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Mocravimod, a Selective Sphingosine-1-Phosphate Receptor Modulator, in Allogeneic Hematopoietic Stem Cell Transplantation for Malignancy

Simone Dertschnig[1,*], Peter Gergely[2], Jürgen Finke[3], Urs Schanz[4], Ernst Holler[5], Udo Holtick[6], Gérard Socié[7], Michael Medinger[8], Jakob Passweg[8], Takanori Teshima[9], Christos Stylianou[10], Stephan Oehen[1,2], Dominik Heim[8], Christoph Bucher[8]

[1] *Priothera SAS, St Louis, France*
[2] *Novartis Institute of Biomedical Research, Basel, Switzerland*
[3] *University of Freiburg Medical Center, Freiburg, Germany*
[4] *Department of Medical Oncology and Hematology, Zurich University and University Hospital, Zurich, Switzerland*
[5] *University Hospital, Regensburg, Germany*
[6] *Department I of Internal Medicine, Medical Faculty and University Hospital of Cologne, University of Cologne, Cologne, Germany*
[7] *APHP Hospital Saint Louis & University of Paris, Paris, France*
[8] *University Hospital, Basel, Switzerland*
[9] *Hokkaido University, Faculty of Medicine, Sapporo, Japan*
[10] *ClinBAY Ltd, Limassol, Cyprus*

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A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the sole curative option for patients with acute myelogenous leukemia. Outcomes are limited by leukemia relapse, graft-versus-host disease (GVHD), and abnormal immune reconstitution. Mocravimod (KRP203) is an oral sphingosine-1-phosphate receptor (S1PR) modulator that blocks the signal required by T cells to egress from lymph nodes and other lymphoid organs. Mocravimod retains T cell effector function, a main differentiator to immunosuppressants. In preclinical models, mocravimod improves survival by maintaining graft-versus-leukemia (GVL) activity while reducing GVHD. In patients undergoing allo-HSCT for hematological malignancies, mocravimod is postulated to prevent GVHD by redistributing allogeneic donor T cells to lymphoid tissues while allowing a sufficient GVL effect in the lymphoid, where malignant cells usually reside. The primary objective of this study was to assess the safety and tolerability of mocravimod in patients undergoing allo-HSCT for hematologic malignancies. Secondary objectives were to determine the pharmacokinetic profiles of mocravimod and its active metabolite mocravimod-phosphate in this patient group, as well as to assess GVHD-free, relapse free survival at 6 months after the last treatment. In this 2-part, single- and 2-arm randomized, open-label trial, we evaluated the safety, tolerability, and pharmacokinetics of mocravimod in allo-HSCT recipients (ClinicalTrials.gov identifier NCT01830010). Patients received either 1 mg or 3 mg mocravimod per day on top of standard of care GVHD prophylaxis with either cyclosporine A/methotrexate or tacrolimus/methotrexate. We found that mocravimod can be safely added to standard treatment regimens in patients with hematologic malignancies requiring allo-HSCT. Mocravimod resulted in a significant reduction of circulating lymphocyte numbers and had no negative impact on engraftment and transplantation outcomes. Our results indicate that mocravimod is safe and support a larger study to investigate its efficacy in a homogeneous acute myelogenous leukemia patient population undergoing allo-HSCT.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is standard of care (SoC) for patients with hematologic malignancies at high risk of relapse [1]. Disease recurrence is prevented in part by alloreactive donor T cells (allo-T) present

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*Correspondence and reprint requests: Simone Dertschnig, Priothera SAS, 57 Av General de Gaulle, 68300 St Louis, France
E-mail address: simone.dertschnig@priothera.com (S. Dertschnig).

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in stem cell product. Although alloreactivity against malignant cells of hematopoietic origin (MCHO) is desired, broader anti-host reactivity, causing graft-versus-host disease (GVHD), limits the overall success of allo-HSCT. Colocalizing allo-T with MCHO while limiting migration of allo-T to non-lymphohematopoietic tissues has shown to improve GVHD-free survival in preclinical models [2,3].

Mocravimod (KRP203) is a novel, synthetic, amino-alcohol, sphingosine-1-phosphate receptor (S1PR) modulator that is phosphorylated in vivo to its active entity mocravimod-phosphate (mocravimod-P). Mocravimod-P targets 4 of the 5 S1PRs, albeit with different potency and efficacy and high selectivity for the S1P1 receptor [4]. Mocravimod has been shown to eliminate the signal required by T cells to egress from lymphoid organs, and thus to prevent effector cell migration to non-lymphohematopoietic tissues [5]. Although redistributing lymphocytes to lymphoid organs results in blood (and non-lymphohematopoietic tissue) lymphopenia, S1PR modulation does not interfere with T cell cytotoxicity [6].

In murine models, pharmacologic modulation of the S1P1 receptor has been shown to efficiently sequester allo-T in lymphoid tissues and consequently reduce GVHD, while maintaining the graft-versus-leukemia (GVL) response and thus reducing mortality [2,7-10]. Here we present the results of the first clinical trial investigating the S1PR modulator mocravimod in the setting of allo-HSCT for the treatment of hematologic malignancies.

METHODS

Study Design and Patient Population

CKRP203A2105 (ClinicalTrials.gov identifier NCT01830010) is a multicenter, phase Ib study of mocravimod in intermediate-risk to high-risk patients undergoing allo-HSCT for hematologic malignancies (Figure 1). Part 1 was a single-arm open-label study to investigate the safety of 3 mg/day orally administered mocravimod added to SoC GVHD prophylaxis with cyclosporine A (CsA)/methotrexate (MTX) (Mo3CsA) in 10 patients. Part 2 was a randomized 2-arm open-label study to assess the safety, pharmacokinetics, and preliminary efficacy of 1 mg/day mocravimod in combination with CsA/MTX (Mo1CsA) or 3 mg/day mocravimod in combination with tacrolimus/MTX (Mo3Tac). Study drug dose adjustments were permitted according to the study protocol, and drug interruptions were allowed based on the judgment of the investigator. Serious adverse events, emergency medical conditions involving or not involving the use of excluded concomitant medications, clinically significant laboratory values or abnormal test or examination results, and patient noncompliance were events that led to study drug interruptions. Adverse events (AEs) were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE version 4.03). Institutional practices were followed to monitor liver safety. Male and female patients age 18 to 65 years with a hematologic malignancy and an HLA-matched donor (9/10 or 10/10) were eligible for the study and were treated for 111 days with mocravimod on top of SoC GVHD prophylaxis. Disease characteristics are summarized in Supplementary Table S1. All patients except the first patient received fully myeloablative conditioning (Table 1). Detailed inclusion and exclusion criteria are listed in Supplementary Table S2.

Treatment

Treatment with mocravimod (on day 1) was initiated 10 days before allo-HSCT (transplantation day 11) and continued for an additional 100 days (day 111). Patients had a follow-up of 2 years post-transplantation. SoC GVHD prophylaxis (with CsA/MTX or tacrolimus/MTX) was initiated in accordance with local practice in compliance with European Society for Blood and Marrow Transplantation guidelines [11].

Assessments and Endpoints

The primary objective of this study was to assess the safety and tolerability of mocravimod in patients undergoing allo-HSCT. Neutrophil engraftment was defined as the first of 3 consecutive days with a neutrophil count $>.5 \times 10^9$/L. Platelet engraftment was defined as the first of 3 consecutive days with a platelet count $>50 \times 10^9$/L. The secondary objectives were to determine the pharmacokinetic profiles of mocravimod and its active metabolite mocravimod-P and to assess GVHD-free, relapse-free survival (GRFS) at 6 months after the last treatment. Serial pharmacokinetic samples were collected on days 1 and 28 in Part 1 and on days 1, 25, and 41 in Part 2. Mocravimod and mocravimod-P concentrations were measured in whole blood using a validated liquid chromatography-tandem mass spectrometry method, with a lower limit of quantification of .05 ng/mL. Pharmacokinetic parameters were calculated using noncompartmental methods. Pharmacodynamic biomarkers included the monitoring of neutrophil recovery, platelet recovery, and absolute lymphocyte count (ALC). Efficacy was explored based on the incidences of GVHD, mortality, and relapse by assessing GRFS at 6 months after the last treatment. GRFS-defining events included death from any cause, relapse, chronic GVHD (cGVHD) requiring systemic treatment, and grade III-IV acute GVHD (aGVHD). The incidence of aGVHD (grade II-IV) and relapse of primary disease were assessed as exploratory endpoints in Part 1 and as secondary efficacy endpoints in Part 2.

Safety

Safety assessments consisted of collecting all AEs and severe AEs (SAEs) and assessing pregnancy and prospective suicidality. Fourteen-day continuous in-house cardiac monitoring, repeat pulmonary function testing,

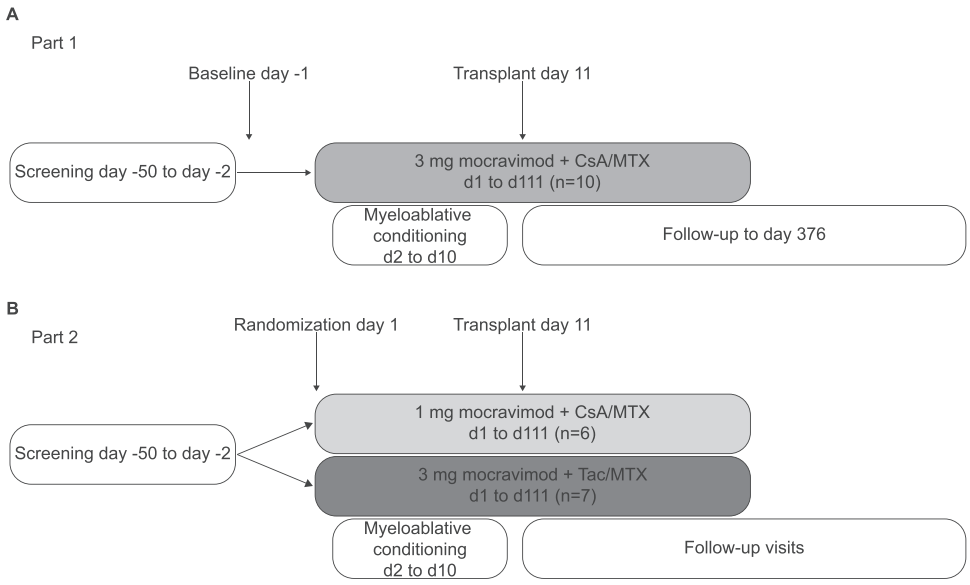

Figure 1. Study design. (A) A total of 10 patients were included in Part 1 of the study. Here 3 mg/kg mocravimod was added to SoC GVHD prophylaxis CsA/MTX (Mo3CsA). (B) A total of 13 patients was included in Part 2 of the study. Six patients were randomized to the study arm with 1 mg/day mocravimod plus CsA/MTX (Mo1CsA), and 7 patients were randomized to the study arm 3 mg/day mocravimod plus tacrolimus/MTX (Mo3Tac).

Table 1
Patient Characteristics

Characteristic	Mo3CsA Arm (N = 10)	Mo1CsA Arm (N = 6)	Mo3Tac Arm (N = 7)	Total (N = 23)
Age, yr, median (range)	49.5 (26-60)	51.0 (35-62)	56 (23-63)	51.0 (23-63)
Male sex, n (%)	5 (50)	5 (83)	4 (57)	14 (61)
Race, n (%)				
Caucasian	9 (90)	6 (100)	5 (71)	20 (87)
Asian	1 (10)	0 (0)	0 (0)	1 (4)
Other	0 (0)	0 (0)	2 (29)	2 (9)
Weight, kg, median (range)	74 (55-80)	84.15 (79-119.1)	76 (56-99.8)	76.3 (55-119.1)
BMI, kg/m^2, median (range)	24.992 (20.66-29.03)	28.407 (24.6-39.34)	25.69 (18.29-33.59)	25.69 (18.29-39.34)
Diagnosis, n (%)				
AML	3 (30)	2 (33.33)	2 (28.57)	7 (30.43)
ALL	3 (30)	1 (16.67)	0 (0)	4 (17.39)
BCL	1 (10)	0 (0)	0 (0)	1 (4.35)
CML	0 (0)	3 (50)	1 (14.29)	4 (17.39)
MDS	2 (20)	0 (0)	1 (14.29)	3 (13.04)
MF	0 (0)	0 (0)	1 (14.29)	1 (4.35)
MM	1 (10)	0 (0)	0 (0)	1 (4.35)
NHL	0 (0)	0 (0)	1 (14.29)	1 (4.35)
HD	0 (0)	0 (0)	1 (14.29)	1 (4.35)
Disease status at transplantation, n (%)				
CR1	4 (40)	3 (50)	3 (42.9)	10 (43.48)
CR2	1 (10)	0 (0)	0 (0)	1 (4.35)
Chronic phase	0 (0)	0 (0)	1 (14.29)	1 (4.35)
Partial remission	1 (10)	0 (0)	0 (0)	1 (4.35)
Partial response	1 (10)	1 (16.67)	1 (14.29)	3 (13.04)
Persistent leukemia	1 (10)	0 (0)	0 (0)	1 (4.35)
PIF	1 (10)	0 (0)	0 (0)	1 (4.35)
Primary refractory	1 (10)	0 (0)	0 (0)	1 (4.35)
Primary refractory/refractory relapse	0 (0)	2 (33.33)	1 (14.29)	3 (13.04)
Untreated	0 (0)	0 (0)	1 (14.29)	1 (4.35)
Conditioning regimen, n (%)				
CyTBI	3 (30)	0 (0)	0 (0)	3 (13.04)
BuCy	2 (20)	0 (0)	1 (14.29)	3 (13.04)
FluBu	2 (20)	1 (16.67)	2 (28.57)	5 (21.74)
FluThioMel	0 (0)	2 (33.33)	3 (42.9)	5 (21.74)
Other myeloablative	2 (20)	3 (50)	1 (14.29)	6 (26.1)
Mini-Seattle FluTBI	1 (10)	0 (0)	0 (0)	1 (4.35)
Donor type, n (%)				
MMUD	2 (20)	0 (0)	0 (0)	2 (8.7)
MSD	5 (50)	2 (33.33)	4 (57.14)	11 (47.83)
MUD	3 (30)	4 (66.67)	3 (42.9)	10 (43.48)

BMI indicates body mass index; **AML, acute myelo**genous **leukemia; ALL, acute lymphoblastic leukemia; BCL, B cell lymphoma; CML, chronic myelo**genous **leukemia; MDS, myelodysplastic syndrome; MF, myelofibrosis; MM, multiple myeloma; NHL, non-**H**odgkin lymphoma; HD**, H**odgkin disease; CR1, first complete remission; CR2, second complete remission; PIF, primary induction failure**; Cy, cyclophosphamide; TBI, total body irradiation; Bu, busulfan; Flu, fludarabine; Thio, thiotepa; Mel, melphalan; **MMUD, mismatched unrelated donor; MSD, matched sibling donor; MUD, matched unrelated donor.**

ophthalmic monitoring, and liver function testing were performed based on the known safety profile of the S1PR modulator class.

Statistical Analysis

All patients who received at least 1 dose of mocravimod were included in the analyses. Descriptive analysis was performed for safety and pharmacokinetic and pharmacodynamic data by treatment and time point. Descriptive visualization of pharmacokinetic data presents the arithmetic mean with error bars representing the standard deviation (SD), and visualization of pharmacodynamic data presents the arithmetic mean with error bars representing the standard error (SE). Where consolidated descriptive visualizations are presented, the errors for both pharmacokinetic and pharmacodynamic data are in terms of SD. Efficacy (time-to-event) data are presented using Kaplan-Meier curves, and where the median time to event is described, it is obtained from Kaplan-Meier estimates. For descriptive summaries of pharmacokinetic parameters, unless stated otherwise, these parameters were summarized using geometric means, except for T_{max} and T_{lag}, which were summarized using the median. The relationship between mocravimod-P and ALC also was analyzed using a sigmoid E_{max} model with a random effect to adjust for patient variability,

$$ALC_{ij} = E_0 - \frac{E_{max} + Conc_{ij}}{EC_{50} + Conc_{ij}} + s_i + \varepsilon_{ij}$$

where ALC_{ij} is the ALC of patient i at observation j, $Conc_{ij}$ is the corresponding metabolite concentration, E_{max} is the maximum decrease, EC_{50} is the metabolite concentration at which the expected benefit is one-half the maximum, and E_0 is the effect at zero metabolite concentration. Safety data were summarized using standard descriptive methods. All statistical analyses were performed using SAS software (SAS Institute, Cary, NC). Analysis of endpoints that may be affected by a competing risk of death were analyzed using cumulative incidence function estimates, unless no effect by competing risks was observed, in which case Kaplan-Meier estimates were used instead.

RESULTS

Patients

Twenty-three patients were enrolled into the study from 2013 to 2017. Ten patients were recruited to the Mo3CsA arm, 6 patients were randomized to the Mo1CsA arm, and 7 patients were randomized to the Mo3Tac arm. Patient malignancies spanned a broad spectrum requiring allo-HSCT (Table 1). The median duration of follow-up was 643 days, and the median duration of exposure to mocravimod was 111 days (Supplementary Figure S1). Three patients in the Mo1CsA arm and 6 patients in the Mo3Tac arm discontinued the study, including 2 during mocravimod treatment (Table 2). The primary reason for study discontinuation was withdrawal of consent (n = 4; 17%), followed by AEs (n = 3; 13%) and death (n = 2; 9%) (Table 2).

Safety

A total of 713 AEs were reported, including 47 SAEs occurring in 15 of the 23 patients (Table 2). Thirteen SAEs occurred in 4 of 6 patients in the Mo1CsA arm, 18 SAEs occurred in 5 of the 10 patients in the Mo3CsA arm, and 16 SAEs occurred in 6 of the 7 patients in the Mo3Tac arm. Bradycardia, a known S1PR modulator-related AE, was reported in 2 patients in the Mo3CsA arm before transplantation, but it did not result in dose adjustment or discontinuation (Table 2). One mocravimod-related AE (cystoid macular edema) was reported within the first 30 days after transplantation in the Mo1CsA arm, and 1 mocravimod-related AE (dyspnea) also was reported in the Mo3CsA arm. After 30 days post-transplantation, 7 mocravimod-related AEs were reported, with no skewing to any of the treatment arms (Mo1CsA: macular and retinal ischemia; Mo3CsA: macular edema, dyspnea, peripheral edema, and pleural effusion; Mo3Tac: hepatic failure, which in a retrospective analysis was considered to not be mocravimod-related but rather a consequence of aGVHD.) Mocravimod was discontinued in 2 patients with macular edema on day 24 and day 86. On mocravimod discontinuation, macular edema resolved in both patients on day 50 and day 107, respectively, and neither

Table 2
Safety

Parameter	Mo3CsA Arm (N = 10)	Mo1CsA Arm (N = 6)	Mo3Tac Arm (N = 7)	Total (N = 23)
Patient disposition, n (%)				
Completed	10 (100)	3 (50)*	1 (14)	14 (61)
Completed 1 yr	2 (20)	2 (33)	1 (14)	5 (22)
Completed 2 yr	8 (80)	0 (0)	0 (0)	8 (35)
Discontinued	0 (0)	3 (50)	6 (86)	9 (39)
Death after completion	2 (20)	0 (0)	0 (0)	2 (9)
Death after discontinuation	0 (0)	0 (0)	3 (43)	3 (13)
Main cause of discontinuation				
AE(s)	0 (0)	1 (17)	2 (29)	3 (13)
Death	0 (0)	1 (17)	1 (14)	2 (9)
Withdrawal of consent	0 (0)	1 (17)	4 (43)	4 (17)
Overall incidence of AEs, nE/nS (%)				
Patients with AEs	179, 10 (100)	267, 6 (100)	267, 7 (100)	713, 23 (100)
AEs of grade 1/mild intensity	120, 10 (100)	117, 6 (100)	106, 5 (71)	343, 21 (91)
AEs of grade 2/moderate intensity	48, 9 (90)	94, 6 (100)	113, 7 (100)	255, 22 (96)
AEs of grade 3/severe intensity	11, 4 (40)	48, 6 (100)	43, 7 (100)	102, 17 (74)
AEs of grade 4/life-threatening intensity	0, 0 (0)	8, 4 (67)	4, 3 (43)	12, 7 (30)
Study drug-related AEs	19, 5 (50)	22, 4 (67)	14, 7 (100)	55, 16 (70)
Serious AEs	18, 5 (50)	13, 4 (67)	16, 6 (86)	47, 15 (65)
Study drug-related serious AEs	3, 1 (10)	4, 2 (33)	4, 3 (43)	11, 6 (26)
AEs leading to discontinuation of study drug	8, 3 (30)	4, 2 (33)	2, 2 (29)	14, 7 (30)
Study drug-related AEs leading to discontinuation of study drug	6, 2 (20)	4, 2 (33)	2, 2 (29)	12, 6 (26)
Mocravimod-related AEs, n (%)				
Time period: from start of treatment to just before allo-HSCT				
Bradycardia	2 (20)	0 (0)	0 (0)	2 (8.7)
Time period: from allo-HSCT up to 30 days post-allo-HSCT				
Cystoid macular edema	0 (0)	1 (17)	0 (0)	1 (4)
Dyspnea	1 (10)	0 (0)	0 (0)	1 (4)
Time period: after 30 days post-allo-HSCT				
Cystoid macular edema	1 (10)	0 (0)	0 (0)	1 (4)
Dyspnea, exertional	1 (10)	0 (0)	0 (0)	1 (4)
Hepatic failure	0 (0)	0 (0)	1 (14)	1 (4)
Macular ischemia	0 (0)	1 (17)	0 (0)	1 (4)
Edema, peripheral	1 (10)	0 (0)	0 (0)	1 (4)
Pleural effusion	1 (10)	0 (0)	0 (0)	1 (4)
Retinal ischemia	0 (0)	1 (17)	0 (0)	1 (4)

Percentages are based on the number of patients. Patients who completed 2 years of the study were not included in the count for completing 1 year.
nE indicates the number of AE events in the category; nS, number of patients with at least 1 AE in the category.
* One patient completed the study on day 275 and thus was not included in the counts for completed 1 year or completed 2 years.

patient had long-term effects with any threat to vision. No long-term AEs were reported in these patients. Overall, mocravimod was well tolerated, as demonstrated by the excellent compliance in the Mo3CsA arm, in which 7 of 10 patients received 111 days of mocravimod treatment (Supplementary Figure S1). Seven patients had AEs that led to mocravimod discontinuation, of which 4 AEs were suspected to be mocravimod-related: macular edema, pleural effusion, and macular and retinal ischemia; others were blepharitis, dyspnea, weight increase, capillary leak syndrome, and increased ALT (Supplementary Table S3). No clinically relevant abnormalities in hematologic and clinical chemistry parameters and electrocardiography findings were observed in any treatment arm.

Seven deaths were reported. One patient in the Mo1CsA arm died from aspiration pneumonia on day 337, 1 patient in the Mo3Tac died from GVHD-related hepatic failure on day 121, and 5 patients died due to relapse: 2 in the Mo3CsA arm on days 542 and 886 and 3 in the Mo3Tac arm on days 89, 168, and 315.

Liver Safety

Mildly elevated liver function test (LFT) results are typical with most S1PR modulators, and increased levels are frequently observed in allo-HSCT recipients [12–16]. Therefore, LFTs were closely monitored in this study (Figure 2). No consistent increase in LFTs beyond the expected range was observed during mocravimod treatment. One noticeable increase in LFTs post-mocravimod treatment was caused by cGVHD. Except for 1 patient in the Mo3Tac arm, total bilirubin remained normal. Importantly, no increases in LFTs were observed on diagnosis of aGVHD. Linear increases of AST and ALT, as observed with increasing doses of fingolimod in multiple sclerosis patients [17–20], did not occur in our allo-HSCT recipients treated with mocravimod. Overall, within the limitations of this small dataset, there is no evidence for mocravimod-induced liver toxicity.

Engraftment

Neutrophil engraftment was confirmed in all 22 patients, at a median of 15 days post-transplantation (range, 11 to 35 days) (Supplementary Figure S2A). The median time to neutrophil recovery was 17 days (range, 15 to 21 days) in the Mo1CsA arm, 13 days (range, 11 to 15 days) in the Mo3CsA arm, and 20 days (range, 12 to 35 days) in the Mo3Tac arm. Platelet engraftment was observed in 20 of the 22 patients, with a median time to engraftment of 14 days post-transplantation (range, 14 to 44 days) (Supplementary Figure S2B). The first patient treated with mocravimod received a nonmyeloablative Mini-Seattle conditioning regimen to mitigate the engraftment-interfering

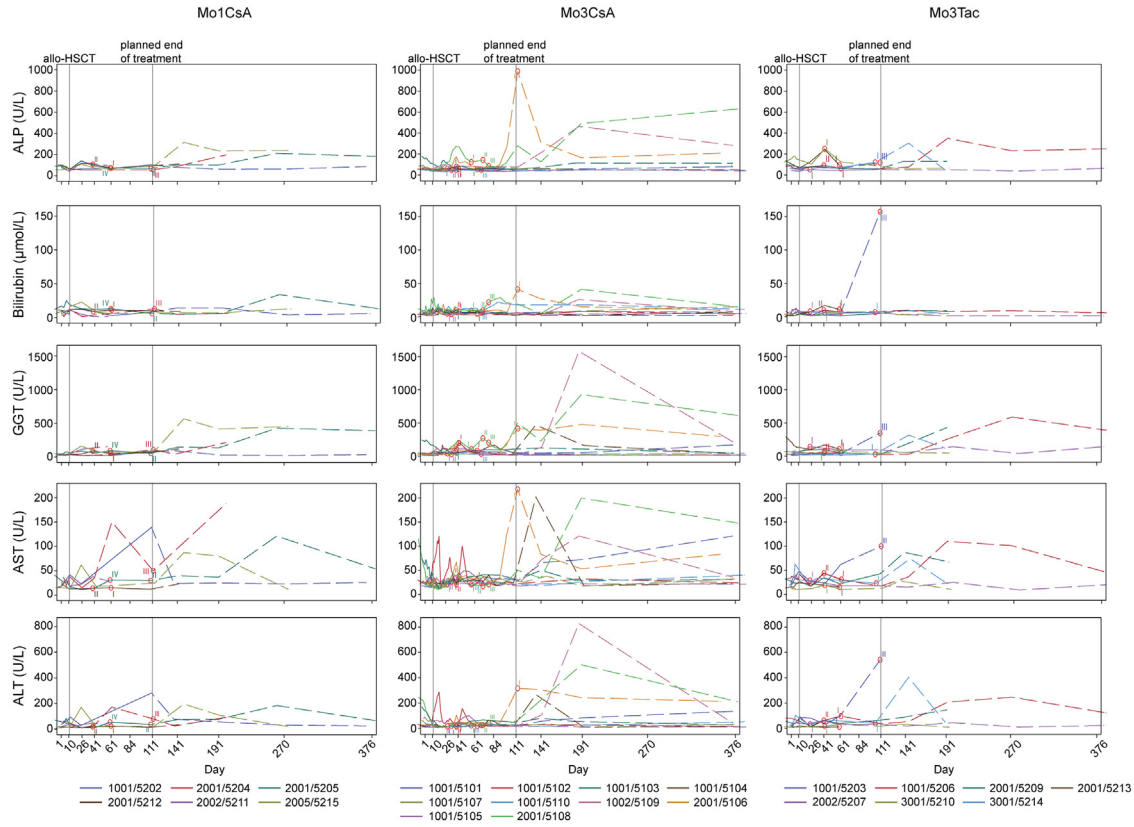

Figure 2. No consistent increase in liver function test results was seen during mocravimod treatment. Serum concentrations of different liver enzymes were measured from baseline to the end of the study. Vertical lines indicate time of allo-HSCT (day 11) and the planned end of mocravimod treatment (day 111). Solid lines indicate that the patient was on mocravimod treatment, and dashed lines indicate that the patient was off mocravimod treatment. Roman numerals indicate time points and grade of aGVHD diagnosis. (A) Serum concentration of alkaline phosphatase (ALP) in the Mo1CsA arm. (B) Serum concentration of ALP in the Mo3Tac arm. (C) Serum concentration of ALP in the Mo3CsA arm. (D) Serum concentration of bilirubin in the Mo1CsA arm. (E) Serum concentration of bilirubin in the Mo3Tac arm. (F) Serum concentration of bilirubin in the Mo3CsA arm. (G) Serum concentration of gamma glutamyl transferase (GGT) in the Mo1CsA arm. (H) Serum concentration of GGT in the Mo3Tac arm. (I) Serum concentration of GGT in the Mo3CsA arm. (J) Serum glutamic oxaloacetic transaminase (SGOT; aspartate transaminase [AST]) concentration in the Mo1CsA arm. (K) SGOT concentration in the Mo3Tac arm. (L) SGOT concentration in the Mo3CsA arm. (M) Serum glutamic pyruvic transaminase (SGPT; alanine transaminase [ALT]) concentration in the Mo1CsA arm. (N) SGPT concentration in the Mo3Tac arm. (O) SGPT concentration in the Mo3CsA arm.

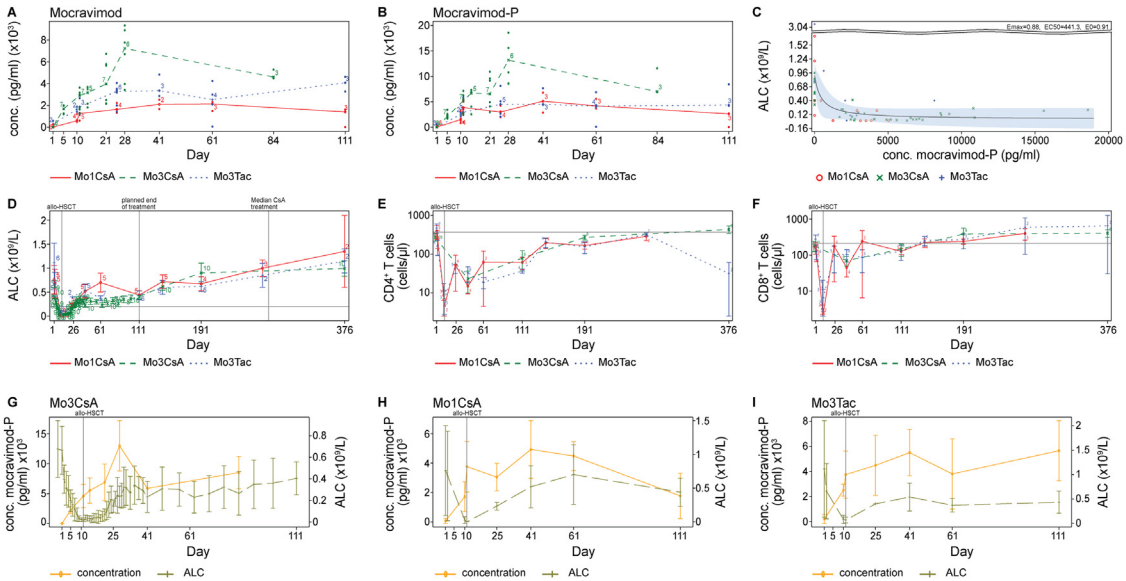

Figure 3. Pharmacokinetics and pharmacodynamics of mocravimod-P. (A) Serum concentration of mocravimod at different time points from day 1 to day 111 of the treatment period. (B) Serum concentration of mocravimod-P at different time points from day 1 to day 111 of the treatment period. The black line depicts the model prediction; blue shading, the 95% CI. (C) Relationship between serum concentration of mocravimod-P and ALC. Only data up to day 30 are included. (D) ALC in the 3 treatment arms from baseline to the end of the study. The horizontal line indicates an ALC safety threshold of .2 × 10^9/L. Vertical lines indicate the time of allo-HSCT (day 11), the planned end of mocravimod treatment (day 111), and the median duration of CsA treatment. (E) Absolute numbers of $CD4^+$ T cells measured in peripheral blood (cells/μL) from baseline to the end of the study. The horizontal line depicts baseline numbers of $CD4^+$ T cells. (F) Absolute numbers of $CD8^+$ T cells measured in peripheral blood (cells/μL) from baseline to the end of the study. The horizontal line depicts baseline numbers of $CD8^+$ T cells. (G) Relationship between serum concentration of mocravimod-P and ALC throughout the period of mocravimod treatment in the Mo3CsA arm. (H) Relationship between serum concentration of mocravimod-P and ALC throughout the period of mocravimod treatment in the Mo1CsA arm. (I) Relationship between serum concentration of mocravimod-P and ALC throughout the period of mocravimod treatment in the Mo3Tac arm.

potential of mocravimod. This patient was excluded from the neutrophil engraftment analysis, because their neutrophil values never decreased. Overall, the median times to neutrophil and platelet engraftment under mocravimod treatment were comparable to the median times reported previously for recipients of allo-HSCT with peripheral blood stem cell grafts, indicating that mocravimod did not affect engraftment [21,22].

Pharmacokinetics

After the initial mocravimod dose, T_{max} was 3 to 12 hours for mocravimod and mocravimod-P (Supplementary Table S4). Despite different sampling time points in the 3 treatment arms, C_{max} and AUCtau for mocravimod and mocravimod-P seemed to be approximately 2- to 3-fold higher in the Mo3CsA arm, supporting a linear dose-exposure relationship as observed in healthy volunteers (Figure 3A,B; Supplementary Table S4; Supplementary Figure S3A-C). A 1:2 ratio of mocravimod to mocravimod-P was observed in all treatment arms.

Following daily mocravimod doses, blood concentrations of mocravimod and mocravimod-P accumulated, and C_{max} and AUCtau increased in a dose-dependent manner (Supplementary Table S4, Supplementary Figure S3A-C). Trough concentrations of mocravimod and mocravimod-P were stable after 14 to 25 days, indicating that the exposure was close to steady state after 14 days, as reported in healthy volunteers (data not shown).

Pharmacodynamics

The pharmacodynamic activity of mocravimod was monitored by 2 different readouts indicative of the redistribution of lymphocytes to lymphoid tissues: ALC in peripheral blood and $CD4^+$ and $CD8^+$ T cell numbers in peripheral blood from baseline until day 376 (Figure 3D-F). In most allo-HSCT recipients, ALC reaches .5 to 2.6 × 10^9/L within 3 months post-transplantation [23]. In our cohort, ALC remained <.5 × 10^9/L during mocravimod treatment and increased steadily thereafter, reaching normal values after 1 year. $CD4^+$ and $CD8^+$ T cell numbers were reduced in response to pretransplantation conditioning and mocravimod treatment to similar levels in all 3 treatment arms. Although $CD4^+$ T cell counts remained below the baseline level for the entire mocravimod treatment period (Figure 3E), $CD8^+$ T cell counts recovered more readily (Figure 3F). Both subpopulations demonstrated a transient fluctuation around day 40. $CD4^+$ T cells recovered and reached pretransplantation levels when mocravimod treatment was stopped.

Because mocravimod-P is the active metabolite, we investigated the relationship between mocravimod-P blood concentration and ALC. Data from days 1 to 30 indicate that blood concentrations of mocravimod-P were inversely correlated with ALC (E_{max} = .88, EC_{50} = 441.3, E_0 = .91) (Figure 3C). In all treatment arms, mocravimod-P blood concentrations increased rapidly up to 10 to 14 days to 4 to 6 pg/μL (Supplementary Figure S3A-C). Although in the Mo3CsA arm, mocravimod-P blood levels continued to increase to 15 pg/μL on day 28, the levels in the Mo3Tac arm remained at roughly the same level after day 25 until mocravimod administration was stopped. ALC remained at low levels (400 to 600 cells/μL) in all treatment arms (Figure 3G-I).

Transplantation Outcomes

Exploratory efficacy endpoints were analyzed by assessing grade III-IV aGVHD-free survival, cGVHD-free survival, relapse-free survival (RFS), overall survival (OS), and the composite endpoint GRFS. Overall, 5 of 23 patients (21.7%) presented with grade III-IV aGVHD (Figure 4A), including 2 of 10 patients (20%; days 55 and 67) in the Mo3CsA arm, 2 of 6 patients (33.3%; days 42 and 65) in the Mo1CsA arm, and 1 of 7

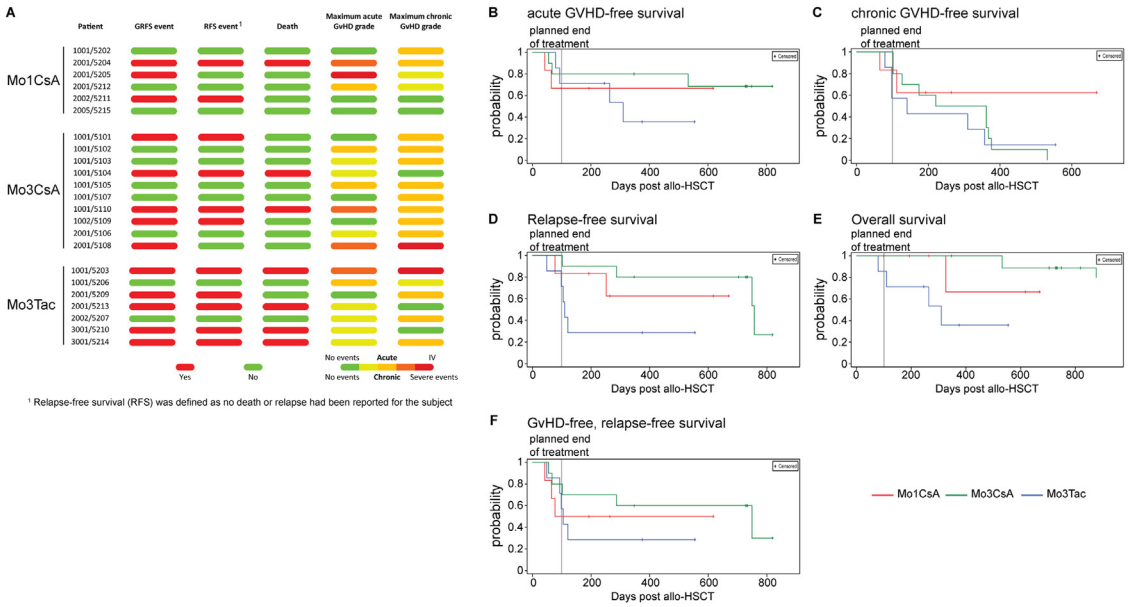

Figure 4. Efficacy outcomes. (A) Table depicting the occurrence of GRFS events, RFS events (relapse or death), and death in each patient. Occurrences of acute and chronic GVHD are shown as maximum diagnosed grade. aGVHD color codes: yellow, grade I; orange, grade II; ochre, grade III; red, grade IV. cGVHD color codes: yellow, mild; orange, moderate; red, severe. (B) Grade III-IV aGVHD-free survival (aGFS). (C) Moderate-severe cGVHD-free survival (cGFS). (D) RFS. (E) OS. (F) GRFS. Vertical gray lines depict the end of mocravimod treatment on day 111.

patients (14.3%; day 93) in the Mo3Tac arm. Lower intestinal tract, liver, and skin were involved in grade III-IV aGVHD. The Mo3Tac arm had the lowest probability of aGVHD-free survival after 1 year, followed by the Mo1CsA and Mo3CsA arms (Figure 4B). The median time to any aGVHD event was 54 days (range, 17 to 102 days) in the Mo3CsA arm and 45 days (range, 14 to 98 days) in the Mo3Tac arm. The median time to any aGVHD could not be estimated for the Mo1CsA arm (22 to 65 days), as >50% of the patients did not have a GVHD event.

Thirteen out of 23 patients (56.5%) presented with moderate cGVHD, and 2 patients (8.7%) presented with severe cGVHD (1 each in the Mo3CsA [day 368] and Mo3Tac [day 98] arms) (Figure 4A). The median time to cGVHD of any severity was 291.5 days (range, 102 to 376 days) in the Mo3CsA arm, 132 days (range, 65 to 215 days) in the Mo1CsA arm, and 137.5 days (range, 98 to 357 days) in the Mo3Tac arm. Moderate to severe cGVHD-free survival is shown in Figure 4C. A total of 10 of the 23 patients (43.5%) reported relapse events post-transplantation, including 2 patients (33.3%; days 77 and 252) in the Mo1CsA arm, 4 (40%; days 102, 287, 749, and 757) in the Mo3CsA arm, and 4 (71.4%; days 49, 98, 105, and 121) in the Mo3Tac arm (Figure 4A; RFS event). Relapse mostly occurred more than 100 days after the end of mocravimod treatment in the Mo3CsA and Mo1CsA arms but coincided with the end of treatment in the Mo3Tac arm (Figure 4D). The median time to relapse was 757 days (range, 102 to 757 days) in the Mo3CsA arm and 121 days (range, 49 to 121 days) in the Mo3Tac arm. The median time to relapse could not be estimated for the Mo1CsA arm (77 to 252 days), because >50% of the patients did not have a relapse event. The probability of relapse at 6 months was highest in the Mo3Tac arm (61.9%), followed by the Mo1CsA (16.6%) and Mo3CsA (10%) arms (Figure 4D).

Seven patients (30.4%) died in the study without being on mocravimod treatment at the time of death (Figure 4A). The OS at 6 months was 100% in the Mo1CsA and Mo3CsA arms and 71.4% in the Mo3Tac arm (Figure 4E).

Thirteen GRFS events (56.5%) were reported until the end of the study (Figure 4A). Figure 4F shows that 3 of 6 patients in the Mo1CsA arm, 7 of 10 patients in the Mo3CsA arm, and 2 of 7 patients in the Mo3Tac arm did not experience any GVHD, death, or relapse and were alive at 6 months post-transplantation.

DISCUSSION

This is the first reported study investigating the effect of an S1PR modulator in patients with hematologic malignancies undergoing allo-HSCT. The primary objective of the study was to evaluate the safety, tolerability, and pharmacokinetics of mocravimod as an adjunctive and maintenance treatment for allo-HSCT. Patients with a broad variety of malignancies were included in this trial. The data suggest that administration of mocravimod in a prophylactic mode from 10 days pretransplantation and then as maintenance therapy to 100 days post-transplantation, along with a broad range of SoC is safe and well tolerated. As reported previously in autoimmune indications, mocravimod treatment caused first dose, transient bradycardia in 2 patients of mild severity with no clinical concern.

Because S1PR signaling is suggested to be involved in the homing and engraftment of hematopoietic stem cells to bone marrow [24], the first patient in our series received nonmyeloablative conditioning (Mini-Seattle) to mitigate the risk of engraftment failure. On confirmation of neutrophil and platelet engraftment, subsequent patients received fully myeloablative conditioning regimens (Supplementary Figure S2), and neutrophil and platelet engraftment swiftly ensued. During mocravimod maintenance treatment, ALC remained low, in a safe range of .4 to .6 $\times$ 10^9/L, similar to that seen in multiple sclerosis patients receiving fingolimod [25]. Notably, once mocravimod treatment was stopped, ALC and T cell numbers recovered to normal values within weeks. All 3 treatment combinations were sufficient to reduce ALC in peripheral blood, whereas counts remained lowest in the Mo3CsA arm during the 111 days of mocravimod treatment. Furthermore, $CD4^+$ T cell counts were reduced more efficiently than $CD8^+$ T cell counts, consistent with data from murine models (data not shown).

Table 3
Efficacy Endpoints

Endpoint	Mo3CsA Arm (N = 10), n (%)	Mo1CsA Arm (N = 6), n (%)	Mo3Tac Arm (N = 7), n (%)	Total (N = 23), n (%)
Acute GVHD	7 (70)	3 (50)	6 (85.7)	16 (69.6)
Grade I	3 (30)	0	4 (57.1)	7 (30.4)
Grade II	2 (20)	1 (16.7)	1 (14.3)	4 (17.4)
Grade III	2 (20)	1 (16.7)	1 (14.3)	4 (17.4)
Grade IV	0	1 (16.7)	0	1 (4.3)
Chronic GVHD	9 (90)	4 (66.7)	5 (71.4)	18 (78.3)
Mild	0	2 (33.3)	1 (14.3)	3 (13)
Moderate	8 (80)	2 (33.3)	3 (42.9)	13 (56.5)
Severe	1 (10)	0	1 (14.3)	2 (8.7)
Relapse	4 (40)	2 (33.3)	4 (57.1)	10 (43.5)
Death	2 (20)	1 (16.7)	4 (57.1)	7 (30.4)

A slight transient elevation of liver enzymes with no long-term impairment was seen during mocravimod treatment. One patient in the Mo3Tac arm died of hepatic failure; however, this event was identified as GVHD-related. Two cases of macular edema were reversible after discontinuation of the study drug.

The desired reduction in circulating T cells did not result in an increased incidence of infections, with cytomegalovirus reactivation occurring in 17.4% of the patients (not shown), consistent with mocravimod not being an immunosuppressant. This supports our hypothesis that recipients continue to be able to mount T cell and B cell responses, which usually are initiated in secondary lymphoid organs, during mocravimod treatment. Therefore, mocravimod likely will not interfere with beneficial GVL and antimicrobial responses. In summary, the data suggest that mocravimod has a similar safety profile in this fragile patient population as mocravimod and other S1PR modulators display in patients with several autoimmune indications.

Mocravimod blood concentrations may be elevated when combined with a strong CYP3A4 inhibitor, such as azole antifungals and CsA, as it is partially metabolized through CYP3A4. Early evidence of elevated mocravimod blood concentrations was found in this study. However, owing to the low patient numbers and widely variable comedications among the patients in our cohort, further drug-drug interaction studies are underway to elucidate the molecular mechanisms that may influence mocravimod and mocravimod-P metabolism.

Exploratory clinical outcomes suggest that S1PR modulation with mocravimod is associated with an aGVHD incidence in a similar range of 30% to 50% as reported previously [26]. It is important to note, however, that the number and heterogeneity of patients in this study are not suitable for drawing final conclusions on GVHD outcomes. Of note, ATG was not used as part of GVHD prophylaxis.

Patients in the Mo3Tac arm performed slightly worse than those in the other arms in terms of efficacy outcomes (RFS and GRFS). The pharmacokinetics of mocravimod and mocravimod-P were similar in the Mo3Tac and Mo1CsA arms, and thus no difference between these arms would be expected based on pharmacokinetics. Relapse rates were slightly better in mocravimod-treated patients compared with a similar patient cohort from the Center for International Blood and Marrow Transplant Research database. Hematologic malignancies were heterogenic with different relapse risks, impeding our ability to draw final conclusions. Nonetheless, this suggests that allo-T, which are sequestered in lymphoid organs during mocravimod treatment, may continue to mediate the GVL effect and remain functional. GVHD may be reduced because allo-T are sequestered in lymphoid organs and thus unable to attack peripheral host tissues.

Overall, the results of this small study support further investigations of mocravimod in a larger homogeneous patient population undergoing allo-HSCT, to confirm safety and assess clinical efficacy, such as its effects on OS, RFS, and GVHD-free survival Table 3.

Authorship statement: P.G. and C.B. developed the idea and conceptualized the study; D.H., J.F., U.S., E.H., U.H., G.S., M.M., J.P., T.T., and C.B. served as investigators and recruited patients into the study; C.S. performed statistical analyses; C.S., S.D., S.O., and C.B. interpreted the data; S.D., S.O. and C.B. wrote the manuscript; and all authors reviewed and approved the manuscript. S.D. and P.G. contributed equally to this work. D.H. and C.B. share senior authorship.

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DECLARATION OF COMPETING INTEREST

S.D. is an employee of Priothera SAS. P.G. is an employee of Novartis. S.O. was an employee of Novartis and is currently an employee of Priothera SAS.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2022.10.029.

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