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Extended CCR5 Blockade for Graft-versus-Host Disease Prophylaxis Improves Outcomes of Reduced-Intensity Unrelated Donor Hematopoietic Cell Transplantation: A Phase II Clinical Trial

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A B S T R A C T

Graft-versus-host disease (GVHD) remains the most common treatment-related complication after allogeneic hematopoietic cell transplantation (allo-HCT). Lymphocyte migration plays a critical role in the pathogenesis of GVHD. A previous phase I/II trial demonstrated that CCR5 blockade with maraviroc in the first 30 days after allo-HCT resulted in a low incidence of early acute GVHD, primarily in visceral organs, but with no impact on late acute or chronic GVHD.

We conducted a phase II trial to examine the efficacy of an extended course of maraviroc, administered through post-transplantation day +90 in addition to standard prophylaxis in 37 recipients of reduced-intensity-conditioned unrelated donor allo-HCT performed to treat hematologic malignancies.

Extended maraviroc treatment was safe and feasible. The primary study endpoint, day +180 rate of grade II-IV acute GVHD, was 22 ± 7%, liver GVHD was not observed, and gut GVHD was uncommon. The day +180 rate of grade III-IV acute GVHD was 5 ± 4%. The 1-year rate of moderate to severe chronic GVHD was 8 ± 5% and that of disease relapse was 30 ± 8%. Overall survival at 1 year was 70 ± 8%. Compared with the previously studied short course of maraviroc, the extended course resulted in a significantly higher GVHD-free, relapse-free survival (adjusted hazard ratio [HR], .45; 95% confidence interval [CI], .25 to .82; $P = .009$) and overall survival (adjusted HR, .48; 95% CI, .24 to .96; $P = .037$). A combined analysis of both trials showed that high maraviroc trough concentrations on the day of hematopoietic cell infusion were associated with lower rates of acute GVHD.

An extended course of maraviroc after reduced-intensity-conditioned unrelated donor allo-HCT is safe and effective in preventing acute and chronic GVHD and is associated with favorable survival.

INTRODUCTION

Graft-versus-host disease (GVHD) remains a significant barrier to successful allogeneic hematopoietic cell transplantation (allo-HCT) and is the most common cause of treatment-related mortality in patients with hematologic malignancies, especially when the gastrointestinal (GI) tract is involved [1]. Acute GVHD complicates approximately 30% to 50% of HLA-matched transplantations from related donors and 50% to 70% of transplantations from unrelated donors with standard prophylaxis regimens [2]. Chronic GVHD further complicates allo-HCT and leads to long-term debilitating symptoms and opportunistic infections in many patients [3]. Therefore, additional strategies to prevent GVHD are desperately needed.

Blocking lymphocyte migration may prevent GVHD without interfering with graft-versus-tumor activity. Donor T cells must home to secondary lymphoid organs and then into target organs to recognize alloantigens presented by antigen-presenting cells and to cause tissue injury [4]. This migration is carefully regulated by adhesion molecules and chemokine receptors expressed by lymphocytes, such as CCR5. In animal models, murine GVHD can be prevented by blocking alloreactive $CCR5^+$ T cell homing [5–7]. We previously reported that brief (up to day +30) CCR5 blockade using maraviroc in patients with hematologic malignancies resulted in a low incidence of acute GVHD and the absence of early liver and gut

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GVHD, although some cases of delayed acute GVHD still occurred, and the incidence of chronic GVHD was unaffected [8,9]. In addition, the effects of treatment were seen mainly in unrelated donor transplantations and not in matched related donor transplantations. Therefore, we conducted a phase II study to test the extended administration of maraviroc in unrelated donor allo-HCT.

METHODS

Trial Design

We conducted a phase II clinical trial to study the role of a 3-month course of maraviroc when added to conventional GVHD prophylaxis after reduced-intensity-conditioned allo-HCT from well-matched or single-antigen-mismatched unrelated donors in patients with hematologic malignancies. A 3-month treatment course parallels the course of calcineurin inhibitors at therapeutic levels on our standard protocols and was not extended further owing to an unknown impact on disease relapse. Our primary objective was to determine the efficacy of an extended course of maraviroc in the prevention of acute GVHD. Study participants were considered eligible if they were at least 18 years old; had an available unrelated donor with at least 7/8 HLA-A, -B, -C and -DRB1 matching by high-resolution typing; and met institutional criteria for performance status and organ function for reduced-intensity allo-HCT. Eligible diseases included acute leukemia in complete remission, chronic myelogenous leukemia or myelodysplastic syndrome with <5% marrow blasts, lymphomas, chronic lymphocytic leukemia, and myeloproliferative neoplasms other than primary myelofibrosis.

All participants were admitted to the Hospital of the University of Pennsylvania and received a uniform conditioning regimen of i.v. fludarabine 120 mg/m^2 and busulfan 6.4 mg/kg (Flu/Bu), followed by the infusion of a granulocyte colony-stimulating factor-mobilized peripheral blood stem cell graft from an unrelated donor on day 0.

All participants received a uniform GVHD prophylaxis regimen of oral tacrolimus 0.06 mg/kg/day in 2 divided doses starting on day −3 and i.v. methotrexate 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11. Trough tacrolimus concentrations were checked at least twice weekly, and doses were adjusted to target levels between 5 and 15 ng/mL.

Maraviroc was administered orally twice daily between day −3 and day +90. An oral suspension served as a substitute for tablets in patients who developed severe mucositis. Dose adjustments were performed with interacting medications listed in the maraviroc package insert; however, no dose adjustments were made for concomitant use of voriconazole or posaconazole, based on pharmacokinetic data from our previous study. Dose reduction was allowed for symptomatic orthostatic hypotension, which is dose-dependent.

Clinical Endpoints

The primary endpoint was the cumulative incidence of grade II-IV acute GVHD by day +180 according to the consensus conference criteria [10]. Safety was monitored and recorded using the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0 criteria. Neutrophil engraftment and platelet engraftment were defined as an absolute neutrophil count $>.5 \times 10^9$/L on the first of 3 consecutive days and a platelet count $>20 \times 10^9$/L on the first of 7 consecutive days without transfusion support, respectively. Time to disease relapse, acute GVHD, nonrelapse mortality (NRM), overall survival (OS), and chronic GVHD were defined as the time from transplantation (day 0) to the event. GVHD-free, relapse-free survival (GRFS) was defined as the time from transplantation to grade III-IV acute GVHD, moderate to severe chronic GVHD, disease relapse, or death, whichever occurred first. Patients were censored at the time of last contact alive and event-free or a second transplantation for all outcomes, and at the time of donor lymphocyte infusion when applicable for GVHD outcomes. Disease relapse was defined as morphologic, cytogenetic, or radiologic evidence of disease demonstrating pretransplantation characteristics. Restaging evaluation, including bone marrow biopsies and appropriate imaging, was routinely performed at day +100 or earlier in patients with signs indicating early relapse. The Consensus Conference criteria and National Institutes of Health criteria were used for acute and chronic GVHD grading, respectively [10,11].

Laboratory Assessment

Donor chimerism levels were measured in whole blood and after immunomagnetic-positive selection of CD3$^+$ cells from peripheral blood and bone marrow samples (STEMCELL Technologies, Vancouver, British Columbia, Canada). IgG levels and CD4$^+$ T cell counts were measured using standard assays. Maraviroc trough concentrations were measured in plasma on day 0 following 6 doses of maraviroc and then again on day +14, as described in Supplementary Methods.

Statistical Analysis

We hypothesized that maraviroc treatment through day +90 would decrease the day +180 rate of grade II-IV acute GVHD to <30% from a historical rate of 52%. The historical rate was established in patients who underwent unrelated donor transplantation with Flu/Bu2 conditioning and tacrolimus/methotrexate prophylaxis at the Hospital of the University of Pennsylvania between 2009 and 2014. A sample size of 31 patients provided 80% power for a 1-sample chi-square test of the null hypothesis that $\pi_0 > .52$ versus $\pi_A < .30$ at a 1-sided 5% significance level. The sample size was increased to 37 patients to account for possible mortality in the first 6 months. The planned follow-up was 1 year. The cumulative incidence function was used to analyze time to GVHD and disease relapse, using death as a competing risk. Relapse was considered a competing risk for NRM and GVHD. OS and GRFS were assessed using the Kaplan-Meier method. An exploratory analysis comparing the outcomes of the current study with the previous study (1 month of maraviroc) were conducted using the Gray test [12] or Cox regression followed by multivariable Cox regression modeling with backward elimination to adjust for significant covariates using $P < .05$ as a criterion for inclusion in the models. For this analysis, long-term follow-up data were used and are presented up to 48 months. OS and time to disease relapse were adjusted for the Disease Risk Index (DRI) [13], GRFS was adjusted for donor age, GVHD outcomes were adjusted for graft CD3$^+$ T cell doses, and NRM had no significant covariates. A similar method was used to compare patients with high versus low day 0 maraviroc concentrations, with the planned treatment duration (1 month versus 3 months) as a fixed covariate. The proportional hazards assumption was tested for each covariate to ensure that this assumption was not violated. Analyses were conducted in R (R Project for Statistical Computing, http://www.r-project.org) or in Stata version 13.1 (StataCorp, College Station, Texas).

Study Approval

The study was approved by the Institutional Review Board of the University of Pennsylvania, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

RESULTS

Patient Characteristics

We enrolled 37 patients who underwent allo-HCT from unrelated donors using Flu/Bu conditioning and peripheral blood stem cells between May 2013 and August 2015. Patient characteristics are presented in Table 1. The median and mean age was 64 years, 84% had a matched unrelated donor, 16% had a 7/8 HLA-mismatched unrelated donor, and 49% had a Hematopoietic Cell Transplantation Comorbidity Index >2. Underlying diseases were predominantly acute leukemia (78%) and myelodysplastic syndrome (16%), and the DRI was high or very high in 49% of patients. At the time of analysis, the median duration of follow-up was 36.1 months. All patients were included in the efficacy and safety analysis.

Safety and Feasibility

The 3-month course of maraviroc was well tolerated. Eight patients did not complete treatment, for the following reasons: disease relapse and early withdrawal of all GVHD prophylactic agents in 5 patients; skin reaction thought to be related to sulfa but with maraviroc was discontinued in 1 patient, early infection-related death in 1 patient, and poor oral tolerance in 1 patient. One patient had a dosage reduction to 150 mg twice daily owing to orthostatic hypotension and completed the course of treatment at the modified dose.

All patients experienced neutrophil engraftment, at a median of 12 days (range, 10 to 24 days), and platelet engraftment at a median of 17 days (range 10 to 43 days) in all patients. Donor chimerism levels in whole blood, CD3$^+$ T cells, and bone marrow (presented in Supplementary Figure 1) did not differ from those in historical controls (data not shown).

Clinical Outcomes

The study met its primary endpoint, day +180 rate of grade II-IV acute GVHD, of 22 ± 7% (95% CI, 8% to 36%) (Figure 1A), rejecting the null hypothesis of a rate of 52%. In addition, the day +180 rate of grade III-IV acute GVHD was 5 ± 4% (Figure 1A). In the first

Table 1
Patient Characteristics

Variable	Value
Recipient age, yr, mean (range)	64 (49-72)
Recipient sex, male, female, %	62/38
Hematopoietic Cell Transplantation Comorbidity Index, n (%)	
Low (0)	7 (19)
Intermediate (1-2)	12 (32)
High (>2)	18 (49)
Diagnosis, n (%)	
AML	27 (73)
Myelodysplastic syndrome	6 (16)
ALL	2 (5)
MPN	1 (3)
NHL	1 (3)
DRI, n (%)	
Low	3 (8)
Intermediate	16 (43)
High/very high	18 (49)
Donor, n (%)	
Matched unrelated	31 (84)
Single-antigen mismatched unrelated	6 (16)
Donor age, yr, mean (range)	32 (19-53)
Donor sex, male/female, %	65/35
Cytomegalovirus serostatus, n (%)	
Recipient positive	18 (49)
Donor positive	9 (24)
Cell doses, mean (range)	
$CD34^+$, $\times 10^6$ cells/kg	6.2 (1.5-20.2)
$CD3^+$, $\times 10^8$ cells/kg	2.2 (0.6-8.1)
$CD4^+$, $\times 10^8$ T cells/kg	1.5 (0.3-5.4)
$CD8^+$, $\times 10^8$ T cells/kg	0.7 (0.1-2.2)

100 days, there were no cases of liver GVHD, 2 patients developed stage 1 upper GI GVHD, and 1 patient developed stage 3 lower GI GVHD together with aggressive relapse with documented leukemic infiltrates in the GI mucosa. At 1 year, the incidence of moderate to severe chronic GVHD was 8 ± 5%, NRM was 11 ± 5%, and disease relapse was 30 ± 8% (Figure 1B to D). The GRFS rate at 1 year was 46 ± 8% and OS rate at 1 year was 70 ± 8% (Figure 1E and F). With a median follow-up of 36.1 months, the median survival has not yet been reached. At the time of this report, 20 of 37 patients are alive and 19 are in complete remission. Six patients received donor lymphocyte infusions owing to disease relapse (5) or incomplete donor engraftment (1). Two of these patients are alive. One patient underwent a second transplantation because of disease relapse. Causes of death are listed in Table 2. Two patients died from infectious complications related to delayed acute GVHD, on days +234 and +245. To date, there have been no deaths due to chronic GVHD.

Immune Reconstitution and Infections

$CD4^+$ and $CD8^+$ T cell counts and IgG levels on days +30, +60, +100, and +180 are displayed in Supplementary Figure 2. A CD4 count $<200/\mu$L was observed in a minority of patients at each time point, ranging from 8% to 23%. Only 2 patients (6%) on day +100 and 5 patients (16%) on day +180 had an IgG level <400 mg/dL. Of 18 CMV-positive recipients, 6 (33%) required treatment for CMV reactivation. Four patients (11%) had a *Clostridium difficile* infection during the first year after transplantation. Other infections occurring during the course of treatment included 8 bacteremias, 2 urinary tract infections, 1 pneumonia and 1 cholecystitis. Of note, antibacterial prophylaxis was not used in any of the patients, and our standard prophylaxis included only antifungal, antiviral and anti-PCP prophylaxis. These results demonstrate that extended maraviroc treatment does not have an adverse effect on immune reconstitution or infectious complications.

Maraviroc Extended Course versus Short Course

We conducted an exploratory analysis comparing the GRFS data in this trial with the previously studied short (1-month) course of maraviroc. We excluded HLA-identical related donor transplantations from the previous study to allow for comparison of similar patients. In this analysis, we examined the duration of maraviroc treatment (ie, 3 months versus 1 month) as the primary variable of interest, with adjustment for significant covariates. GRFS was significantly better for the 3-month course compared with the 1-month course (adjusted HR, .45; 95% CI, .25 to .82; $P = .009$; Figure 2A) and the GRFS rates at 1 year were 46 ± 8% versus 8 ± 6%. In a detailed analysis of the components of the GRFS, there were no significant differences in the incidence rate of acute GVHD, but the incidence of disease relapse was significantly lower with the extended treatment course, with adjustment for the DRI (adjusted HR, .41; 95% CI, .19 to .89; $P = .025$; Figure 2B). The incidence rates of chronic GVHD and NRM were also lower for the extended course, but the differences did not reach statistical significance (Figure 2B). Importantly, the extended course of maraviroc was associated with an OS advantage (adjusted HR, .48; 95% CI, .24 to .96; $P = .037$; Figure 2C), and the OS at 1 year was 70 ± 8% for the extended course and 50 ± 10% for the short course.

Maraviroc Blood Concentration Correlates with Efficacy

We combined data from both trials to examine the impact of day 0 trough maraviroc concentration on outcomes. Data were evaluable for 67 patients. There was notable variability in trough concentrations between patients (median, 65 ng/mL; range, 12 to 316 ng/mL) and a similarly high variability was also demonstrated on days +7 and +14 (Supplementary Figure 3). Maraviroc concentrations were not associated with age or sex and were not significantly different between the 2 trials (data not shown). To analyze the independent impact of day 0 trough drug concentration on outcomes, we included the treatment duration (short versus extended) as a fixed covariate in all multivariable models. We found that maraviroc concentrations above the median were associated with lower incidence of grade II-IV acute GVHD (4% versus 36% at 6 months; adjusted HR, .33; 95% CI, .12 to .87; $P = .025$; Figure 3A). The rate of grade III-IV acute GVHD was also lower for higher maraviroc concentrations (0% versus 11% at 6 months; Figure 3B), but the difference did not reach statistical significance in multivariable analysis. Similarly, a lower relapse rate at 1 year (25% versus 54%; Figure 3C) and higher GRFS rate at 1 year (36% versus 20%; Figure 3D) were achieved with higher maraviroc concentrations, but again the differences lacked statistical significance. Maraviroc day 0 concentrations had no association with chronic GVHD, NRM, or OS. Interestingly, among 21 patients with a day 0 trough concentration >100 ng/mL, only 1 patient developed delayed acute GVHD at 7 months after transplantation, and no other cases of acute GVHD were observed.

DISCUSSION

Our group previously conducted the first proof-of-concept study with the CCR5 antagonist maraviroc, demonstrating biological activity in blocking lymphocyte chemotaxis and a clinical benefit manifesting as a low rate of early severe acute GVHD, primarily in visceral organs [8,9]. However, even though 1 month of maraviroc is biologically active, long-term outcomes might not be significantly improved with this brief course of treatment, as also has been suggested in a recent multicenter phase II study comparing maraviroc-treated patients with a contemporary control cohort [14]. In the

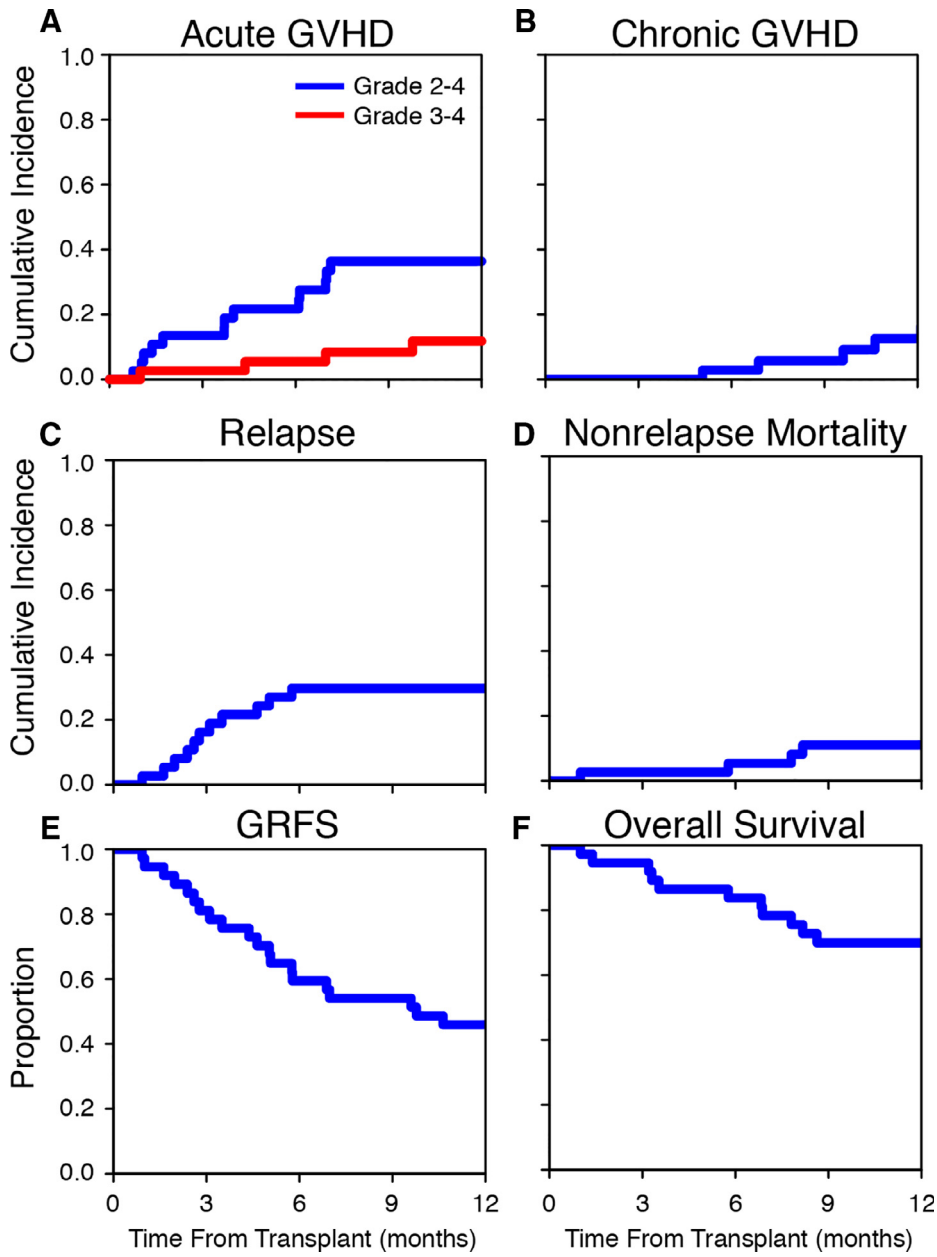

Figure 1. Clinical outcomes with extended course of maraviroc. Shown are cumulative incidence plots of grade II-IV acute GVHD (A), grade III-IV acute GVHD (A), moderate to severe chronic GVHD (B), disease relapse (C), and nonrelapse mortality (D) and Kaplan-Meier plots of GRFS (E) and OS (F).

present study, we examined whether longer treatment, specifically in recipients of unrelated donor transplantations, would further benefit patients by decreasing the risks of delayed acute and chronic GVHD. We found that the longer treatment duration was well tolerated, and that the composite endpoint of GRFS was improved compared to our previous study, leading to a 1-year GRFS rate of 46%. This rate compares favorably with a recent CIBMTR analysis that showed a GRFS of 23% in >5000 patients treated with standard tacrolimus and methotrexate [15]. It is also higher than a recent University of Minnesota analysis in which the 1-year GRFS rate in a heterogeneous group of adult patients who received calcineurin-inhibitor-based GVHD prophylaxis was 24% overall and 26% after reduced-intensity conditioning [16]. Although our cross-trial comparison of 3-month versus historical 1-month treatment is exploratory, these data strongly suggest that the extended course of CCR5 blockade is associated with improved transplantation outcomes.

Table 2
Causes of Death

Cause of Death	Number (%)
Disease relapse	12 (32)
Acute GVHD	2 (5)
Infection	2 (5)
Second malignancy	1 (3)

Surprisingly, the high GRFS rate in our study was driven by low rates of disease relapse and chronic GVHD, whereas the rate of acute GVHD was not significantly different from that associated with the shorter course of maraviroc. Importantly, this study also demonstrates favorable long-term survival

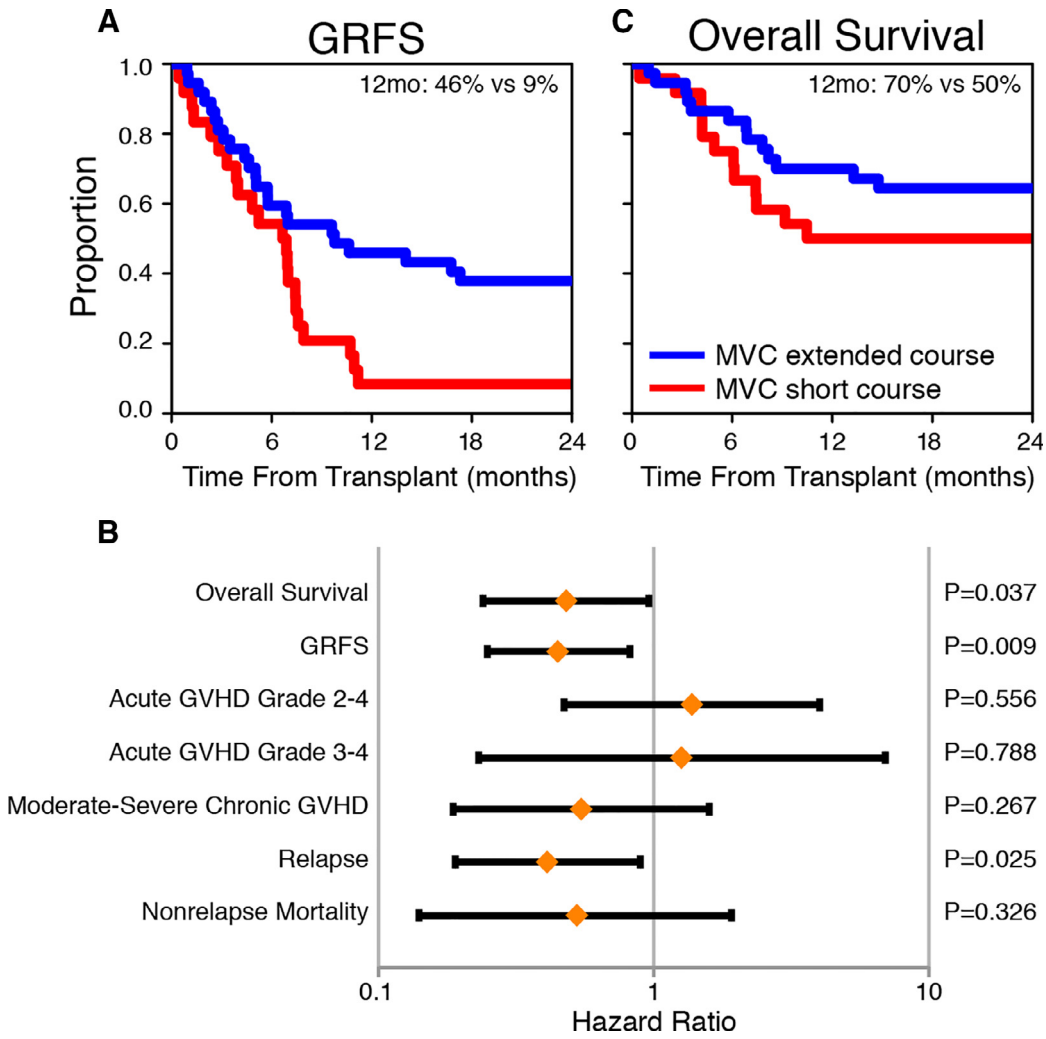

Figure 2. Comparison of outcomes of short-course (1 month) and extended-course (3 months) of maraviroc. (A and C) Kaplan-Meier plots of GRFS (A) and OS (C) with a comparison of 1-year estimates. (B) Forest plot showing adjusted HRs, standard errors, and *P* values from multivariable analyses of transplantation outcomes.

outcomes, with the median survival not reached after a median follow-up of 3 years. Considering the patients' older age, high-risk disease mix, and high Hematopoietic Cell Transplantation Comorbidity Index values, these survival outcomes compare favorably to the published data on reduced-intensity-conditioned HCT [17].

Although a historical comparison of relapse risk across studies is limited and highly exploratory, the relapse risk not only was lower for the extended course of treatment, but also was independently increased with high maraviroc concentrations when analyzing both trials together. These findings support the possibility of a direct antitumor mechanism of maraviroc, either by potentiating the graft-versus-tumor response or through a direct cytotoxic effect. It has been previously shown that absence of CCR5 delays the growth of murine melanoma and enhances dendritic cell-based vaccination [18]. CCR5 antagonists have demonstrated a direct antimetastatic effect in models of CCR5-expressing prostate cancer and breast cancer, and synergism with DNA-damaging cytotoxic agents has been demonstrated [19–21]. CCR5 blockade also has been shown to modify the tumor microenvironment in colorectal and pancreatic cancer models, an effect confirmed in early-phase clinical trials [22–24]. The potential therapeutic effect of maraviroc in cancer is actively explored in clinical trials (NCT03274804 and NCT01736813).

This study confirms previous findings showing that the impact of CCR5 blockade is primarily in visceral organs, as demonstrated by absence of liver GVHD in the first 100 days and the low rate and severity of gut GVHD. We also have previously shown that low levels of the gut biomarker Reg3α accompany the clinical protective effect [9]. The gut-protective effect of maraviroc is particularly important considering the high morbidity, mortality, and cost associated with gut GVHD. This study also confirms the safety profile of maraviroc, which in both studies was not associated with graft failure, an increase in infectious complications, or poor immune reconstitution.

We observed a strong and independent association between maraviroc blood concentrations on day 0 and the incidence of subsequent acute GVHD. In vitro, chemotaxis blockade by maraviroc is dose-dependent [8]. The effect of chemotherapy on maraviroc bioavailability has not been formally explored. In addition, maraviroc metabolism involves cytochrome P450-3A4 and is potentially affected by common genomic polymorphisms [25]. Finally, we have previously described pharmacodynamic variability by conducting CCR5 phosphoflow in real time, which also was correlated with

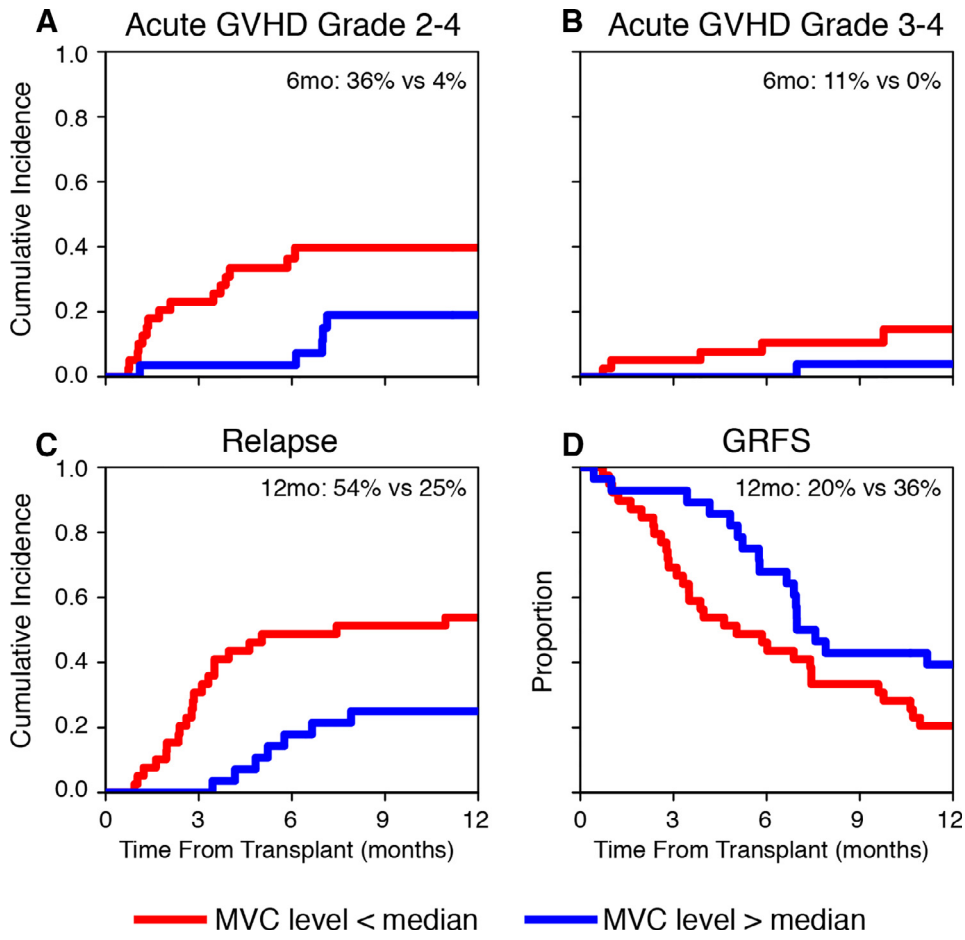

Figure 3. Clinical outcomes in patients with high (>median) versus low (<median) day 0 trough maraviroc concentrations. Shown are cumulative incidence plots of acute GVHD grade II-IV (A), acute GVHD grade III-IV (B), and relapse (C) and a Kaplan-Meier plot of GRFS (D).

outcomes [26]. Together, these factors may explain the heterogeneity in responses to maraviroc, resulting in inferior efficacy in some patients. The importance of adequate levels of GVHD prophylaxis agents early after hematopoietic cell infusion has been previously demonstrated for tacrolimus and cyclosporine [27–29] and potentially mirrors the findings in murine models indicating that T cell activation and migration into tissues is an early event occurring within the first 24 to 72 hours after transplantation [30]. Further studies are needed to characterize the impact of conditioning on maraviroc absorption and to define the optimal maraviroc concentration and a dosing strategy that targets this concentration.

Although this study adds critical information about lymphocyte chemotaxis blockade as a strategy in preventing GVHD, we acknowledge some limitations. This was a single-arm study conducted in a single center. Although we limited the study to a single conditioning regimen and to unrelated donors, it was still a heterogeneous population in terms of disease type and HLA matching. Whereas a comparison with our initial trial using a short course of maraviroc provides important insights, these were sequential studies and not a randomized controlled trial. In particular, the comparison of relapse rates is potentially confounded by variables not captured by the DRI (eg, somatic mutations). In addition, we and others have focused on maraviroc and CCR5 as a proof of concept [8,31], inspired by work in mouse models that lends support to this approach [5–7,32], but other chemokine receptors and adhesion molecules have been implicated, some of which are currently being explored in clinical trials [33–42]. Finally, the results of this study should be examined in the context of the recent BMT-CTN 1203 phase 2 study, which did not identify a benefit for a short course of maraviroc compared with nonrandomized contemporary controls [14]. Our present results suggest that the short course of maraviroc in the 1203 study was suboptimal, but additional differences between these studies should be noted. The 1203 study allowed both unrelated and HLA-identical related donors, whereas the benefit in our phase 1/2 study was exclusive to unrelated donor transplantations. The 1203 study also allowed several conditioning regimens and mouse models have previously shown that the role of CCR5 in GVHD strongly depends on conditioning intensity and may become redundant with more intensive conditioning regimens than Flu/Bu2 [7].

In summary, an extended course of maraviroc is safe and effective and results in a high rate of GFRS. Pharmacologic variables may impact maraviroc concentration and thereby its efficacy, and thus merit prospective evaluation.

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Authorship statement: R.R. and D.L.P. designed and conducted the clinical trial, analyzed and interpreted data, and wrote the manuscript; A.G. and E.P.A. analyzed pharmacokinetic data; R.B., L.C., and J.M. collected and analyzed clinical information; E.O.H., A.W.L., S.M.L., J.M., and E.A.S. treated patients on the clinical trial; J.A.H. and R.H.V. consulted on scientific design and interpreted data; and R.M. consulted on statistical design and data analysis and presentation. All authors reviewed the final manuscript.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.09.034.

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