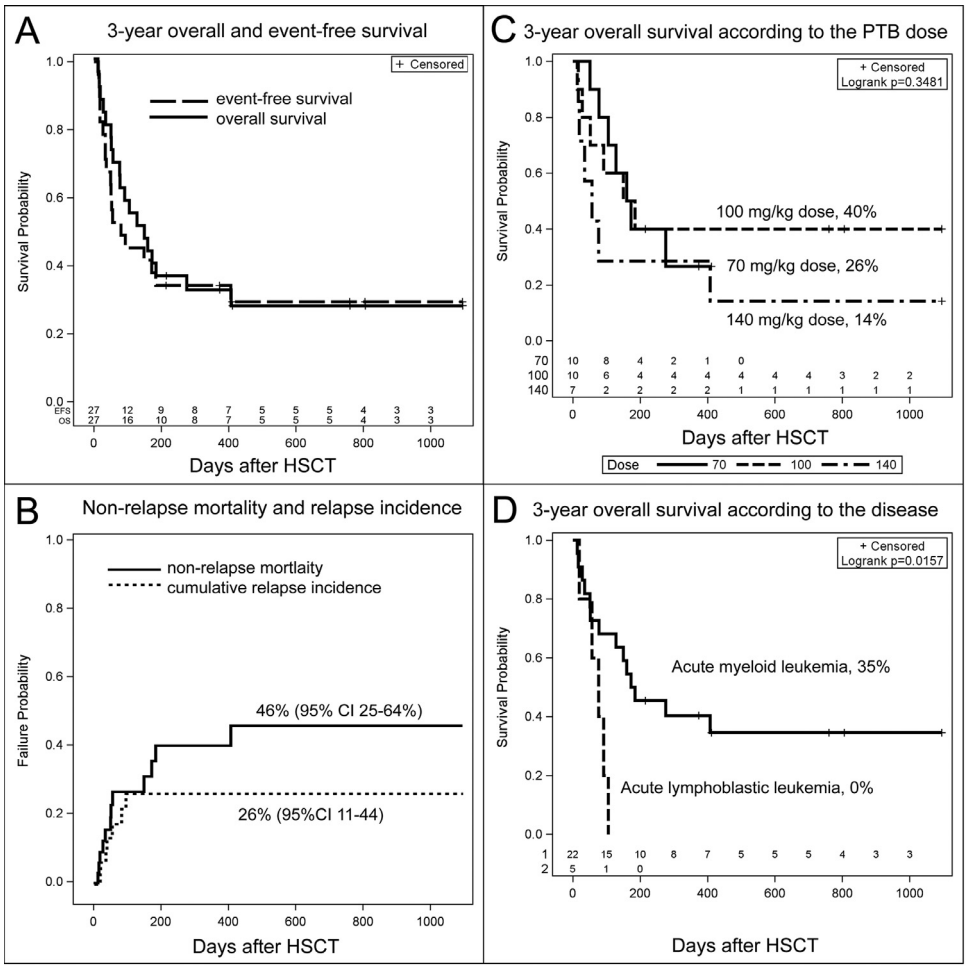

Figure 2. (A) OS and EFS in the study group. (B) NRM and relapse incidence in the study group. (C) OS in the groups with different PTB dose levels. (D) OS in AML and ALL patients.

Figures S9 and S10). In this subset of patients, we analyzed the presence of myeloid-derived suppressor cells, but their prevalence was relatively low after engraftment, only 0.84 ± 1.01% of nucleated cells. On the other hand, we observed a large subpopulation of $CD16^-$ granulocytes, composing 29 ± 19% of nucleated cells. There were too few observations to perform a statistical analysis, but this population of granulocytes was significantly diminished in patients who developed CRS and GVHD (Supplementary Figure S11). We also observed high expression of PD-1L, both on monocytes (66 ± 30% of PD-1L-positive monocytes) and on this $CD16^-$ subpopulation (10 ± 10% of PD-1L-positive granulocytes). PD-1L was also expressed to a lesser extent on lymphocytes (7 ± 7% of PD-1L-positive lymphocytes) (Supplementary Figure S12). Because fresh samples were used for flow cytometry, these subpopulations were not further characterized retrospectively.

DISCUSSION

The present study partially confirmed the results of the preclinical mouse study [11] but also yielded some unexpected results. On the one hand, it confirmed a more rapid engraftment than with PTCY prophylaxis [22]. Moreover, the rate of engraftment was higher than reported previously for refractory leukemia [9]. Another confirmation of preclinical results was the ability of PTB to suppress aGVHD even as a single agent. Several patients from the single-agent cohort did not receive additional immunosuppression after receipt of matched PBSC allografts and did not develop aGVHD. Owing to additional immunologic complications, we did not have the possibility to fully assess the activity of PTB as a single agent, but limited observations indicate that PTB is likely comparable to single-agent PTCY in terms of aGVHD-preventive activity [23]. Nonetheless, all long-term survivors after single-agent PTB therapy developed severe cGVHD, and thus tacrolimus and MMF were added starting on day +5 for all subsequent patients. Unlike PTCY, PTB cannot be used without additional immunosuppression even in the low-risk GVHD population, such as recipients of matched related BM transplantation.

On the other hand, we observed an unexpected immunologic complication: CRS with early signs of macrophage activation. Although CRS has been previously described in replete haploidentical allografts [24-26], there are differences in clinical presentation compared with PTCY prophylaxis. After PTCY, CRS symptoms usually develop immediately or shortly after graft transfusion; however, after PTB, clinical and laboratory signs of CRS usually appeared after graft infusion, indicating that PTB promotes the development of CRS rather than preventing its development after transfusion. Furthermore, the clinical manifestations of CRS differ from those after PTCY, in which the most common symptoms are hypotension, diarrhea, and respiratory distress. Severe CRS is also very uncommon after PTCY [25,26]. In the present study, the most frequent manifestations besides fever were

skin vasculitis, elevated liver function tests, elevated serum amylase, mucositis, colitis, and polyserositis. These features are more characteristic of macrophage activation syndrome in rheumatic diseases [27,28]. This syndrome is associated with significantly increased mortality, especially after haploidentical transplantations. Although NRM is high in the majority of studies focusing on refractory leukemia [8], here it was almost twice as high, reaching 46%. Thus, additional approaches to prevent and effectively treat CRS should be studied before the clinical application of PTB. These may include prophylactic JAK inhibitors [29], prophylactic tocilizumab as in some CAR T cell therapy studies [30], or combinations of PTCY and PTB, as in the original study by Katsanis et al. [12] but in a different proportion.

Besides CRS, we observed some deep and durable responses in the AML patients, which may be a sign of an augmented GVL effect. The rate of complete response was much higher than that with conventional transplantation approaches in refractory leukemia, where it is usually around 50% to 60%. Moreover, the relapse incidence was lower compared with the reported 40% to 50% during the first 2 years [8,9]. There were no late relapses beyond day +100, which is very uncommon for refractory AML and indicates a persistent GVL effect, which can be explained in part by the development of cGVHD in some patients. Because there was no association between EFS and the occurrence of moderate to severe CRS, it is likely that the immunologic mechanisms behind CRS and GVL with this type of prophylaxis might not be the same. The absence of durable remissions in B cell ALL can be explained in part by the previous blinatumomab failure and selection of immunotherapy-resistant patients for the study. Too few patients with T cell ALL were included in the study to allow for any conclusions.

The laboratory studies performed in this study were too limited to determine the exact mechanisms of CRS and GVL induction with PTB prophylaxis. However, we did confirm that, as in CAR T cell-induced CRS [31] and COVID-19-induced CRS, IL-6 plays a significant role here [32]. The response to tocilizumab in one-half of the patients in this study indicates that IL-6-mediated activation of macrophages is one of the mechanisms behind CRS, but the high number of failures indicates that other mechanisms may be in play as well. Our limited flow cytometry studies showed a prominent expansion of NK cells, reaching 30% of nucleated cells in some patients. Thus, NK cells can be a driver of CRS and GVL. The clear association of CRS with HLA-mismatch confirms this hypothesis, as NK cells are always activated after allografts with HLA disparity [33]. The mechanisms behind tolerance induction also seem to be different from those in currently used strategies, but our flow cytometry studies allowed for only speculations about the role of M2 macrophages and PD-1L expression.

In conclusion, PTB represents a promising option to augment the GVL effect in refractory AML. Combination immunosuppression with tacrolimus and MMF is a viable option to control the complications with the use of HLA-matched allografts. Given the high rate of severe CRS after haploidentical HSCT, additional studies are needed before the implementation of PTB in HLA-mismatched HSCT. Although the ALL group was extremely small, no clear benefit of PTB could be demonstrated in these patients. The expansion phase study is now commencing in a refractory AML population with a 100 mg/kg dosing regimen.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2021.03.032.

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