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A Phase Ib/II Trial of the First-in-Class Anti-CXCR4 Antibody Ulocuplumab in Combination with Lenalidomide or Bortezomib Plus Dexamethasone in Relapsed Multiple Myeloma

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ABSTRACT

Purpose: Ulocuplumab (BMS-936564) is a first-in-class fully human IgG4 monoclonal anti-CXCR4 antibody that inhibits the binding of CXCR4 to CXCL12.

Patients and Methods: This phase Ib/II study aimed to determine the safety and tolerability of ulocuplumab alone and in combination with lenalidomide and dexamethasone (Arm A), or bortezomib and dexamethasone (Arm B), in patients with relapsed/refractory multiple myeloma.

Results: Forty-six patients were evaluated (median age, 60 years; range, 53–67). The median number of prior therapies was 3 (range, 1–11), with 70% of subjects having received ≥3. This trial had a dose-escalation and a dose-expansion part. Using a 3+3 design on both arms of the trial, ulocuplumab's dose was escalated to a maximum of 10 mg/kg without reaching MTD. The most common treatment-related adverse events (AE) were neutropenia (13 patients, 43.3%) in Arm A and thrombocytopenia (6 patients, 37.5%) in Arm B. No deaths related to study drugs occurred. The combination of ulocuplumab with lenalidomide and dexamethasone showed a high response rate (PR or better) of 55.2% and a clinical benefit rate of 72.4%, even in patients who had been previously treated with immunomodulatory agents (IMiD).

Conclusions: This study showed that blockade of the CXCR4–CXCL12 axis by ulocuplumab is safe with acceptable AEs and leads to a high response rate in combination with lenalidomide and dexamethasone in patients with relapsed/refractory myeloma, making CXCR4 inhibitors a promising class of antimyeloma drugs that should be further explored in clinical trials.

Introduction

Multiple myeloma is a heterogeneous plasma cell malignancy that resides in the bone marrow. Even though survival rates have improved, most patients with myeloma eventually relapse, despite timely diagnosis and advances in treatment (1). The bone marrow microenvironment has been shown to be particularly important in response to treatment and dissemination of disease, and is thus an area of active investigation in drug development for multiple myeloma. CXCR4, in particular, has attracted attention for its role in disease progression and treatment response (2). *CXCR4* is a chemokine receptor that is overexpressed in more than 75% of cancer cells, including myeloma (3), whereas its pathway, the CXCR4/CXCL12 (SDF-1) axis, is a known critical regulator of tumor proliferation, cell dissemination, and migration in and out of the bone marrow (4, 5). Although activating mutations in *CXCR4* are not common in multiple myeloma, increased expression is achieved through external stimulation by high levels of stroma-secreted CXCL12, suggesting CXCR4 is important for myeloma growth and sustenance (6).

We, and others, have shown that CXCR4 is critical for cell dissemination in and out of the bone marrow, and constitutes a major regulator of extramedullary dissemination through the acquisition of an "epithelial–mesenchymal-like" activation phenotype (6, 7). That makes CXCR4 a unique target for therapeutic interventions in patients with extramedullary disease and end-stage relapsed/refractory multiple myeloma. In fact, we have previously demonstrated *in vitro* and *in vivo* that downregulation of *CXCR4* by knockdown or therapeutic targeting can lead to the deadhesion of multiple myeloma cells from the bone marrow, which renders them more sensitive to other therapeutic agents like bortezomib (8). Those results suggest that not only is CXCR4 inhibition relevant in myeloma treatment as monotherapy, but it could also enhance the activity of other drugs, thus justifying testing its use in therapeutic combinations.

Ulocuplumab (BMS-936564) is a first-in-class fully human IgG4 monoclonal anti-CXCR4 antibody that inhibits the binding of CXCR4 to CXCL12 (9). It has been shown to induce apoptosis in multiple myeloma cell lines with high CXCR4 expression and has single-agent activity *in vivo* in multiple myeloma tumor xenograft models (9). It is thus reasonable to assume that ulocuplumab could potentially improve the overall response rate to standard therapy, through several mechanisms of action, including mobilization and apoptosis of malignant plasma cells. A prior phase I trial in relapsed/refractory acute myelogenous leukemia (AML) was conducted using ulocuplumab in

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

This clinical trial tested ulocuplumab, a first-in-class fully human IgG4 monoclonal anti-CXCR4 antibody in relapsed/refractory multiple myeloma. The combination of ulocuplumab with lenalidomide and dexamethasone showed a high response rate (PR or better) of 55.2% and a clinical benefit rate of 72.4%, even in patients who had been previously treated with immunomodulatory agents (IMiD). This study showed that blockade of the CXCR4–CXCL12 axis by ulocuplumab is safe and leads to a high response rate in combination with lenalidomide and dexamethasone in patients with relapsed/refractory myeloma, making CXCR4 inhibitors a promising class of antimyeloma drugs that should be further explored in clinical trials.

combination with mitoxantrone, etoposide, and cytarabine (MEC) in patients and showed a 51% overall complete remission and complete remission with incomplete blood count recovery rate (CR/Cri; ref. 10). The adverse effects of ulocuplumab in combination with MEC was similar to MEC alone.

This study aimed to determine the safety, tolerability, and clinical activity of ulocuplumab alone and in combination with lenalidomide/low-dose dexamethasone or bortezomib/dexamethasone in subjects with relapsed/refractory multiple myeloma.

Patients and Methods

This was a phase Ib/II open-label, multicenter study with the primary objective to evaluate the safety profile, tolerability, pharmacokinetics, pharmacodynamics of ulocuplumab, either alone (first 14 days of cycle 1) or in combination with either lenalidomide and dexamethasone (Arm A) or bortezomib and dexamethasone (Arm B). From October 2011 to March 2014, patients were enrolled at Dana Faber Cancer Institute (Boston, MA), the H. Lee Moffitt Cancer Center and Research Institute (Tampa, FL), the University of Kansas Cancer Center and Medical Pavilion (Westwood, KS), and the University of Washington School of Medicine (Seattle, WA).

Patients were eligible for this trial if they were 18 years of age or older, with relapsed or relapsed/refractory multiple myeloma after having received at least two prior lines of treatment. Subjects who had previously failed lenalidomide or bortezomib were not excluded from retreatment. Other eligibility criteria included: Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and at least one of the following: IgG, IgA, IgM M-protein $\geq$0.5 g/dL, IgD M-protein $\geq$0.05 g/dL, monoclonal light chain in the urine protein electrophoresis of $\geq$200 mg/24 hours, abnormal serum-free light chain ratio $\geq$10. Patients also had to have demonstrated the following laboratory values within 28 days prior to dosing: absolute neutrophil count (ANC) $\geq$1,000/mm^3, platelets (PLT) $\geq$50,000/mm^3, hemoglobin (HGB) $\geq$8.0 gm/dL, direct bilirubin $\leq$2.0 $\times$ upper limit of normal (ULN), aspartate and alanine aminotransferase (AST/ALT) $\leq$3 $\times$ ULN, and an estimated creatinine clearance >50 mL/min/1.73 m^2. Patients with uncontrolled medical disorders or active infection, gastrointestinal diseases or condition that could impact the absorption of orally administered drug, inability to swallow oral medication or be administered intravenous medications, uncontrolled or significant cardiovascular diseases, clinically significant coagulation or platelet function disorder, other malignancies, acute diffuse infiltrative pulmonary and pericardial diseases, HIV, or hepatitis B or C infection were excluded.

This phase Ib/II study used 3+3 design for the phase I dose-escalation portion and a two-stage outcome design to assess the efficacy and tolerability of ulocuplumab in combination with lenalidomide or bortezomib in patients with relapsed/refractory MM. The primary objectives of the phase I and the phase II studies were to ascertain the safety and MTD of the combination therapies and to determine the overall response rates of those combinations, respectively. In addition, the phase II study was also designed to determine the safety of the combination therapies at the phase II level as well as to determine the duration of response (DOR), time to progression (TTP), and progression-free survival (PFS).

There was no specific subject assignment across the two arms. The two arms were two separate studies and should not be compared with each other. In general, subjects who most recently failed lenalidomide were preferentially enrolled on the bortezomib/dexamethasone arm and subjects who most recently failed bortezomib were preferentially enrolled on the lenalidomide plus dexamethasone arm based on physician discretion. Subjects who had failed but were not refractory to lenalidomide and bortezomib were not excluded from retreatment with the same regimen.

All patients provided written informed consent. The review boards of all participating centers approved the study in accordance with the Declaration of Helsinki and the International Conference of Harmonization Guideline for Good Clinical Practice (ICH GCP).

Study treatment

For the dose-escalation portion of the study, patients were assigned to a dose level in the order of study entry for each of the two arms. In the dose-escalation scheme, 1, 3, or 10 mg/kg/dose of ulocuplumab was given as monotherapy on days 1 and 8 of the first 14 days of cycle 1 in both arms. For Arm A, from day 15 to 42 of the 42-day cycle 1, the assigned dose of ulocuplumab was given on days 15, 22, 29, and 36, in combination with both 25 mg of lenalidomide on days 15 to 35 and 40 mg of oral dexamethasone on days 15, 22, 29, and 36. For Arm B, from day 15 to 35 of the 35-day cycle 1, the assigned dose of ulocuplumab was given on days 15, 22, and 29, in combination with both 1.3 mg/m^2 of intravenous push bortezomib on days 15, 18, 22, and 25 and 20 mg of oral dexamethasone on days 15,16, 18, 19,22, 23, 25, and 26. For cycle 2 and subsequent cycles of Arm A, the assigned dose of ulocuplumab was given on days 1, 8, 15, and 22, in combination with both 25 mg of lenalidomide on days 1 to 21 and 40 mg of oral dexamethasone on days 1, 8, 15, and 22 of the 28-day cycles. For cycle 2 and subsequent cycles of Arm B, the assigned dose of ulocuplumab was given on days 1, 8, and 15, in combination with both 1.3 mg/m^2 of intravenous push bortezomib on days 1, 4, 8, and 11 and 20 mg of oral dexamethasone on days 1, 2, 4, 5, 8, 9, 11, and 12 of the 21-day cycles.

For the dose-expansion portion of the study, patients were enrolled to each arm at the established maximum dose of ulocuplumab of 10 mg/kg/dose given that there were no DLTs observed. For both Arm A and Arm B, cycle 1 consisted of a 28-day cycle of ulocuplumab monotherapy given at days 1, 8, 15, and 22. For cycle 2 and subsequent cycles of Arm A, ulocuplumab was given on days 1, 8, 15, and 22, in combination with both 25 mg of lenalidomide on days 1 to 21 and 40 mg of oral dexamethasone on days 1, 8, 15, and 22 of the 28-day cycles. For further details, see consort diagrams (Supplementary Fig. S1).

Determination of the MTD and expansion cohort

In deriving the recommended phase II dose ranges and schedules, consideration was given to the rate and nature of delayed toxicities

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beyond cycle 1. After cycle 1 (for escalation) and cycle 2 (for expansion) of treatment, the timing, nature, and severity of the toxicities were reviewed by the sponsor/monitor and investigators.

In the dose-escalation portion of the study, cohorts of 3 patients were sequentially enrolled at each dose based on a 3+3 design on each arm. Adverse events (AE) experienced during the first cycle of treatment determined the dose-escalation proceedings. The dose was escalated if none of the first 3 patients in the cohort or 1 of the 6 patients experienced dose-limiting AEs during the first treatment cycle. If two or more patients experienced dose-limiting AEs, then dose escalation was halted. The MTD of ulocuplumab and the combination partners was defined as the highest dose at which less than one-third of the subjects experienced a dose-limiting toxicity (DLT) in the first cycle of treatment. DLTs were defined as any of the following events attributed to either ulocuplumab alone or in combination: ANC < 500 cells/mm^3 for more than 5 days or febrile neutropenia, PLT < 10,000 cells/mm^3

Table 1. Baseline patient characteristics of relapsed or refractory MM.

Characteristics	Total (n = 46)	Arm A (n = 30)	Arm B (n = 16)
Median age (y, range)	60 (53–67)	60 (55–69)	60 (50–66)
Male	24 (52.2)	16 (53.3)	8 (50.0)
Race			
White	41 (89.1)	28 (93.3)	13 (81.3)
African American	3 (6.5)	1 (3.3)	2 (12.5)
Asian	1 (2.2)	1 (3.3)	0 (0.0)
Others	1 (2.2)	0 (0.0)	1 (6.3)
Disease status			
Relapsed and refractory	15 (32.6)	8 (26.7)	7 (43.8)
Primary refractory	2 (4.4)	2 (6.7)	0 (0.0)
Relapsed	29 (63.0)	20 (66.7)	9 (56.3)
Heavy-chain type			
IgG	24 (52.2)	16 (53.3)	8 (50.0)
IgA	11 (23.9)	9 (30.0)	2 (12.5)
No heavy chain	9 (19.6)	4 (13.3)	5 (31.3)
Light-chain type			
Kappa	26 (56.5)	20 (66.7)	6 (37.5)
Lambda	16 (34.8)	8 (26.7)	8 (50.0)
Soft-tissue plasmacytomas	11 (23.9)	6 (20.0)	5 (31.3)
ECOG performance status			
0	12 (26.1)	9 (30.0)	3 (18.8)
1	28 (60.9)	18 (60.0)	10 (62.5)
2	6 (13.0)	3 (10.0)	3 (18.8)
Median no. of regimens (range)	3 (1–11)	3 (1–9)	4 (2–11)
Prior treatments			
Bortezomib	42 (91.3)	28 (93.3)	14 (87.5)
Dexamethasone	42 (91.3)	27 (90.0)	15 (93.8)
Lenalidomide	40 (87.0)	24 (80.0)	16 (100.0)
Melphalan	20 (43.5)	14 (46.7)	6 (37.5)
Cyclophosphamide	27 (58.7)	16 (53.3)	11 (68.8)
Thalidomide	17 (37.0)	13 (43.3)	4 (25.0)
Stem cell transplantation	29 (63.0)	20 (66.7)	9 (56.3)
Drug resistance			
Bortezomib	24 (52.2)	15 (50.0)	9 (56.3)
Lenalidomide	23 (50.0)	10 (33.3)	13 (81.3)
Median plasma cell percentage (range)	21 (1–97)	22 (1–97)	20 (5–74)
β2-microglobulin, mg/dL (range)	2.9 (1.3–8.6)	3.2 (1.7–7.2)	2.7 (1.3–8.6)
Albumin (range)	3.8 (2.0–5.1)	3.9 (3.0–5.1)	3.7 (2.0–5.0)
ISS stage			
I	25 (54.4)	15 (50.0)	10 (62.5)
II	11 (23.9)	9 (30.0)	2 (12.5)
III	9 (19.6)	6 (20.0)	3 (18.8)
Disease duration before enrollment (range), months	55.5 (0.2–299.4)	56.9 (0.2–299.4)	55.4 (7.3–91.2)
Cytogenetics			
Del 13q	17 (37.0)	12 (40.0)	5 (31.3)
Del 17p13	4 (8.7)	2 (6.7)	2 (12.5)
t(4; 14)	4 (8.7)	3 (10.0)	1 (6.3)
t(11; 14)	12 (26.1)	9 (30.0)	3 (18.8)
t(14; 16)	4 (8.7)	3 (10.0)	1 (6.3)
1q21 amplification	12 (26.1)	9 (30.0)	3 (18.8)
High-risk[a]	16 (34.8)	12 (40.0)	4 (25.0)

[a]High-risk cytogenetics are defined by the presence of t(4;14), t(14;16), 17p deletion, and +1q amplification.

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more than once, grade 3 or higher nausea, vomiting, or diarrhea despite intervention, QTcF 500 msec or greater confirmed, grade 3 or higher cardiac ischemia/infarction, LVEF decrease by >10 percentage points from baseline, grade 3 or higher total bilirubin, grade 3 or higher ALT or AST, grade 3 or higher creatinine, and any other grade 3 or higher drug-related toxicity. Dose modification of combination partners was permitted after cycle 1. After cycle 1, subjects in both the dose expansion and dose escalation were able to receive additional cycles (cycle 2 and subsequent) of combination therapy until progressive disease (PD), intolerability, unacceptable toxicity, withdrawal of consent, or achievement of complete response (CR).

To further characterize the safety and efficacy of ulocuplumab in combination with lenalidomide or bortezomib, at least 20 subjects (6 in the dose-escalation cohort + 14 in the expansion cohort) were allowed to be enrolled in each Arm at the MTD or the highest dose tested if the MTD was not identified. Given the early responses observed in Arm A more than Arm B, a decision was made to move forward with phase II expansion of Arm A only and not of Arm B.

Efficacy and safety assessments

Toxicities were monitored throughout the study and for up to 30 days after the last dose of study drug. AEs were graded according to the National Cancer Institute Common Terminology Criteria for AEs (version 3.0). In addition, neuropathy symptoms were assessed using the FACT/GOG-Neuropathy questionnaire (Version 4.0).

Pharmacokinetic and cell trafficking assessments

Pharmacokinetic and flow cytometry data were collected during the first cycle and scheduled at 0 (predose), 1.5, 2, 4, 6, 24, 48, and 72 hours after the first dose; 0 (predose) on day 8; 0 (predose), 6, 24, and 72 hours after the third dose, on day 15; 0 (predose), 1.5, 2, 4, 6, 24, 48, and 72 after the fourth dose, on day 22; and 0 (predose), 1.5, 2, 4, 6, 24, 48, 72, and 168 hours after the fifth dose, on day 29 of cycle 1. Pharmacokinetic parameters for ulocuplumab, including maximum observed serum concentration (C_{max}), time of maximum observed serum concentration (T_{max}), and the area under the concentration–time curve (AUC) were derived from plotting serum concentration over time. Actual times were used for the analyses. In addition, exploratory assessment of dose proportionality was based on a power model and a confidence interval around the slope was calculated.

Whole blood was collected and the mononuclear cell populations were measured for the specific cell types using flow cytometry. Multiple myeloma cells were enumerated with a custom validated assay using fluorescently labeled antibodies specific for CD45, CD19, CD56, CD38, and CD138 (BD Biosciences) as $CD38^+$ $CD138^+CD45DIMCD19^-CD56^+$ cells. T cells, B cells, and NK cells were enumerated using the 6-color IVD TBNK assay from BD Biosciences. $CD34^+$ stem cells were enumerated using the BD stem count assay as required by the manufacturer. All samples were acquired using BD FACSCanto flow cytometers and data were analyzed using FACSvDIVA software. CXCR4 expression on multiple myeloma cells was also assessed by flow cytometry (BD Biosciences). Flow cytometry sampling was performed at 0 (predose), 2, 4, 6, 24, 48, and 72 hours after the first dose; 0 (predose), 2, 4, 5, 24 on day 8; and 0 (predose), 2, 4, 6, 24, and 72 hours after the third dose, on day 15 of cycle 1.

Statistical analysis

Study data are reported for all patients by stratification into study groups (Arms A and B) as well as for all patients combined. A summary of baseline patient characteristics was reported as number (%) of patients and median and range of values. Single-agent and combination therapy treatment responses were generated as proportions with 95% exact binomial confidence intervals. All time-to-event intervals were calculated using Kaplan–Meier estimates with 95% confidence intervals determined by Greenwood's variance estimator.

Time to response (TTR) was determined from treatment initiation to the date that the response had been originally observed. DOR was calculated from the start of the response until PD or death and was censored from analysis for patients who failed to progress using the date of the last disease assessment. TTP and PFS were determined from the start of treatment to the date of an event (PD for TTP; PD or death for PFS). Patients without an event were censored using the date of last disease assessment for both TTP and PFS. In the incident that nonprotocol therapy (excluding erythropoietin) had been added before an event, patients were censored in the time-to-event analyses at the start of that added therapy. Kaplan–Meier estimates were used to determine time to event whereas a long-rank test compared between estimates of time to event. All statistical analysis was computed using SAS software, SAS (version 9.3, SAS Institute Inc.) and R version 3.1.1. All results with P values <0.05 were considered statistically significant. Data cutoff was July 2016.

Results

Patients and treatment

Between October 2011 and March 2014, 30 patients were enrolled in Arm A and 16 patients were enrolled in Arm B (Supplementary Fig. S1 consort diagram). The number of patients on each dose level of the phase I dose escalation are described in Supplementary Fig. S1. The median age at enrollment for Arm A was 60 years (range, 55–69). The median number of prior therapies was 3 (range, 1–9), including prior

Table 2. Treatment-related AEs ≥ 10% frequency for grade levels and all events for grade 3 or 4.

	Arm A (n = 30)		Arm B (n = 16)	
Adverse events	**Any Grade**	**Grade 3–4**	**Any Grade**	**Grade 3–4**
Infections				
Upper respiratory infection	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
Shingles	0 (0.0)	0 (0.0)	2 (12.5)	0 (0.0)
Nervous system disorders				
Dizziness	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
Neuropathy	0 (0.0)	0 (0.0)	2 (12.5)	0 (0.0)
General				
Fatigue	7 (23.3)	1 (3.3)	4 (25.0)	0 (0.0)
Metabolic disorders				
Hyperglycemia	4 (13.3)	1 (3.3)	2 (12.5)	0 (0.0)
Hypokalemia	4 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
Hypomagnesemia	4 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
Hypophosphatemia	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gastrointestinal disorders				
Constipation	4 (13.3)	0 (0.0)	3 (18.8)	0 (0.0)
Diarrhea	10 (33.3)	1 (3.3)	0 (0.0)	0 (0.0)
Nausea	5 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
Hematologic toxicities				
Anemia	6 (20.0)	2 (6.7)	4 (25.0)	1 (6.3)
Neutropenia	13 (43.3)	9 (30.0)	3 (18.8)	1 (6.3)
Thrombocytopenia	10 (33.3)	5 (16.7)	6 (37.5)	3 (18.8)
Others				
CPK increased	0 (0.0)	0 (0.0)	2 (12.5)	0 (0.0)
Lipase increased	0 (0.0)	0 (0.0)	2 (12.5)	2 (12.5)

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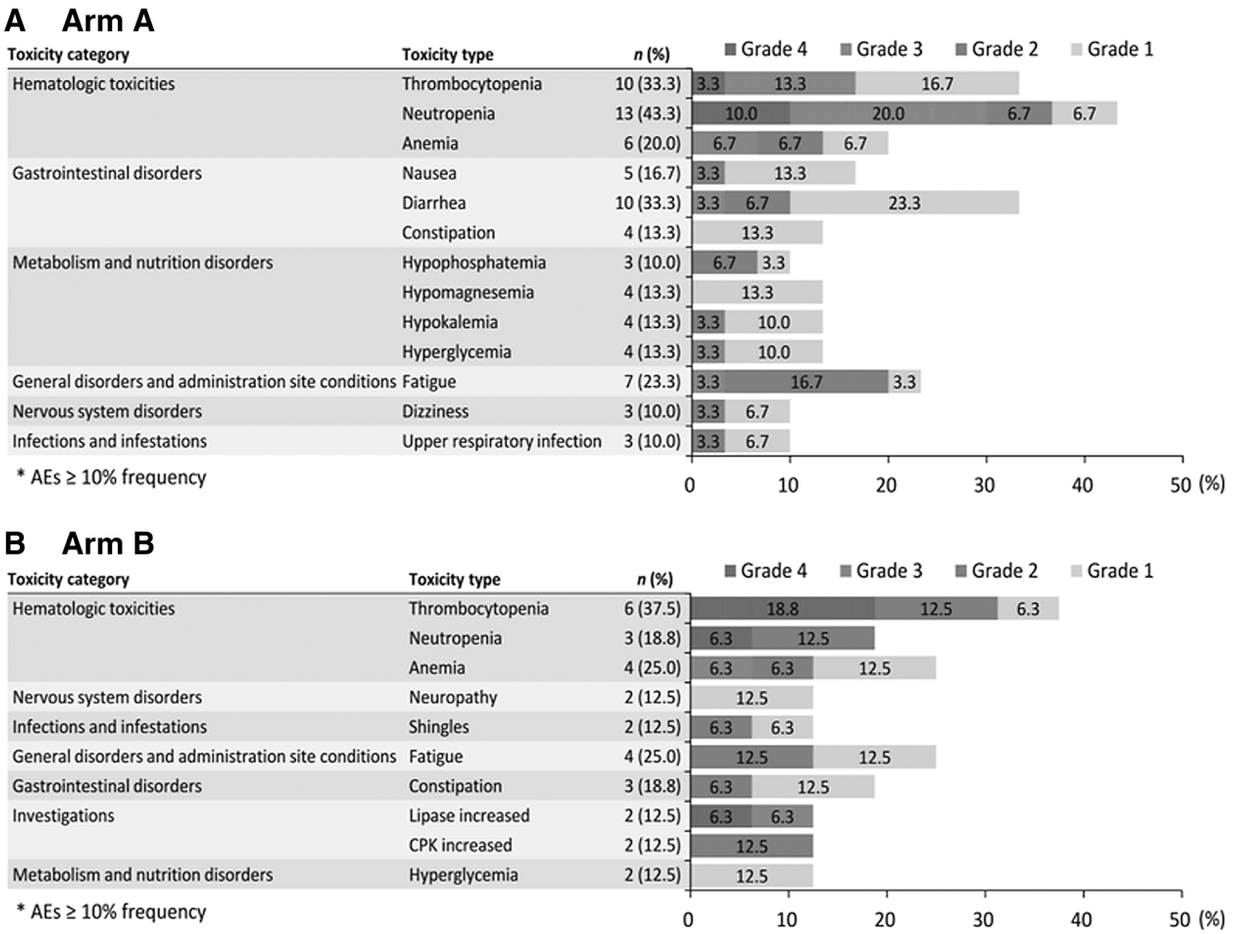

Figure 1.

Treatment-related AEs. Treatment-related AEs ≥10% frequency for any grade and all events for grades ≥3 are listed for Arm A (**A**) and Arm B (**B**) according to the toxicity categories.

bortezomib in 28 patients (93.3%). The median age at enrollment for Arm B was 60 years (range, 50–66) and the median number of prior therapies was 4 (range, 2–11), including prior bortezomib in 14 patients (87.5%). The median duration of treatment was 7.2 months (range, 0.7–48.8 months). Cytogenetics were available in all patients; of them, 40.0% patients in Arm A and 25.0% patients in Arm B had high-risk cytogenetics defined as patients with 17p deletion, t(4;14), t(14;16), and +1q amplification. The baseline characteristics of patients who were enrolled in Arms A and B are listed in **Table 1**.

Pharmacokinetics, safety, and tolerability

Ulocuplumab was administered at three dose levels (1, 3, and 10 mg/kg/dose). No DLT or MTD was identified. Therefore, the ulocuplumab dose level up to 10 mg/kg/dose was used for the expansion cohort. The median time to maximum serum concentration was 2 (range 1–24) hours following ulocuplumab administration. Patients who received 10 mg of ulocuplumab achieved higher concentration peak and larger dose-corrected area under curve (both $P < 0.001$). Pharmacokinetics analyses are listed in Supplementary Table S10 and Supplementary Fig. S2.

Treatment-related AEs with ≥10% frequency are summarized in **Table 2** and **Fig. 1**. In Arm A, the most common treatment-related AEs of any grade were neutropenia (13 patients, 43.3%), diarrhea (10 patients, 33.3%), thrombocytopenia (10 patients, 33.3%), and fatigue (7 patients, 23.3%). Fifteen patients (50.0%) had ≥ grade 3 toxicities. The most common grade 3 AEs were neutropenia

Table 3. Efficacy summary.

IMWG Response	Arm A ($n = 29$)	Arm B ($n = 16$)
Complete response	1 (3.5)	0 (0.0)
Very good partial response	4 (13.8)	2 (12.5)
Partial response	11 (37.9)	2 (12.5)
Minimal response	5 (17.2)	4 (25.0)
Stable disease	6 (20.7)	7 (43.8)
Progression	2 (6.9)	1 (6.3)
Clinical benefit rate (MR or better)	21 (72.4)	8 (50.0)
Response rate (PR or better)	16 (55.2)	4 (25.0)

*Evaluable patients, $n = 45$ (one patient in Arm A withdrew the consent before complete evaluation); clinical response rate includes complete response, very good partial response, partial response, and minimal response; response rate includes complete response, very good partial response, and partial response.

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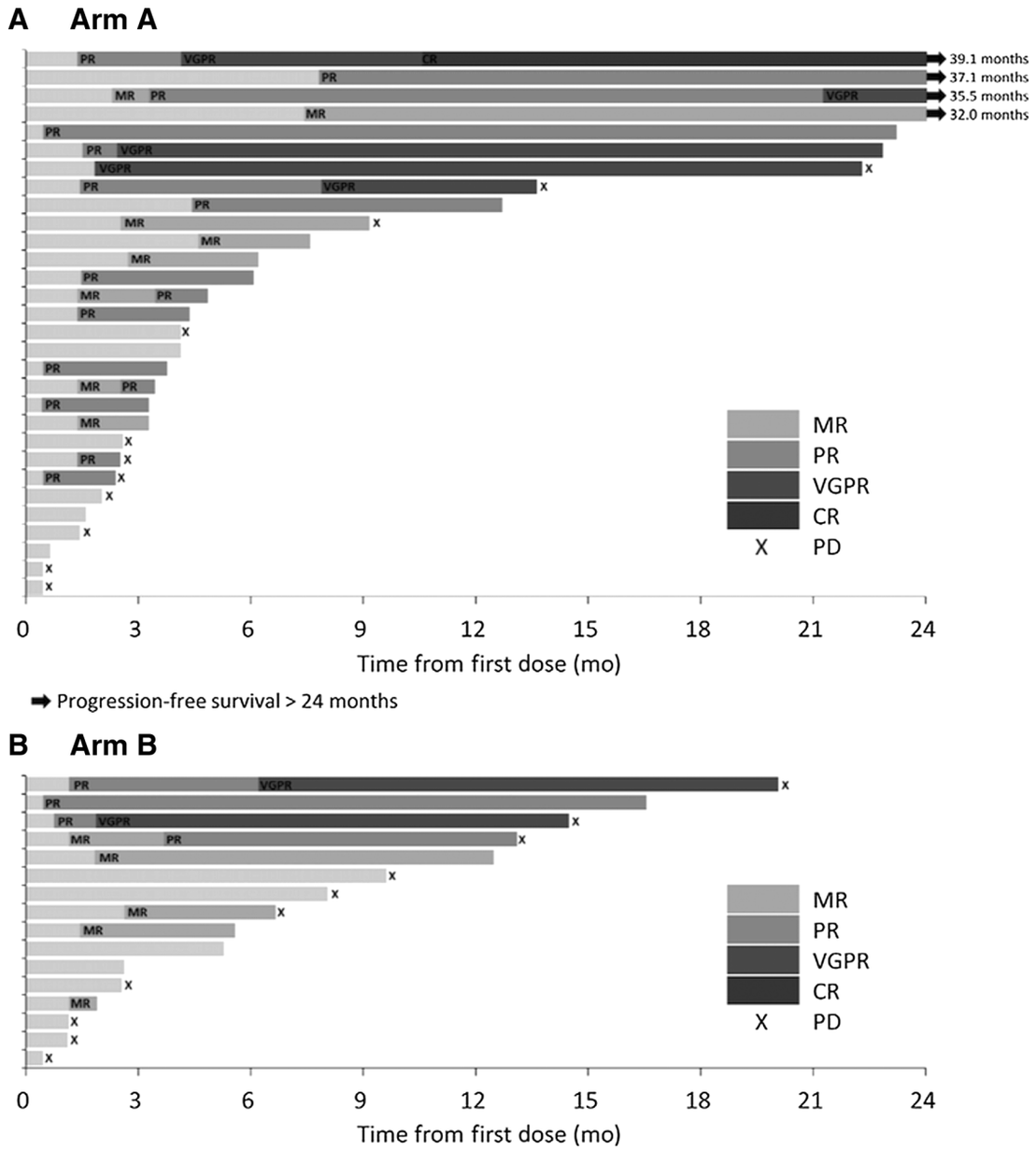

Figure 2.

Swim-lane plot of patients' responses. The timing and depth of treatment responses for Arm A (**A**) and Arm B (**B**) are shown in a swim-lane plot. X indicates disease progression. The evaluation of responses was based on IMWG uniform response criteria for multiple myeloma (MR, minimal response; PR, partial response; VGPR, very good partial response; CR, complete response; PD, progressive disease).

(6 patients, 20.0%) and thrombocytopenia (4 patients, 13.3%). Neutropenia (3 patients, 10.0%) was the most common AE in grade 4, followed by lymphopenia (1 patient, 3.3%), and thrombocytopenia (1 patient, 3.3%). In Arm B, the most common treatment-related AEs of any grade were thrombocytopenia (6 patients, 37.5%), fatigue (4 patients, 25.0%), and anemia (4 patients, 25.0%). Seven patients (37.5%) had $\geq$ grade 3 toxicities and four (25.0%) had grade 4 toxicities. No deaths related to study drugs occurred. The most common grade 3 or 4 AEs were thrombocytopenia (3 patients, 18.8%) and increased lipase (2 patients, 12.5%). Sensory peripheral neuropathy was reported in 2 patients, one in Arm A with grade 1 neuropathy and one in Arm B with grade 2. Two patients (6.7%) in Arm A encountered grade 2 infusion reaction; one patient (6.3%) in Arm A had grade 1 infusion reaction. All the reactions were limited in the first two cycles. Grade 3 or 4 sensory peripheral neuropathy was not observed. Twenty-five (83.3%) patients required dose modifications in Arm A, and 15 (93.8%) patients required dose modifications in Arm B. Four patients in Arm A discontinued treatment due to treatment-related toxicities, whereas none discontinued in Arm B for treatment-related toxicities.

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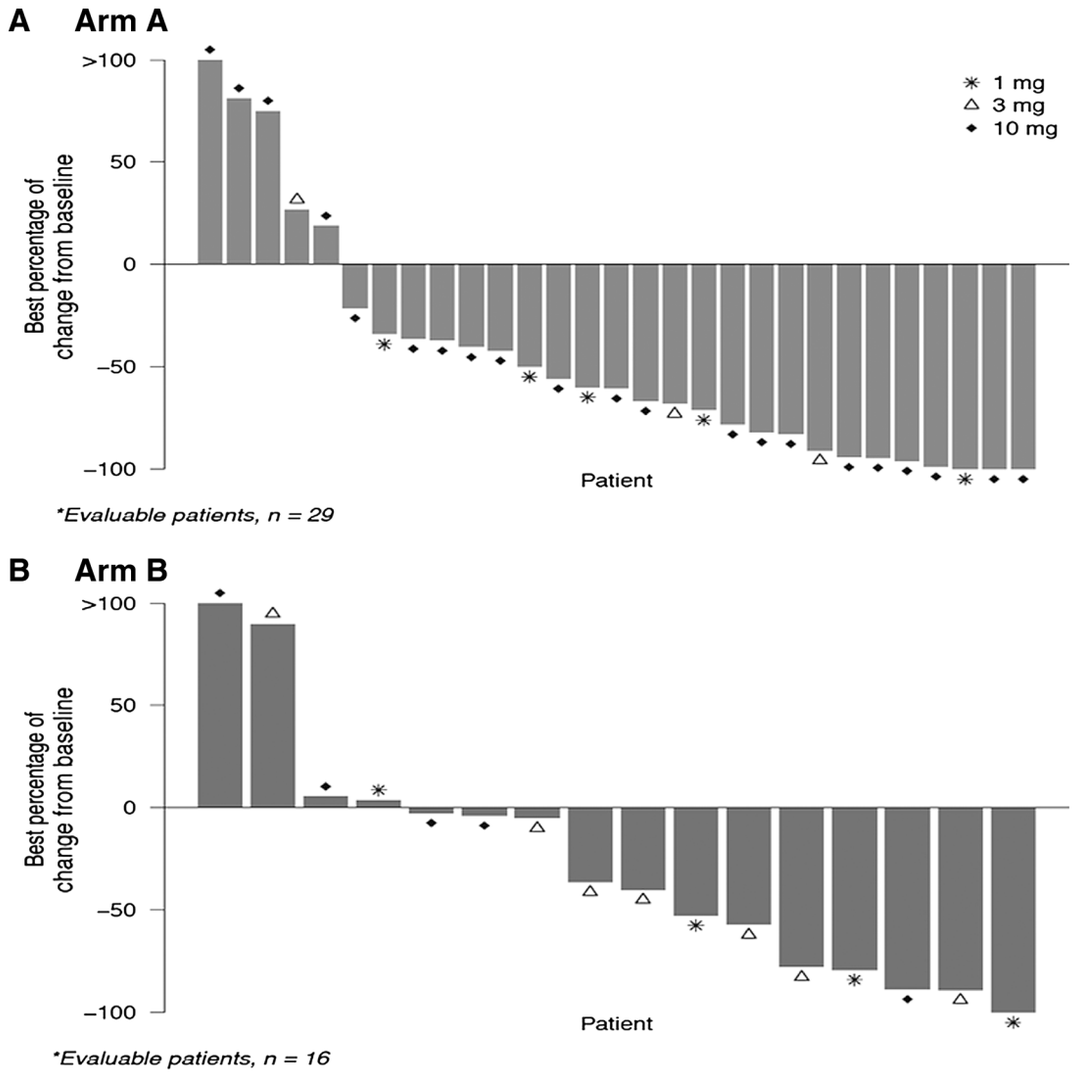

Figure 3.
Waterfall plot of the maximum M-protein change from baseline. The maximum change from baseline in the level of M-protein shown for Arms A (**A**) and B (**B**).

Efficacy

Response assessment is summarized in **Table 3** and **Figs. 2** and **3**. One patient in Arm A withdrew consent before completing the first cycle of treatment so that the total number of evaluable patients was 45 (29 in Arm A and 16 in Arm B). For Arm A patients, the overall response rate (PR or better) was 55.2% ($n = 16$) and the clinical benefit rate was 72.4% (21/29), with 1 patient (3.5%) achieving complete response (CR), 4 patients (13.8%) achieving very good partial response (VGPR), 11 patients (37.9%) achieving partial response (PR), 5 patients (17.2%) achieving minimal response (MR), and 6 patients (20.7%) maintaining stable disease (SD). There was no difference in the overall response rate when we assessed response only in patients who received the 10 mg/kg dose, $n = 21$ patients at the MTD dose, with 3 of 21 (14%) achieving VGPR, 8 of 21 (38%) achieving PR and 4 of 21 (19%) achieving MR. The overall clinical benefit rate was 15 (71%) and the overall response rate was observed in 11 (52%). For Arm B patients, the overall response rate was 25.0% ($n = 4$) and the clinical benefit rate was 50.0%, with 2 patients (12.5%) achieving VGPR, two patients achieving PR, 4 patients achieving MR, and 7 patients (43.8%) maintaining SD. Only 5 patients received the 10 mg/kg dose and again, there was no difference observed in the overall response rate in those patients compared with all patients treated on Arm B, with 1/5 (20%) achieving PR or better in these 5 patients.

Of the patients who responded, the median time to response to PR or better (min and max) was 1.5 months (range, 0.4–7.8 months) for Arm A and 1.0 month (range, 0.5–3.7 months) for Arm B. The number of prior lines of therapy did not affect response in these patients ($P = 0.131$). **Figure 2** demonstrates the time and depth of response for patients who achieved at least PR. The maximum change in M-protein levels from baseline is shown in **Fig. 3**.

Time-to-event analysis

Patients received a median of 5 cycles in Arm A (range, 1–50) and 9 cycles in Arm B (range, 1–65).

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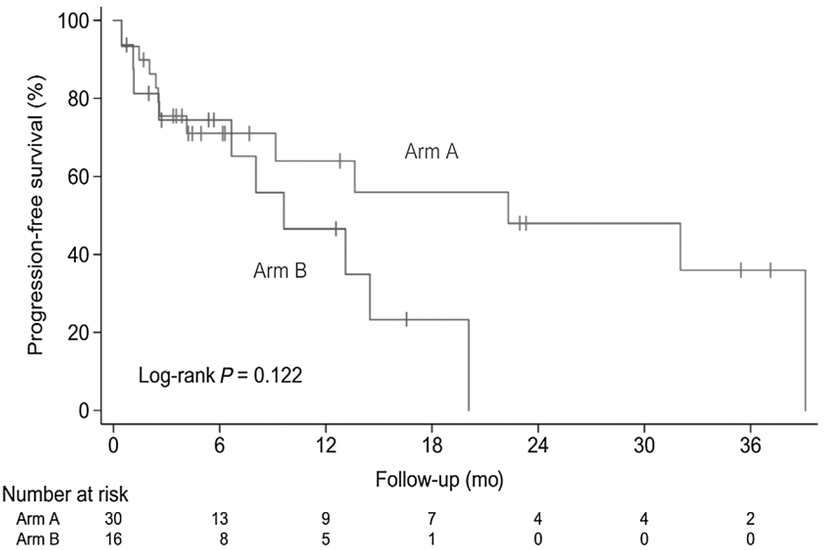

Figure 4.
Cumulative probability of progression among patients in Arms A and B. The Kaplan–Meier method was used for estimation of cumulative incidence of progression. The median PFS was 22.31 (95% CI, 9.17–not reached) months and 9.63 (95% CI, 2.56–not reached) months in Arms A and B, respectively.

The median PFS were 22.31 (95% CI, 9.17–not reached) months and 9.63 (95% CI, 2.56–not reached) months in Arms A and B, respectively. The Kaplan–Meier curve of PFS is shown in **Fig. 4**.

In a *post hoc* analysis, we evaluated PFS in patients who had previously received bortezomib and lenalidomide therapy. The median PFS was 9.2 (95% CI, 4.1–22.3) months for patients who were refractory to bortezomib, and 20.1 (13.6–not reached) months for those who were not ($P = 0.103$). The median (95% CI) PFS of patients who were refractory to lenalidomide was 8.0 (95% CI, 2.6–14.5) months, while it was 22.3 (9.2–not reached) months for those who were not ($P = 0.045$).

Plasma and immune cell mobilization in response to ulocuplumab

To assess the effect of CXCR4 inhibition by ulocuplumab on cell mobilization, we performed flow cytometry on peripheral blood samples drawn during cycle 1. Flow cytometry showed that ulocuplumab administration lead to a successful mobilization of hematopoietic stem cells and to high variability in numbers of circulating plasma cells (Supplementary Fig. S3A–S3D). Interestingly, whereas the fluctuating levels of circulating plasma cells persisted during cycle 1 in nonresponding group, a slight trend to more robust mobilization of plasma cells after day 8 was observed in the responding group (Supplementary Fig. S3D). Moreover, we found significantly increased numbers of circulating B cells, $CD3^+$ T cells and CD14-expressing monocytes in peripheral blood following ulocuplumab administration (Supplementary Fig. S4), demonstrating an efficient mobilization of microenvironmental cells from the bone marrow environment upon CXCR4 inhibition. Patient response or adverse effects were not significantly correlated to the reported changes in the numbers of circulating plasma or immune cells in the peripheral blood.

Discussion

The bone marrow microenvironment is suspected to play a role in response to treatment and disease dissemination in many cancers (11–14). Particularly, CXCR4, a chemokine receptor related to homing of tumor cells to the bone marrow, has been shown to be associated with metastasis and poor prognosis in cancers like AML, breast, colorectal, ovarian, pancreatic and non–small cell lung cancer (15–20). In multiple myeloma, an incurable malignancy where most patients eventually relapse, the role of the bone marrow microenvironment and especially CXCR4 is an area of active investigation, with preclinical data supporting a role for CXCR4 in disease dissemination and response to treatment. Specifically, CXCR4 expression was found to be increased in patients with multiple myeloma with extramedullary plasmacytoma, linking CXCR4 to extramedullary dissemination of disease (21), whereas CXCR4 blockade was shown to inhibit tumor growth and dissemination of multiple myeloma cells *in vivo* (6). We have previously shown that ulocuplumab, a first-in-kind monoclonal anti-CXCR4 antibody, can inhibit CXCR4-mediated cell migration and dissemination, including EMT-like transcriptional regulation, as well as inhibit intracellular signaling pathways that regulate drug resistance like the Akt/mTOR pathway, therefore, rendering the cells more sensitive to drugs (8). This phase Ib/II study was designed to evaluate the safety and efficacy of a three-drug combination of the first fully human monoclonal anti-CXCR4 antibody with lenalidomide plus dexamethasone or bortezomib plus dexamethasone in relapsed/refractory multiple myeloma.

The study proved the combination to be safe, with no DLTs observed with full dosing of the combination in patients with relapsed/refractory multiple myeloma. The major grade 3 and 4 hematologic toxicities were thrombocytopenia and neutropenia, which were expected and are related to bortezomib or lenalidomide. With minimal infusion reactions, the safety profile of this antibody makes it ideal for therapeutic combinations in myeloma, even for patients with significant cytopenias and end-stage disease.

In terms of efficacy, our study demonstrated a high response rate, with an overall response rate (PR or better) of 55.2% and a clinical benefit rate of 72.4% in the combination arm of ulocuplumab/lenalidomide/dexamethasone, even in patients who had been previously treated with immunomodulatory agents (IMiD). This led to a prolonged PFS of over 22 months in this relapsed/refractory population. This could indicate that the antibody synergizes with IMiDs. Although we cannot compare across trials, recent studies using lenalidomide and dexamethasone such as in the ELOQUENT-2 study had a median PFS of 14.9 months in the control arm and 19.4 months in the elotuzumab, lenalidomide, and dexamethasone arm (22). The response rate was 66% in the lenalidomide arm (22). Our study showed a median PFS of 22.31 months in the lenalidomide arm, indicating that this

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combination is active and further considerations of larger studies should be evaluated. Although the role of ulocuplumab in immune cell regulation in multiple myeloma has not been studied, CXCR4 is expressed on the surface of many immune cells (23, 24), indicating that ulocuplumab could be involved in immune cell activation. Indeed, in our study, we observed mobilization of some immune cells, including T cells, B cells and monocytes, following ulocuplumab administration. Previous *in vivo* and *in vitro* data showed that blockade of CXCR4 mitigates $CD4^+$ T-cell exhaustion (25), reverts the suppressive activity of T-regulatory cells (26), modulate immunotherapy with anti-PD-1 (27) and activates cytotoxicity of immune cells (23), emphasizing the immunomodulatory function of ulocuplumab. Blocking CXCR4 attenuates differentiation of CD14-expressing monocytes to the tumor-supportive M2-polarized macrophages, which are known to be increased in multiple myeloma bone marrow microenvironment (28). Moreover, higher levels of CD19 B cells have been correlated with longer event-free and overall survival in multiple myeloma (29), thus implicating the potential antimyeloma response of mobilized B cells in our study. Further studies are warranted to better define the mechanisms of synergy between CXCR4 regulation and immune cell activation as a potential mechanism of antimyeloma activity.

To conclude, this study demonstrated that ulocuplumab in combination with lenalidomide and dexamethasone led to a high response rate even in heavily pretreated relapsed/refractory patients. Although further studies to explore the synergy between ulocuplumab and lenalidomide are warranted, combinations of ulocuplumab with novel agents, like mAbs against CD38, SLAMF7, and BCMA could also be investigated, especially in the treatment of extramedullary multiple myeloma, an area of unmet clinical need in multiple myeloma therapeutics.

Disclosure of Potential Conflicts of Interest

I.M. Ghobrial is a paid consultant/advisory board member of GSK, Sanofi, Janssen, Takeda, Celgene, Karyopharm, AbbVie, GNS, Cellectar, Medscape, Genetech, Adaptive, BMS, Aptitude, Curio Science, Magenta, and Oncopeptides. R. Baz reports receiving commercial research grants from Bristol-Myers Squibb, Karyopharm, Celgene, AbbVie, Janssen, and Merck. P.G. Richardson is an employee/paid consultant for Karyopharm, Oncopeptides, Celgene, Takeda, Amgen, Janssen, and Sanofi and reports receiving commercial research grants from Oncopeptides, Celgene, Takeda, and Bristol-Myers Squibb. K.C. Anderson is an employee/paid consultant for Bristol-Myers Squibb, Celgene, Millennium Takeda, Janssen, Sanofi-Aventis, and Gilead and holds ownership interest as scientific founder of C4 Therapeutics and Oncopep. M.D. Robbins is an employee/paid consultant for Bristol-Myers Squibb. P.S. Becker is an employee/paid consultant for Accordant Health Services/Caremark and McKesson. No potential conflicts of interest were disclosed by the other authors.

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References

1. Bustoros M, Mouhieddine TH, Detappe A, Ghobrial IM. Established and novel prognostic biomarkers in multiple myeloma. Am Soc Clin Oncol Educ Book 2017;37:548–60.
2. Peled A, Klein S, Beider K, Burger JA, Abraham M. Role of CXCL12 and CXCR4 in the pathogenesis of hematological malignancies. Cytokine 2018; 109:11–6.
3. Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. Semin Cancer Biol 2004;14:171–9.
4. Domanska UM, Kruizinga RC, Nagengast WB, Timmer-Bosscha H, Huls G, de Vries EG, et al. A review on CXCR4/CXCL12 axis in oncology: no place to hide. Eur J Cancer 2013;49:219–30.
5. Sacco A, Roccaro AM, Ma D, Shi J, Mishima Y, Moschetta M, et al. Cancer cell dissemination and homing to the bone marrow in a zebrafish model. Cancer Res 2016;76:463–71.
6. Alsayed Y, Ngo H, Runnels J, Leleu X, Singha UK, Pitsillides CM, et al. Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)-dependent migration and homing in multiple myeloma. Blood 2007;109:2708–17.
7. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423–37.
8. Azab AK, Runnels JM, Pitsillides C, Moreau AS, Azab F, Leleu X, et al. CXCR4 inhibitor AMD3100 disrupts the interaction of multiple myeloma cells with the bone marrow microenvironment and enhances their sensitivity to therapy. Blood 2009;113:4341–51.
9. Kuhne MR, Mulvey T, Belanger B, Chen S, Pan C, Chong C, et al. BMS-936564/MDX-1338: a fully human anti-CXCR4 antibody induces apoptosis *in vitro* and shows antitumor activity *in vivo* in hematologic malignancies. Clin Cancer Res 2013;19:357–66.
10. Becker PS, Foran JM, Altman JK, Yacoub A, Castro JE, Sabbatini P, et al. Targeting the CXCR4 pathway: safety, tolerability and clinical activity of ulocuplumab (BMS-936564), an anti-CXCR4 antibody, in relapsed/refractory acute myeloid leukemia. Blood 2014;124:386.
11. Passaro D, Di Tullio A, Abarrategi A, Rouault-Pierre K, Foster K, Ariza-McNaughton L, et al. Increased vascular permeability in the bone marrow microenvironment contributes to disease progression and drug response in acute myeloid leukemia. Cancer Cell 2017;32:324–41.
12. Shafat MS, Gnaneswaran B, Bowles KM, Rushworth SA. The bone marrow microenvironment - home of the leukemic blasts. Blood Rev 2017;31: 277–86.
13. Manier S, Sacco A, Leleu X, Ghobrial IM, Roccaro AM. Bone marrow microenvironment in multiple myeloma progression. J Biomed Biotechnol 2012;2012: 157496.
14. Jalali S, Ansell SM. Bone marrow microenvironment in waldenstrom's macroglobulinemia. Best Pract Res Clin Haematol 2016;29:148–55.
15. Spoo AC, Lubbert M, Wierda WG, Burger JA. CXCR4 is a prognostic marker in acute myelogenous leukemia. Blood 2007;109:786–91.
16. Hiller DJ, Meschonat C, Kim R, Li BD, Chu QD. Chemokine receptor CXCR4 level in primary tumors independently predicts outcome for patients with locally advanced breast cancer. Surgery 2011;150:459–65.
17. Ottaiano A, Franco R, Aiello Talamanca A, Liguori G, Tatangelo F, Delrio P, et al. Overexpression of both CXC chemokine receptor 4 and vascular endothelial

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growth factor proteins predicts early distant relapse in stage II-III colorectal cancer patients. Clin Cancer Res 2006;12:2795–803.
18. Spano JP, Andre F, Morat L, Sabatier L, Besse B, Combadiere C, et al. Chemokine receptor CXCR4 and early-stage non–small cell lung cancer: pattern of expression and correlation with outcome. Ann Oncol 2004;15: 613–7.
19. Jiang YP, Wu XH, Shi B, Wu WX, Yin GR. Expression of chemokine CXCL12 and its receptor CXCR4 in human epithelial ovarian cancer: an independent prognostic factor for tumor progression. Gynecol Oncol 2006;103:226–33.
20. Marechal R, Demetter P, Nagy N, Berton A, Decaestecker C, Polus M, et al. High expression of CXCR4 may predict poor survival in resected pancreatic adenocarcinoma. Br J Cancer 2009;100:1444–51.
21. Roccaro AM, Mishima Y, Sacco A, Moschetta M, Tai YT, Shi J, et al. CXCR4 regulates extra-medullary myeloma through epithelial-mesenchymal-transition-like transcriptional activation. Cell Reports 2015;12:622–35.
22. Dimopoulos MA, Lonial S, Betts KA, Chen C, Zichlin ML, Brun A, et al. Elotuzumab plus lenalidomide and dexamethasone in relapsed/refractory multiple myeloma: extended 4-year follow-up and analysis of relative progression-free survival from the randomized ELOQUENT-2 trial. Cancer 2018;124:4032–43.
23. Han AR, Lee JY, Kim HJ, Min WS, Park G, Kim SH. A CXCR4 antagonist leads to tumor suppression by activation of immune cells in a leukemia-induced microenvironment. Oncol Rep 2015;34:2880–8.
24. Jung K, Heishi T, Incio J, Huang Y, Beech EY, Pinter M, et al. Targeting CXCR4-dependent immunosuppressive Ly6C(low) monocytes improves antiangiogenic therapy in colorectal cancer. PNAS 2017;114:10455–60.
25. Ramonell KM, Zhang W, Hadley A, Chen CW, Fay KT, Lyons JD, et al. CXCR4 blockade decreases CD4+ T cell exhaustion and improves survival in a murine model of polymicrobial sepsis. PLoS ONE 2017;12: e0188882.
26. Santagata S, Napolitano M, D'Alterio C, Desicato S, Maro SD, Marinelli L, et al. Targeting CXCR4 reverts the suppressive activity of T-regulatory cells in renal cancer. Oncotarget 2017;8:77110–20.
27. Chen Y, Ramjiawan RR, Reiberger T, Ng MR, Hato T, Huang Y, et al. CXCR4 inhibition in tumor microenvironment facilitates anti-programmed death receptor-1 immunotherapy in sorafenib-treated hepatocellular carcinoma in mice. Hepatology 2015;61:1591–602.
28. Chatterjee M, von Ungern-Sternberg SN, Seizer P, Schlegel F, Buttcher M, Sindhu NA, et al. Platelet-derived CXCL12 regulates monocyte function, survival, differentiation into macrophages and foam cells through differential involvement of CXCR4-CXCR7. Cell Death Dis 2015;6:e1989.
29. Kay NE, Leong T, Kyle RA, Greipp P, Billadeau D, Van Ness B, et al. Circulating blood B cells in multiple myeloma: analysis and relationship to circulating clonal cells and clinical parameters in a cohort of patients entered on the Eastern Cooperative Oncology Group phase III E9486 clinical trial. Blood 1997;90:340–5.

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Clinical Cancer Research

A Phase Ib/II Trial of the First-in-Class Anti-CXCR4 Antibody Ulocuplumab in Combination with Lenalidomide or Bortezomib Plus Dexamethasone in Relapsed Multiple Myeloma

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