Clinical Cancer Research

Phase I and Biomarker Study of Plerixafor and Bevacizumab in Recurrent High-Grade Glioma

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Abstract

Purpose: Although antiangiogenic therapy for high-grade glioma (HGG) is promising, responses are not durable. Correlative clinical studies suggest that the SDF-1α/CXCR4 axis may mediate resistance to VEGFR inhibition. Preclinical data have demonstrated that plerixafor (a reversible CXCR4 inhibitor) could inhibit glioma progression after anti-VEGF pathway inhibition. We conducted a phase I study to determine the safety of plerixafor and bevacizumab in recurrent HGG.

Patients and Methods: Part 1 enrolled 23 patients with a 3 × 3 dose escalation design to a maximum planned dose of plerixafor 320 μg/kg subcutaneously on days 1 to 21 and bevacizumab 10 mg/kg intravenously on days 1 and 15 of each 28-day cycle. Cerebrospinal fluid (CSF) and plasma samples were obtained for pharmacokinetic analyses. Plasma and cellular biomarkers were evaluated before and after treatment. Part 2 enrolled 3 patients and was a surgical study to determine plerixafor's penetration in tumor tissue.

Results: In Part 1, no dose-limiting toxicities were seen at the maximum planned dose of plerixafor + bevacizumab. Treatment was well tolerated. After plerixafor 320 μg/kg treatment, the average CSF drug concentration was 26.8 ± 19.6 ng/mL. Plerixafor concentration in resected tumor tissue from patients pretreated with plerixafor was 10 to 12 μg/g. Circulating biomarker data indicated that plerixafor + bevacizumab induces rapid and persistent increases in plasma SDF-1α and placental growth factor. Progression-free survival correlated with pretreatment plasma soluble mesenchymal–epithelial transition receptor and sVEGFR1, and overall survival with the change during treatment in $CD34^+$ progenitor/stem cells and CD8 T cells.

Conclusions: Plerixafor + bevacizumab was well tolerated in HGG patients. Plerixafor distributed to both the CSF and brain tumor tissue, and treatment was associated with biomarker changes consistent with VEGF and CXCR4 inhibition.
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Introduction

High-grade gliomas (HGG) often develop resistance to treatment targeting the VEGF/VEGF receptor (VEGFR) pathway within months of starting therapy (1). Once patients progress on one bevacizumab regimen, median progression-free survival (PFS) on a second bevacizumab regimen is only 30 to 38 days (2, 3). This indicates that targeting VEGF pathway–driven angiogenesis alone is not sufficient to achieve durable responses (4). Tumor vasculature can also arise by colonization of bone marrow–derived vascular progenitors (referred to as vasculogenesis; ref. 5). Although the predominant mechanisms of resistance to antiangiogenic therapies are still being elucidated, there is evidence that signaling by the chemokine receptor CXCR4, a mediator of progenitor/stem cell and inflammatory cell trafficking and retention in tissues, as well as a driver of cancer cell invasion, may be important. In a phase II study of cediranib, an oral pan-VEGFR small-molecule inhibitor, for recurrent HGG, plasma obtained at the time of tumor recurrence showed statistically significant increases in plasma levels of stromal cell–derived factor 1α (SDF-1α; ref. 6). This suggested that this chemokine may help mediate resistance to VEGFR inhibition. There was also a significant positive correlation between SDF-1α levels and tumor blood vessel size as measured by MRI.

Preclinical data provided compelling evidence to support the hypothesis that the SDF-1α/CXCR4 axis plays an important role in HGG progression and treatment resistance (7–9). CXCR4 is not known to be mutationally activated in cancer, and thus receptor activation requires engagement of SDF-1α (also known as CXCL12; ref. 8). CXCR4 RNA and protein are highly expressed in HGG samples from patients (8, 9), and protein expression correlates with tumor grade in astrocytomas (10). *In vitro*, CXCR4 activation promotes tumor cell proliferation, inhibits apoptosis, and mediates cellular migration (8, 11). In preclinical models and in patient-derived tumor samples, CXCR4-expressing tumor cells distribute around SDF-1α–expressing endothelial cells, a paracrine interaction that may be critical for tumor growth (8, 9). Evidence from glioma models shows that SDF-1α is both necessary and sufficient to induce vasculogenesis (12) and that SDF-1α mediates the cross-talk between HGG and endothelial cells to enhance tumor invasion (13). Inhibition of CXCR4 with

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Translational Relevance

Responses to anti-VEGF therapies such as bevacizumab in high-grade glioma (HGG) are not durable. Signaling by CXCR4 may play an important role in resistance to anti-VEGF therapies. Prolonged inhibition of tumor neovascularization may require combination therapy targeting both VEGF and CXCR4 pathways. In addition, CXCR4 inhibition by plerixafor in preclinical HGG models resulted in diminished tumor growth. Therefore, we performed a phase I and biomarker study of plerixafor in combination with bevacizumab in patients with recurrent HGG. This is the first clinical trial of a CXCR4 inhibitor with extensive biomarker data in a solid tumor. We demonstrate that plerixafor distributes into the cerebrospinal fluid and brain tumor tissue at CXCR4-inhibitory concentrations. Although treatment was well-tolerated, efficacy was limited. The role of VEGF-independent pathways (HGF/MET) and immune evasion in treatment resistance will need to be established in future studies.

plerixafor caused a 2.7-fold increase in apoptosis in HGG xenografts (8) and resulted in diminished growth of orthotopic tumors (8, 14). In an irradiated HGG model, inhibition of SDF-1α/CXCR4 axis using plerixafor (AMD3100) prevented the influx of bone marrow–derived cells and inhibited tumor growth (14). These findings show that CXCR4 is a potential target in HGG and that prolonged inhibition of tumor neovascularization may require combination therapy targeting both VEGF and CXCR4 pathways.

Plerixafor is an FDA-approved inhibitor of CXCR4. Preclinical studies have shown that plerixafor can cross the blood–brain barrier (BBB), and a small but measurable amount is detected in the cerebrospinal fluid (CSF). However, no such studies have been performed in patients with solid cancers. We performed a phase I and biomarker study of plerixafor in combination with standard anti-VEGF antibody treatment (bevacizumab) in patients with recurrent HGG.

Patients and Methods

Patient eligibility

Adults (≥18 years old) with histologically confirmed glioblastoma (GBM), gliosarcoma, anaplastic astrocytoma, anaplastic oligodendroglioma, or anaplastic oligoastrocytoma with unequivocal tumor recurrence by MRI scans were eligible. A baseline MRI was performed within 14 days of registration on a stable or decreasing steroid dosage for ≥5 days. Any number of prior relapses on non–anti-VEGF(R)-containing regimens were allowed, although only one prior relapse on a bevacizumab or anti–VEGF(R)-containing regimen was allowed. Additional eligibility criteria included Karnofsky performance score (KPS) ≥ 60, as well as adequate bone marrow, renal, and hepatic function. Due to potential teratogenicity of plerixafor and bevacizumab, all patients of childbearing potential were required to use adequate birth control. Exclusion criteria included pregnancy; uncontrolled intercurrent medical illnesses such as uncontrolled hypertension; known coagulopathy; history of a clinically significant hemorrhage; history of myocardial infarction, unstable angina, stroke, or transient ischemic attack (TIA) within 6 months; and history of gastrointestinal perforation, abdominal fistula, or intraabdominal abscess. Patients whose MRI scan demonstrated intratumoral or peritumoral hemorrhage were not eligible if deemed significant by the treating physician. For patients enrolled on the surgical cohort, patients were required to be appropriate surgical candidates. For the first 20 patients registered on trial, no anticoagulation was allowed. However, for all subsequent patients, patients requiring therapeutic or prophylactic therapy with a low-molecular-weight heparin at baseline were allowed to participate on study.

The study was approved by the Institutional Review Board of Dana-Farber/Harvard Cancer Center and conducted in accordance with institutional and federal guidelines for human investigations as well as the Declaration of Helsinki. Patients were informed of the investigational nature of this study and provided Institutional Review Board–approved informed written consent before enrollment.

Treatment plan

The study was divided into two parts: a nonsurgical cohort (Part 1) and a surgical cohort (Part 2). In Part 1, plerixafor was administered once daily subcutaneously on days 1 to 21 of each 28-day cycle, (i.e., 3 weeks on, 1 week off) together with bevacizumab 10 mg/kg intravenously on days 1 and 15. Dose escalation of plerixafor occurred in a standard 3 × 3 design and evaluated 3 planned dose levels (160, 240, and 320 μg/kg). The FDA indication for plerixafor is for 240 μg/kg once daily for up to 4 consecutive days for hematopoietic stem cell mobilization in autologous transplantation. Since this study was the first investigation of continuous plerixafor treatment in any tumor, there was very limited experience with prolonged administration of the agent. In addition, vasovagal reactions can occur at plerixafor doses above 240 μg/kg (15). Therefore, the maximum planned dose was 320 μg/kg. The adverse event (AE) grade was defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). Dose-limiting toxicity (DLT) was determined during the initial 4 weeks of therapy and defined as any drug-related grade 3 nonhematologic toxicity despite maximal medical therapy lasting >7 days with the exception of grade 3 proteinuria (which was considered a DLT if lasting >14 days); any grade 4 related toxicity with or without maximal therapy; any grade 3 thrombocytopenia lasting more than 7 days; grade 4 thrombocytopenia of any duration; grade 4 anemia lasting more than 7 days; grade ≥ 3 neutropenia complicated by fever or infection; grade 4 neutropenia lasting more than 7 days; and failure to recover from toxicities to be eligible for retreatment with plerixafor and bevacizumab within 28 days of the last dose of either drug.

Once the maximum dose from Part 1 was established, Part 2 (surgical cohort) opened. These patients received plerixafor monotherapy for 5 to 9 days prior to surgery at the maximum dose established in Part 1 of the study. Following recovery from surgery, they resumed treatment with plerixafor at the maximum dose from Part 1 on days 1 to 21 together with bevacizumab 10 mg/kg administered on days 1 and 15 of each 28-day cycle.

All patients underwent clinical evaluation weekly for the first 4 weeks and then every 4 weeks thereafter. Brain MRI with contrast was performed at baseline and then after every 2 cycles (8 weeks) thereafter; when feasible, dynamic contrast-enhanced and diffusion MRI were also obtained at these same imaging time-points.

Table 1. Patient characteristics

Patient characteristic	$N = 26$
Median age, years (range)	59 (23–72)
Median KPS (range)	90 (70–100)
Gender, female	11 (42.3%)
Race/ethnicity	
Caucasian	26 (100%)
Histology	
GBM	17 (65.4%)
AA	5 (19.2%)
AO	1 (3.9%)
AOA	3 (11.5%)
Number of prior therapies, median (range)	1 (1–7)
Previously received bevacizumab	5 (19.2%)

Abbreviations: AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma.

Local investigators used RANO-GBM criteria (16) for response assessment.

Pharmacokinetic and pharmacodynamic biomarker studies

Serial plasma pharmacokinetics (PK) assessments for measurement of plerixafor were performed on days 1 before and after treatment, 2, 16, 21, and 22 of cycle 1. Plasma and cellular biomarker studies were performed in the Clinical Laboratory Improvement Amendments–certified core of the Steele Laboratories, Massachusetts General Hospital. Plasma for circulating biomarkers and whole blood for circulating lymphocyte and myeloid cell populations and circulating precursor cells were obtained on days 1 before treatment, 2, and 15 of cycle 1 as well as on day 1 of subsequent cycles and off treatment. Plasma measurements were performed for free VEGF, placental growth factor (PlGF), sVEGFR1, basic fibroblast growth factor (bFGF), VEGF-C, VEGF-D, and sTIE2 using the Human Angiogenesis Panel 1 V-PLEX Kit (catalog number K15190D; Meso-Scale Discovery; ref. 17); for IL1β, TNFα, IL6, and IL8 using the Human ProInflammatory II 4-Plex Ultra-Sensitive Kit (catalog number K15025C; MesoScale Discovery); and single analyte ELISA for SDF-1α and CAIX (R&D Systems), collagen IV (USCN/CloudClone Corp.), and soluble mesenchymal–epithelial transition receptor (sMET; Invitrogen). Cell biomarkers were evaluated by flow cytometry using antibodies for the following cell surface markers: CD3, CD4, CD8, CD11b, CD14, CD25, CD31, CD34, CD45, CD56, CD127, and CD133 and an LSR-II cytometer (BD). At each dose level in Part 1, if deemed safe by the treating physician, CSF was collected on day 15 (±7 days) of the first and second cycles, approximately 2 to 3 hours after the last dose of plerixafor, for measurement of plerixafor levels. For the surgical cohort, there were additional PK assessments while on plerixafor monotherapy prior to surgery on day 1 before and after treatment, day 2, and day of surgery. Plasma PK, CSF PK, and tissue PK samples were analyzed by sensitive (LLOQ 5 ng/mL) and validated LC/MS/MS methods developed by Covance.

Statistical analysis

The primary objective of Part 1 was to determine the recommended phase II dose of plerixafor, 3 weeks on and 1 week off, in combination with bevacizumab, 10 mg/kg every other week in patients with HGG. For Part 2, the primary objective was to obtain preliminary information about whether plerixafor penetrates tumor tissue in HGG patients. Descriptive statistics were used for safety, tumor response, PK, and PD. The Kaplan–Meier method was used for estimating overall survival (OS) and PFS values. Percent change from baseline in biomarkers values was evaluated using the Sign-Rank test. Relationship between biomarker values at baseline or percent changes from baseline and OS and PFS outcomes were tested with the Cox Proportional Hazard model.

Plerixafor PK parameters were determined using standard noncompartmental methods. Peak concentrations (C_{max}) were determined by inspection of each patient's concentration–time curve. Elimination rate constants were estimated by linear regression analysis of the last two time-points of the log-concentration versus time curve. Terminal half-lives ($t_{1/2}$) were calculated by dividing 0.693 by the elimination rate constant. The AUC was estimated using the linear trapezoidal rule up the last sampling time (AUC_{0-24}), then extrapolated to infinity (AUC). Systemic clearance (CL/F) was determined by dividing dose by AUC. Volume of distribution (Vd/F) was calculated by dividing CL by the elimination rate constant. Changes in kinetic parameters over time were evaluated using the Sign-Rank test. Linear relationships were determined by the Spearman rank correlation.

Results

Patient characteristics

The trial accrued 26 subjects (23 in Part 1 and 3 in Part 2) between December 2011 and January 2016. The study closed early during Part 2 (surgical cohort) due to poor enrollment. Patient characteristics are summarized in Table 1. Median age was 59 (range, 23–73), and median KPS was 90 (range, 70–100). Most patients had a histologic diagnosis of GBM (65.45%). The median number of prior therapies was 1 (range, 1–7) with 5 patients having received prior bevacizumab (3 in Cohort 1).

Safety

Treatment with plerixafor in combination with bevacizumab was administered at the three planned dose levels. There were no DLTs at the maximum planned dose of plerixafor (320 μg/kg) 3 weeks on, 1 week off combined with bevacizumab (10 mg/kg)

Table 2. Number of patients experiencing each AE at least possibly related to bevacizumab and/or plerixafor

	Bevacizumab			Plerixafor		
Toxicity	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4
aPTT prolonged	1	–	–	–	–	–
Fatigue	–	–	–	1	–	–
Headache	1	–	–	1	–	–
Hypertension	4	–	–	–	–	–
Hypophosphatemia	2	1	–	2	1	–
Increased WBC	–	–	–	1	–	–
Insomnia	–	–	–	1	–	–
Intracranial hemorrhage	1	–	–	–	–	–
Rectal fistula	–	1	–	–	–	–
Wound complication	1	–	–	–	–	–

Table 3. Plasma PK parameters in recurrent HGG patients after plerixafor and bevacizumab

	Dose level		
PK parameters[a] average (±SD)	**160 μg/kg ($n = 3$)**	**240 μg/kg ($n = 8$)**	**320 μg/kg ($n = 15$)**
C_{max} (ng/mL)	452 (±56.0)	709 (±162)	974 (±271)
Medium T_{max} (h) (range)	1 (0.5–1)	0.5 (0.5–1.5)	0.5 (0.5–1)
$T_{1/2}$ (h)[b]	6.17 (±1.68)	7.40 (±2.40)	7.07 (±2.42)
AUC_{0-24} (μgxh/mL)	2.52 (±0.300)	4.17 (±0.541)	5.72 (±0.968)
AUC (μgxh/mL)	2.68 (±0.225)	4.66 (±0.671)	6.44 (±1.22)
AUC D_{21}/AUC D_1 ratio	1.28 (±0.242)	1.39[c] (±0.318)	1.54[d] (±0.578)
CL/F (L/h)	4.29 (±0.716)	3.79 (±0.544)	3.91 (±1.03)
Vd/F (L)	40.83 (±15.6)	43.17 (±12.3)	42.28 (±10.70)

[a]Cycle 1, day 1.
[b]Harmonic mean.
[c]$n = 6$.
[d]$n = 11$.

every 2 weeks. The cohort was expanded to ensure a total 12 patients were treated at this dose level. Treatment-related toxicities are summarized in Table 2. One grade 3 hypophosphatemia and one grade 3 rectal fistula were reported. Four patients were taken off study due to unacceptable toxicity: 1 due to the grade 3 rectal fistula, 1 due to a grade 1 stroke, 1 due to grade 2 dysphasia (initially believed related to a TIA by the treating investigator but later deemed unrelated to study treatment), and 1 due to grade 2 intracranial hemorrhage.

Pharmacokinetics

The PK profiles for plerixafor were characterized in 26 patients (Table 3). Dose proportional increases in C_{max} ($R = 0.70$, $P < 0.002$) and AUC ($R = 0.663$, $P < 0.002$) were observed between 160 and 320 μg/kg. Clearance was independent of dose ($R = -0.64$, $P > 0.20$). There was a small but statistically significant difference ($P = 3.0^{e-5}$) between day 1 clearance values (4.0 ± 0.81 L/h) and day 21 (2.9 ± 0.65 L/h) resulting in a 1.5-fold increase in AUC values between days 1 and 21. At the 320 μg/kg dose level, the 24-hour trough levels were consistently above the IC_{50} for CXCR4 inhibition (18).

In Part 1, CSF was collected by LP approximately 2 to 3 hours after patient's last dose on C1D15 and C2D15 with concomitant plasma levels on C1D15 (Fig. 1). At the recommended phase II dose (320 μg/kg), the average CSF concentration was 26.8 ng/mL (SD ± 19.6). There was a good relationship between dose and CSF concentration ($R = 0.76$), but there was no relationship between dose to the CSF/plasma ratio ($P > 0.2$). The CSF levels between C1D15 and C2D15 were comparatively similar (Fig. 1).

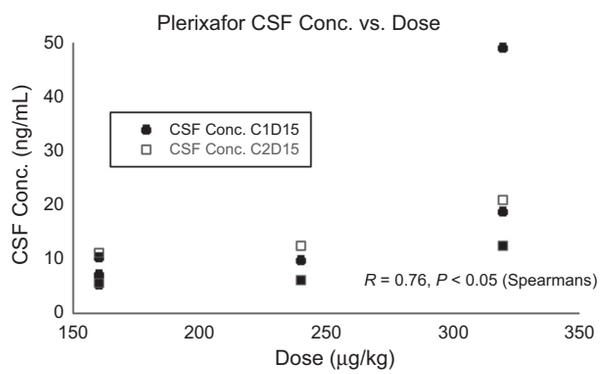

Figure 1.
CSF concentrations of plerixafor in recurrent HGG patients receiving plerixafor plus bevacizumab.

In part 2, patients were pretreated with plerixafor (320 μg/kg) for 5 to 9 days prior to tumor resection. Tumors were resected approximately 3 hours after their last dose with a concurrent plasma level obtained. Data obtained from 2 patients revealed tumor-to-plasma ratios of 15.9 and 16.5, respectively. Adequate tumor tissue was not available for this assessment from the third patient.

Clinical outcomes

For all patients enrolled in Part 1 (nonsurgical cohort), median OS was 7.11 months [95% confidence interval (CI), 5.6–9.2], and median PFS was 2.87 months (95% CI, 1.9–3.8). PFS6 was 14.5% (95% CI, 3.9–32.9). For the GBM subset enrolled in Part 1, median OS was 6.4 months (95% CI, 3.2–7.4), and median PFS was 1.9 months (95% CI, 1.7–6.4). Of the 20 patients with evaluable measurable enhancing tumor in Part 1, 3 patients (all of whom were bevacizumab-naïve at enrollment) had a partial response and 9 patients had stable disease (SD) as their best response. Of the 3 patients in Part 1 who had received prior bevacizumab, only 1 patient (with an anaplastic astrocytoma) achieved SD but progressed after 4 cycles of treatment. Of the 3 patients enrolled in Part 2 (surgical cohort), OS times were 1.78, 8, and 10.3 months, and times to progression were 1.09, 3.2, and 7.43 months, respectively.

Circulating cellular and protein biomarkers

Plerixafor plus bevacizumab treatment induced a significant but transient increase in the fraction of circulating $CD3^+$ lymphocytes (at day 2) and $CD14^+$ monocytes (at day 15) and induced a persistent increase in plasma PlGF and SDF-1α (at all time-points; Table 4). Combination treatment also persistently decreased plasma-free VEGF (unbound to bevacizumab, at all time-points) and transiently decreased plasma Ang-2, sMET, bFGF, and IL8 (Table 4). The other molecular and cellular biomarkers did not show any significant changes after combination treatment. When analyzed for correlation with survival outcomes, a longer PFS correlated with lower sVEGFR1 and IL6 and higher sMET at baseline, greater increases in PlGF (at day 2) and IL6 (at all time-points), and decreases in Ang-1 (at day 60; Table 5). In addition, a longer OS correlated with lower CAIX and higher sMET at baseline, and greater increases in PlGF, IL6, and bFGF (at days 2 and 15; Table 5). For cellular biomarkers, although there was no correlation with PFS, a longer OS correlated with lower

Table 4. Medians and interquartile range for circulating plasma biomarker levels and circulating cell biomarker fractions at baseline and percent changes after plerixafor plus bevacizumab treatment in recurrent HGG patients

Plasma biomarker	Baseline (pg/mL; $n = 23$)	Cycle 1 day 2 (% change, $n = 23$)	Cycle 1 day 15 (% change; $n = 21$)	Cycle 2 day 1 (% change; $n = 19$)	Cycle 3 day 1 (% change; $n = 14$)
VEGF (free)	78.35 (48.17–121.01)	**−70.4 (−80.8 to −51.8)**	**−66.9 (−77.9 to −50.9)**	**−66.9 (−77.9 to −36.3)**	**−64.0 (−79.7 to −51.1)**
***P* value**	N/A	**<0.0001**	**<0.0001**	**<0.0001**	**0.0002**
SDF-1α	1,525.29 (1,256.45–1,671.86)	**31.1 (17.7–41.1)**	**35.8 (28.4–45.4)**	**13.4 (−0.8 to 25.4)**	**15.6 (8.2–21.0)**
***P* value**	N/A	**<0.0001**	**<0.0001**	**0.0082**	**0.0002**
PlGF	42.31 (33.47–49.70)	**13.4 (−2.9 to 39.6)**	**51.3 (31.5–75.4)**	**70.8 (33.2–124.8**	**91.9 (50.2–117.0)**
***P* value**	N/A	**0.00315**	**<0.0001**	**<0.0001**	**0.0001**
Ang-2	1,941.40 (1,368.00–2,300.20)	3.5 (−5.5 to 13.2)	**−9.8 (−36.9 to −3.2)**	−10.3 (−20.3 to 20.4)	−12.7 (−20.7 to 16.7)
***P* value**	N/A	0.2197	**0.004**	0.89	0.587
IL8	5.36 (4.09–12.22)	**−33.6 (−41.9 to −2.1)**	**−19 (−36.9 to −3.2)**	4.9 (−11.1 to 58.6)	1.1 (−10.1 to 12.9)
***P* value**	N/A	**0.0716**	**0.004**	0.0545	0.855
bFGF	23.70 (12.62–53.54)	15.6 (−27.1 to 41.9)	−17.5 (−37.0 to 47.3)	37.3 (−29.0 to 59.5)	−33.8 (−52.5 to −3.6)
***P* value**	N/A	0.477	0.801	0.15	0.1189
sMET	1,194.90 (1,119.30–1,259.90)	0.62 (−5.6 to 5.6)	**−4.8 (−9.7 to −0.9)**	−3.4 (−6.6 to 9.7)	−6.8 (−12.3 to 5.4)
***P* value**	N/A	0.7237	**0.0186**	0.7982	0.426
Cell biomarkers	**Baseline (%PBMC; $n = 23$)**	**Cycle 1 day 2 (% change, $n = 23$)**	**Cycle 1 day 15 (% change; $n = 21$)**	**Cycle 2 day 1 (% change; $n = 17$)**	**Cycle 3 day 1 (% change; $n = 14$)**
$CD14^+$ monocytes	33 (25–44)	10 (−5 to 34)	**29 (2–51)**	−11 (−30 to 23)	7 (−2 to 79)
***P* value**	N/A	0.13	**0.041**	0.45	0.42
$CD3^+$ lymphocytes	30 (14–47)	**34 (8–100)**	47 (−12 to 55)	−2 (−75 to 108)	2 (−23 to 113)
***P* value**	N/A	**0.013**	0.42	1.0	1.0

NOTE: *P* value is from the Wilcoxon Sign-Rank test for percent change after treatment. The numbers in bold represent statistically significant values.
Abbreviation: Ang, angiopoietin.

fractions of $CD34^+$ progenitor cells and NKT cells, and greater increases in $CD8^+$ T cells (at day 2) and decreases in $CD34^+$ progenitor cells (at day 15; Table 5). Tissue analysis in the tumor specimens collected in Part II of this study from patients treated preoperatively with plerixafor showed high expression of CXCR4 and SDF-1α.

Discussion

This is the first study, to our knowledge, evaluating continuous dosing of plerixafor to target CXCR4 as a mechanism of resistance to antiangiogenic therapy in any solid tumors. Treatment at the maximum planned dose of plerixafor (320 μg/kg 3 weeks on, 1 week off combined with standard bevacizumab) was well-tolerated without any DLTs. The PK parameters measured after 1 cycle of plerixafor are consistent with previously published studies in patients with varying degrees of renal impairment (19) and patients with non-Hodgkin lymphoma or multiple myeloma (20). At the 320 μg/kg dose level, the 24-hour trough levels were consistently above the IC_{50} for CXCR4 inhibition (18). CSF studies from Part 1 and surgical resection specimens in patients pretreated with plerixafor from Part 2 demonstrate distribution of plerixafor into the CSF and brain tumor tissue at CXCR4-inhibitory concentrations.

Biomarker evaluations confirmed potential pharmacodynamic changes for the two targeted therapies, including an increase in plasma PlGF and decreases in free VEGF and Ang-2 (as expected after efficient VEGF blockade). Interestingly, dual inhibition of VEGF and CXCR4 induced significant changes in inflammatory biomarkers such as sustained increases in plasma SDF-1α, transient increases in circulating $CD3^+$ lymphocytes and $CD14^+$ monocytes, and decreases in IL8. Of note, chronic bevacizumab + plerixafor treatment did not result in a significant increase in circulating progenitor/stem cells at any time-point. Finally, combination treatment transiently decreased plasma Ang-2 and bFGF (both proangiogenic markers), and sMET (an endogenous inhibitor of the HGF/MET pathway involved in cancer invasion). Several biomarkers and their changes associated with survival outcomes. Interestingly, although high baseline levels of biomarkers of inflammation (IL6) and hypoxia (CAIX) associated with poor PFS and OS, respectively, an increase in these biomarkers on treatment was associated with more favorable outcomes. In a preclinical study in liver cancer, we previously showed that CXCR4 inhibition can prevent the changes in the immune microenvironment that lead to evasion from antiangiogenic therapy (21). In addition, in GBM models in mice, we showed that CXCR4 inhibition decreases cancer cell invasion and vessel cooption without increasing survival with anti-VEGFR agents alone (22). Thus, the effects of CXCR4 inhibition may explain the lack of correlation with a poor outcome for biomarkers usually associated with inflammation and anti-VEGF treatment resistance such as CAIX, IL6, or SDF-1α (which was increased at all time-points).

Our correlative studies also offered new insight into why the combination may have failed to produce better outcomes. High pretreatment plasma sMET (an inhibitor of HGF pathway) and low sVEGFR1 (an inhibitor of VEGF pathway) were associated with longer PFS. In addition, a shorter OS was associated with higher baseline fractions of circulating $CD34^+$ progenitor/stem cells and NKT cells, with an increase in $CD34^+$ progenitor/stem cells at day 15 and a decrease in $CD8^+$ T cells at day 2. HGF/MET and progenitor cells have been linked with hypoxia-induced

Table 5. Plasma and cellular circulating biomarkers associated with PFS and OS at baseline and on treatment after plerixafor plus bevacizumab treatment in recurrent HGG patients

	Pretreatment		Change at day 2		Change at day 15		Change at day 30		Change at day 60	
Biomarker	**PFS**	**OS**	**PFS**	**OS**	**PFS**	**OS**	**PFS**	**OS**	**PFS**	**OS**
PlGF	1.020	1.021	**0.986**	**0.982**	0.986	**0.982**	0.999	0.998	1.001	0.998
***P* value**	0.17	0.099	**0.12**	**0.024**	0.12	**0.024**	0.90	0.74	0.85	0.73
sVEGFR1	**1.004**	1.003	0.999	1.003	1.000	1.000	0.998	0.999	0.999	0.999
***P* value**	**0.039**	0.051	0.87	0.65	0.67	0.50	0.33	0.56	0.49	0.51
bFGF	1.000	1.010	0.993	**0.990**	0.993	**0.990**	0.999	0.999	1.011	1.002
***P* value**	0.96	0.26	0.15	**0.042**	0.15	**0.042**	0.64	0.56	0.074	0.55
Ang-1	1.000	1.000	0.996	1.000	0.998	0.997	0.997	0.997	**1.011**	1.003
***P* value**	0.16	0.86	0.16	0.90	0.21	0.090	0.056	0.062	**0.017**	0.38
sMET	**0.996**	**0.996**	1.035	1.006	1.035	1.006	1.032	1.012	0.997	0.980
***P* value**	**0.041**	**0.043**	0.092	0.81	0.092	0.81	0.17	0.61	0.85	0.33
CAIX	1.001	**1.010**	0.993	0.995	1.005	0.998	0.994	0.993	**0.986**	0.992
***P* value**	0.85	**0.042**	0.27	0.35	0.34	0.76	0.19	0.10	**0.022**	0.15
IL6	**1.064**	1.025	**0.983**	**0.982**	**0.983**	**0.983**	**0.981**	0.997	**0.976**	0.993
***P* value**	**0.025**	0.36	**0.018**	**0.035**	**0.035**	**0.018**	**0.017**	0.70	**0.021**	0.57
$CD34^+$ progenitor/stem cells	2.525	**4.123**	1.000	1.000	1.001	**1.003**	N/A	N/A	N/A	N/A
***P* value**	0.070	**0.0054**	0.91	0.89	0.54	**0.026**	N/A	N/A	N/A	N/A
$CD3^+CD56^+$ NKT cells	0.844	**1.794**	0.999	1.005	1.002	1.001	N/A	N/A	N/A	N/A
***P* value**	0.28	**0.0070**	0.82	0.35	0.63	0.84	N/A	N/A	N/A	N/A
$CD8^+$ T cells	0.963	1.033	0.989	**0.971**	1.002	1.000	N/A	N/A	N/A	N/A
***P* value**	0.14	0.23	0.47	**0.038**	0.83	1.0	N/A	N/A	N/A	N/A

NOTE: Data shown as HR from Cox regression. HR was calculated as the increase/decrease in the chance of progression or death per increase of one unit in the biomarker tested. The numbers in bold represent statistically significant values.
Abbreviations: Ang, angiopoietin; CAIX, carbonic anhydrase 9; NKT, natural killer T cells.

increase in GBM invasion in mouse models (23, 24). sVEGFR1 is a potential biomarker of inherent resistance to anti-VEGF therapy (25). The potential roles of HGF/MET pathway, pretreatment sVEGFR1, and immune evasion mechanisms related to changes in progenitor/stem cells and $CD8^+$ T cells in treatment resistance will need to be established in future studies as these pathways and cells are clinically actionable with existing drugs. These insights will be critical for future combination studies of anti-CXCR4 agents with antiangiogenics or other therapies, such as chemotherapy (26).

The CXCR4 pathway has long been studied and is a target of interest in several human solid and hematologic tumors, including HGG. This is the first clinical trial of a CXCR4 inhibitor with extensive biomarker data in a solid tumor. Combination treatment with bevacizumab and plerixafor was well tolerated, and plerixafor distributed to both the CSF and brain tumor tissue. The biomarker data indicate significant changes in PD biomarkers consistent with anti-VEGF and anti-CXCR4 activity, as well as an association of certain potential biomarkers of treatment response and resistance, which should be validated in larger studies. However, the clinical outcomes in these small number of patients were limited, with median OS and PFS were similar to prior studies of bevacizumab-containing regimens (27).

Disclosure of Potential Conflicts of Interest

D.G. Duda reports receiving commercial research grants from Bayer, Bristol-Myers Squibb, Exelixis, Leap, and Merrimack, and is a consultant/advisory board member for Bayer, Bristol-Myers Squibb, Tilos, and twoXAR. D.A. Reardon is a consultant/advisory board member for AbbVie, Agenus, Bristol-Myers Squibb, Celldex, EMD Serono, Genentech/Roche, Inovio, Merck, Merck KGaA, Monteris, Novocure, Oncorus, Oxigene, Regeneron, Stemline, and Taiho Oncology. A.D. Norden is an employee of Cota Healthcare. T.T. Batchelor is a consultant/advisory board member for Genomicare. R.K. Jain is an employee of Tekla Life Sciences, holds ownership interest (including patents) in Enlight Biosciences, Ophthotech, and SynDevRx, and is a consultant/advisory board member for Merck, Ophthotech, Pfizer, SPARC, and SynDevRx. No potential conflicts of interest were disclosed by the other authors.

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