A follow-up study to add whole brain radiotherapy (WBRT) to standard temozolomide chemo-radiotherapy in newly diagnosed glioblastoma (GBM) treated with 4 weeks of continuous infusion Plerixafor

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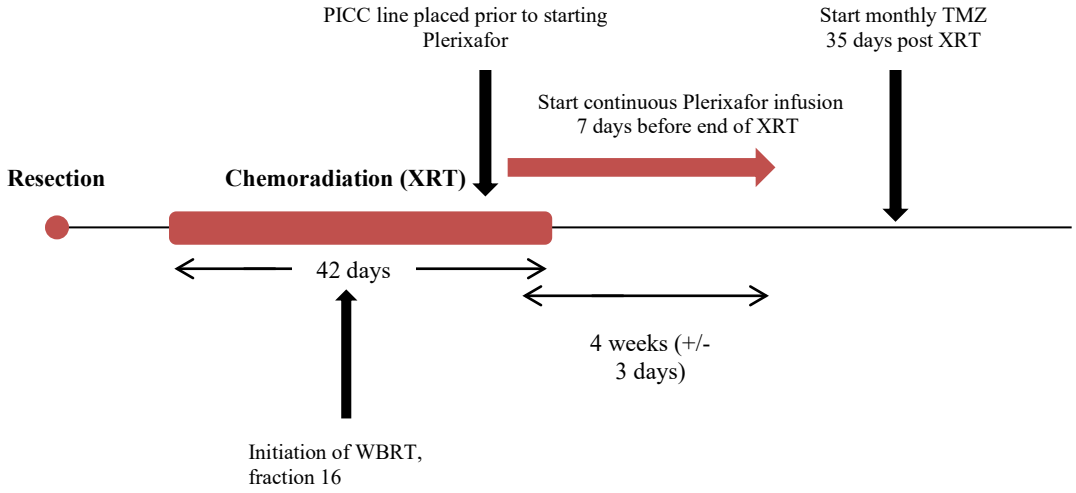
PROTOCOL SYNOPSIS

TITLE	A follow-up study to add whole brain radiotherapy (WBRT) to standard temozolomide chemo-radiotherapy in newly diagnosed glioblastoma (GBM) treated with 4 weeks of continuous infusion Plerixafor
STUDY PHASE	II
INDICATION	Newly diagnosed glioblastoma
INVESTIGATIONAL PRODUCT OR PROCEDURE	Continuous infusion of Plerixafor
PRIMARY OBJECTIVE(S)	Efficacy as measured by progression free survival at 6 months from the start of Chemoradiation
SECONDARY OBJECTIVE(S)	(i) Overall survival (ii) Toxicity (iii) Recurrent tumor patterns of failure
EXPLORATORY OBJECTIVE	Neurocognitive outcomes & Patient-reported health-related QOL
TREATMENT SUMMARY	Patients will be treated within a conventional framework of maximal safe surgical resection followed by a 6 week course of irradiation administered concomitantly with 75 mg/m^2 temozolomide x 42 days with two additions: (i) Plerixafor will be administered at 400 micrograms per kilogram per day for four weeks beginning one week before the end of radiation; and (ii) Whole brain radiotherapy (WBRT) – Radiotherapy consists of standard 30 Gy of IMRT to the tumor bed followed by 30 Gy to the whole brain. Plerixafor infusion will begin during this WBRT component. After completion of Plerixafor infusion, patients will be administered six cycles of monthly TMZ
SAMPLE SIZE	20 patients
STATISTICAL CONSIDERATIONS	For efficacy, a greater than 50% PFS at 6 months will be considered a positive signal and warrant further study. A secondary endpoint will consider a median survival greater than 32 months as a measure of especially meaningful significance.

SCHEMA


PICC line placed prior to starting Plerixafor
Start monthly TMZ 35 days post XRT
Start continuous Plerixafor infusion 7 days before end of XRT
Resection
Chemoradiation (XRT)
42 days
4 weeks (+/- 3 days)
Initiation of WBRT, fraction 16

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ABW	Actual Body Weight
ADL	Activities of daily living
AE	Adverse event
CBC	Complete blood count
CMAX	Maximum concentration of drug
CNS	Central nervous system
CRF	Case report/Record form
CR	Complete response
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
GBM	Glioblastoma
GI	Gastrointestinal
HIV	Human Immunodeficiency Virus
IBW	Ideal Body Weight
IMRT	Intensity Modulated Radiation Therapy
IRB	Institutional Review Board
IV	Intravenous
KPS	Karnofsky Performance Score
MTPI	Modified Toxicity Probability Interval
OS	Overall survival
PLT	Platelet
PD	Progressive disease
PFS	Progression free survival
PR	Partial response
QOL	Quality of Life
RANO	Revised Assessment in Neuro-oncology
RR	Response rate
RT	Radiotherapy
SAE	Serious adverse event
SD	Stable disease
SOC	Standard of care
SLD	Sum of the longest diameter
SPD	Sum of the products of the diameter
TMZ	Temozolomide
TTP	Time to progression
ULN	Upper limit of normal
UNK	Unknown
WBC	White blood cell
WBRT	Whole brain radiotherapy
XRT	Chemoradiation

1. OBJECTIVES

1.1. Primary Objectives

- To assess the 6 month PFS (post initiation of radiation) of continuous infusion Plerixafor beginning one week prior to the end of concurrent chemotherapy with Temozolomide and a modified radiation regimen that includes a component of whole brain radiation therapy (WBRT) in patients with newly diagnosed GBM.

1.2 Secondary Objectives

- To assess the median survival of patients treated with continuous infusion Plerixafor/WBRT
- To assess the toxicities both short and long term of continuous infusion Plerixafor/WBRT.
- To assess the patterns of failure (in and out of irradiated brain field, out of brain) of continuous infusion Plerixafor/WBRT.

1.3 Exploratory Objectives

- To assess the neurocognitive outcomes and patient-reported health-related quality of life after continuous infusion Plerixafor/WBRT.

2. BACKGROUND

2.1 Introduction and Rationale for Follow-up Study of Continuous Infusion Plerixafor in Glioblastoma

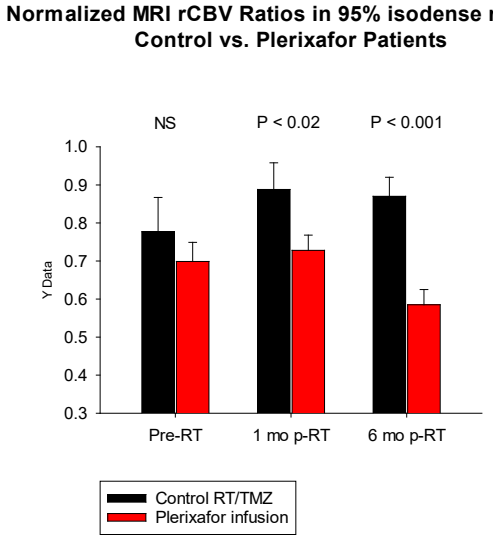
Although radiotherapy delays the recurrence of most glioblastomas (GBMs), these tumors invariably regrow and prove fatal by two years after diagnosis in more than half the patients. Importantly, 80% of first recurrences occur within the field of high dose radiation, underscoring the importance of local control in improving survival [1-4].

Our preclinical work demonstrated that brain cancer recurrence after irradiation resulted in large part through vasculogenesis rather than angiogenesis (which is significantly inhibited by RT). Vasculogenesis, the growth of tumor blood vessels from circulating bone marrow-derived cells, is fueled in large part by SDF-1 (CXCL12), which is secreted by the injured tumor bed and results in recruitment of peripheral monocytes that promote vascularization. As described below, blocking SDF-1 with Plerixafor prevented this repopulation and resulted in cures of several different models of experimental glioblastoma.

In July 2014, with the generous assistance of Sanofi, we launched a Phase I/II study that assessed the effects in newly diagnosed GBM patients of a 4-week continuous infusion of Plerixafor that started one week prior to the end of standard focal radiotherapy (i.e. 6 weeks radiation, 60 Gy to a field encompassing the original tumor bed plus a 2 cm margin). The timing of initiation was chosen based on experimental evidence that SDF-1 levels were highest in the immediate post-RT period. Enrollment criteria allowed us to enroll patients between 18 and 75 years old who only needed biopsy confirmation of diagnosis and whose KPS was 60 or better; criteria that were quite liberal relative to other studies that examined treatments for GBM.

We determined in Phase I that a four-week infusion of 400 μg/kg/day was well tolerated without any Grade 3 toxicities and resulted in a consistent elevation of Plerixafor levels and intravascular myeloid cells. An additional 20 patients were evaluated in the Phase II companion

study. All 29 patients have met their primary and secondary endpoints with the following observations:



Graph 1. Normalized rCBV in tissue receiving 95% of maximum RT in controls and Plerixafor treated patients. Note values for 1 and 6 mo. are significantly lower in the Plerixafor group. Areas outside the 95% field were not significantly different.

(i) We have noted a marked decrease in cerebral blood volume and blood flow as determined by dynamic susceptibility contrast (DSC)-MRI in the irradiated field that extends out to six months after irradiation. This decrease is noted only in the field that received at least 95% of the maximal RT dosage. CBV's outside of this area as well as CBV's within the 95% fields of non-Plerixafor infused treated controls are not impacted (**Graph 1**);
(ii) Our estimated median overall survival as assessed by Kaplan Meier analysis is 20 months (compared to the historical control figure of 14 months), despite the fact that the entry criteria into this study were very liberal (i.e., age up to 75, KPS of 60 or greater), and that there was biopsy only in over 20% of the patients; and;
(iii) While the KM estimate alone is encouraging, it is even more compelling in light of a high incidence of first recurrences occurring out of the irradiated field and in the meninges (10/12 to date compared to a standard expectation that 80% of recurrences will occur within the irradiation field). This is an unprecedented result and suggests that widening the radiation field may be a key element in markedly improving overall survival using this strategy.

These results are promising and suggest that the incorporation of end-of-treatment Plerixafor may represent a "one size fits all" radiation amplifier that may apply not only to GBM but also to other solid cancers. Furthermore, specific to malignant glioma, it leads to the crucial next experiment: given that local tumor control within the irradiation field is seen with Plerixafor, what are the outcomes if the entire brain is included in the irradiation field?

We are therefore proposing to treat an additional 20 newly diagnosed GBM patients with a four-week Plerixafor infusion that will begin one week after a modified irradiation regimen that will be designed to administer whole brain irradiation during the last three weeks of a six week treatment. It is our hope that this increased field will significantly decrease out of field recurrences in the brain and believe that this will markedly increase survival, a result that can be established with even this small cohort. Furthermore, it is important to note that widening the field does not necessarily mean that this will be associated with increased neurotoxicity; we and others have previously shown that the same treatment that blocks macrophages and produces radiation enhancement of tumor response not only produces no enhancement of normal skin and GI tract reactions to irradiation but produces a significant radioprotection including radioprotection against neurocognitive dysfunction [5-7].

2.2 Plerixafor

Plerixafor is a reversible inhibitor of the binding of stromal cell derived factor - 1α (SDF-1α), also known as chemokine (C-X-C motif) ligand 12 (CXCL12) to its cognate receptor chemokine (C-X-C motif) receptor 4 (CXCR4).

The FDA has not approved the drug or biologic for treatment of this indication to date.

2.3 Rationale

Our experimental studies suggest that post-irradiation tumor recurrences can be prevented or markedly delayed by blocking the influx of circulating proangiogenic cells including CD11b+ monocytes and endothelial cells into the tumor [5, 8]. Plerixafor is a reversible inhibitor of SDF-1 binding that prevents or markedly delays the influx of proangiogenic cells thereby preventing post-irradiation tumor recurrences in glioblastoma.

2.4 Study Design

For clinicaltrials.gov and Stanford Clinical Trials Directory compliance

This is an open-label, non-randomized, single-arm trial.

The primary purpose of this Phase II study is to evaluate the efficacy of Plerixafor administered with a modified radiation regimen that includes a component of WBRT. The **primary endpoint** is 6-month progression free survival post initiation of Chemoradiation..

The **secondary endpoints** are the rate of patients surviving 32 months; toxicity of treatment; patterns of treatment failure; and cognitive outcome.

2.5 Correlative Studies Background

Using transplanted glioblastoma tumors in mice, we have developed and validated a new paradigm to enhance the therapeutic effect of radiation: Namely that post-irradiation tumor recurrences can be prevented or markedly delayed by blocking the influx of circulating proangiogenic cells- CD11b+ monocytes and endothelial cells into the tumor [5, 8]. The significance of our findings and therapeutic strategy to prevent GBM recurrence has been highlighted in recent commentaries in prominent biomedical journals [9]. Our findings of the importance of CD11b+ monocytes and/or macrophages in a tumor response to irradiation have now been confirmed by others [10].

Our findings of relevance to the present proposal are summarized as follows:
• We have shown that the clinically approved drug Plerixafor (AMD3100), which inhibits the interaction of stromal cell-derived factor-1 (SDF-1, CXCL-12) with its receptor, CXCR4, on bone marrow derived, proangiogenic, CD11b+ monocytes [11] both inhibits the radiation-induced influx of the CD11b+ monocytes and prevents tumor recurrence following single or fractionated doses of irradiation **(Figure 1A,C).** We have confirmed the importance of the SDF-

1/CXCR4 pathway for tumor recurrences by demonstrating that neutralizing antibodies to CXCR4 also inhibit tumor recurrence after irradiation **(Figure 1D).** In addition we have shown that neutralizing antibodies to CD11b+ monocytes can inhibit the recurrence of a human head and neck cancer in nude mice, as well as demonstrating that tumors in mice genetically deficient in CD11b+ cells are radiosensitive [8].

• We have demonstrated that blockage of angiogenesis following irradiation with the anti-VEGFR2 neutralizing antibody DC101 is not as effective in potentiating the response to irradiation as is AMD3100 either by tumor recurrence or by inhibition of tumor blood flow following irradiation **(Figure 1E, F).**

• We have shown that radiation selectively depletes the tumor vasculature thereby inducing tumor hypoxia and upregulating the transcription factor hypoxia inducible factor-1 (HIF-1), which in turn trans activates SDF-1, the key chemokine responsible both for the mobilization of bone marrow derived proangiogenic cells and their retention in the irradiated tumor. [5, 8].

• We have demonstrated the importance of CD11b+ monocytes in tumor recurrences after irradiation in patients by showing that, as in mice, GBM recurrences in patients following Irradiation have higher levels of CD11b+ monocytes than prior to therapy **(Figure 2).**

• We have demonstrated that irradiated tumors express elevated levels of SDF-1 and this Chemokine is found at higher levels in the plasma of rats and patients with irradiated Brain tumors.

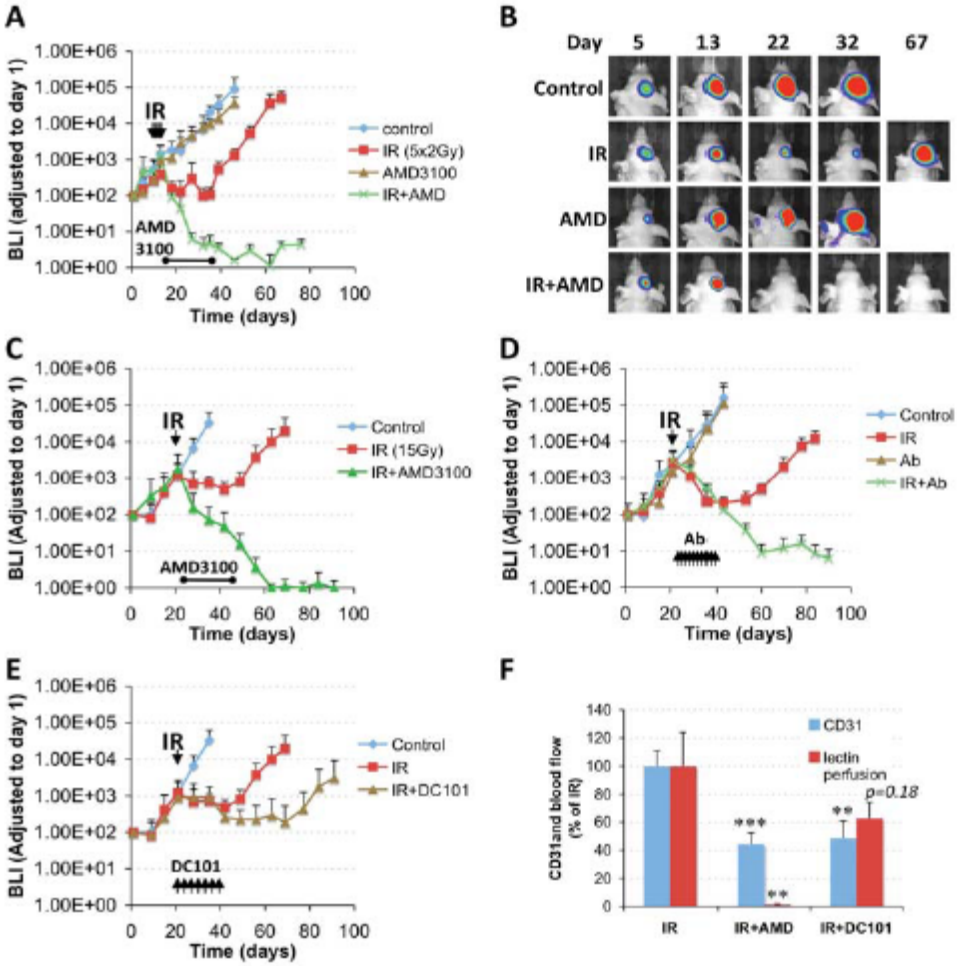


Figure 1. Inhibition of the interaction of SDF-1 with CXCR4 with AMD3100 prevents tumor recurrence post irradiation. A Growth curves of i.c. U251 by bioluminescent imaging (BLI) following whole brain fractionated irradiation (5 daily doses of 2 Gy starting on day 11 after transplantation) with AMD3100 (5mg/kg/day for 21 days) started immediately after the last irradiation, **B**. BLI images following fractionated irradiation (5 x 2 Gy) treated with or without AMD3100. (**CD**) Growth curves of i.c. U251 following a single dose of irradiation (15 Gy on day 22 following transplantation) treated with AMD3100 (21 day infusion); **C**) or treated with neutralizing anti-CXCR4 Ab (**D**), starting immediately following irradiation. **E,** growth curves of U251 i.c. tumor following 15 Gy irradiation treated with DC101. Arrow-heads indicate the treatment of DC101 (started immediately following irradiation and maintained for 21 days). **F,** AMD3100 is more effective than DC101 in reducing tumor blood flow by irradiation. Quantification of endothelial cells and functional vessels in U251 s.c. tumors following 15 Gy irradiation and combined with AMD3100 or DC101. Samples were taken 17 days after irradiation. Errors indicates S.E.M. **p<0.01, ***p<0.001 (*versus* IR). From Kioi et al [12]

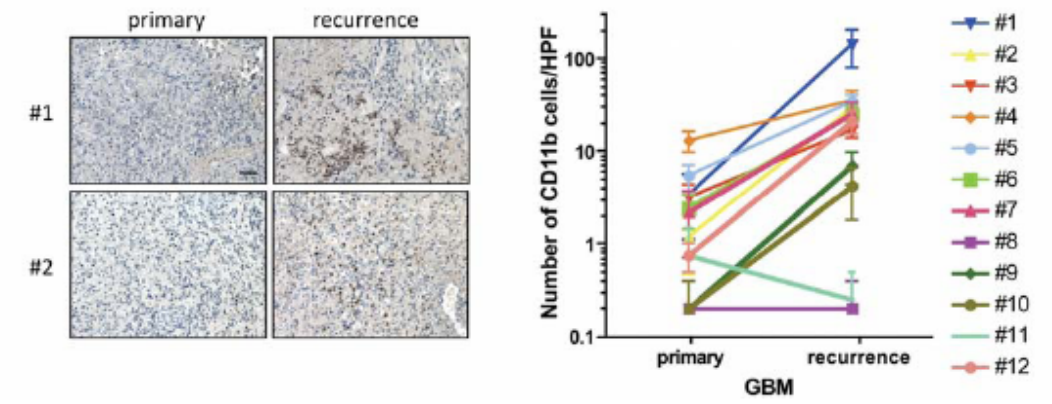


Figure 2. CD11b+ cells are increased with GBM tumor recurrences in patients., Left: IHC staining for CD11b of GBM clinical samples both before treatment (primary) and after recurrence **Right** Significant increased levels of CD11b+ cells in the recurrent human GBMs compared to the untreated tumors. Quantification of CD11b based on IHC with CD11b staining. Ten of twelve samples showed increases of CD11b cells in recurrent GBMs (Error SEM). Bar; 50 μm. From Kioi et al[12]

Because the CXCR4 serves as a co-receptor, along with CD4, for the binding of human immunodeficiency virus type 1 (HIV-1) [12], initial Plerixafor clinical trials were conducted for the treatment of HIV-1 infection [13]. In follow-up to the observation that Plerixafor given to healthy volunteers and HIV-1 infected patients elicited increases in white blood cell counts, studies have been done in healthy volunteers to assess the effect on circulating peripheral blood stem cells (PBSCs) [14]. Coincidentally, data emerged demonstrating that homing of CXCR4-expressing stem cells to bone marrow is regulated, at least in part, through a chemoattractant effect of SDF-1α that is produced locally by bone marrow stromal cells [15]. In fact, disruption of the SDF-1α/CXCR4 through G-CSF exposure [16] or with chemotherapy, results in the appearance of both mature and pluripotent cells in the systemic circulation. Unlike the modes of action of either chemotherapy or cytokine growth factors, however, Plerixafor exerts its effect on PBSC mobilization as a direct consequent of its antagonism of CXCR4 [17]. Subsequent PBSC mobilization studies have shown Plerixafor to have a synergistic effect on the number of circulating progenitor cells when administered with G-CSF in patients with non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) [18, 19].

Pharmacokinetics of Plerixafor

The kinetics of dosing of Plerixafor was first explored in humans in a phase I bioavailability study in 17 healthy volunteers; 12 by intravenous (IV) infusion (three subjects each at 10, 20, 40, and 80 μg/kg), 5 by subcutaneous (SC) injection (two subjects at 40 μg/kg and three at 80 μg/kg) [20]. In this study, the C_{max} and $AUC_{0-\infty}$ demonstrated dose proportionality across the four dose levels. However, a higher Cmax for IV administration was noted compared to SC administration (IV: 292.8 ± 67.0 and 503.9 ± 29.6, SC: 123.5 ± 27.9 and 238.3 ± 17.3) for the 40 and 80 μg/kg dose levels, respectively. The bioavailability of Plerixafor was determined to be 80-90%. The pharmacokinetic behavior of Plerixafor is characterized by elimination from the plasma in a bi-exponential manner with a terminal elimination half-life of approximately 3.5-5 hours following a single dose. Plerixafor absorption following subcutaneous administration is rapid and essentially complete, with peak plasma levels occurring within 0.5–1

hour of dosing. The exposure-response relationship of Plerixafor in mobilizing CD34 + cells when administered as a single agent was also independently explored at doses ranging from 80-320 μg/kg in 32 healthy volunteers [21] and from 40–320 μg/kg in 29 additional healthy volunteers [22]. In both studies, Plerixafor exhibited linear pharmacokinetics (PK) over the tested dose range (up to 320 μg/kg), consistent with previously reported PK results. Plerixafor is extensively protein bound to both human serum albumin and 1-acid glycoprotein; however, protein binding does not appear to have a major influence on either antiviral activity, effect on stem cell mobilization or toxicity. Saturation of protein binding sites may occur at plasma Plerixafor concentrations in excess of those likely to be achieved in any ongoing or planned clinical studies.

The safety and the pharmacokinetics and pharmacodynamics of Plerixafor with G-CSF in patients with non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) was also evaluated in a phase II, open-label, single-arm study [23]. The patients were given G-CSF (10 μg/kg/day SC) for 4 days in the morning and Plerixafor 240 μg/kg SC on the evening before each day of apheresis. The PK profile of Plerixafor was characterized in 13 patients (5 with NHL and 8 with MM) and, overall, parameters were comparable in the patients with NHL and those with MM. Plerixafor was rapidly absorbed after SC administration with no observable lag time, with peak plasma concentrations occurring 0.5 hour after administration in most patients. Plerixafor was rapidly cleared, with a median terminal half-life of 4.6 hours. The median maximum increase in the number of circulating cells from baseline was 4.2-fold (range, 3.0- to 5.5-fold); with the maximum fold increase occurring approximately 10 hours after Plerixafor injection for all patients. The Plerixafor PK and PD profiles in the study patients were consistent with those in healthy volunteers and support the current dosing regimen and timing of apheresis.

The primary route of elimination of Plerixafor is through the kidneys. A Phase I open-label study in healthy subjects was conducted to evaluate the pharmacokinetic characteristics of Plerixafor in subjects with renal impairment [24]. All subjects received a single 240 μg/kg subcutaneous dose of Plerixafor. Subjects were stratified into 4 cohorts based on creatinine clearance determined from a 24-hour urine collection: control (>90 mL/min), mild renal impairment (51-80 mL/min), moderate renal impairment (31-50 mL/min), and severe renal impairment (<31 mL/min, not requiring dialysis). Eleven women (48%) and 12 men (52%), ranging in age from 35 to 73 years, were enrolled. Plerixafor clearance was reduced in subjects with renal impairment and was positively correlated with creatinine clearance. The mean area under the concentration-versus-time curve from time 0 to 24 hours post dose of Plerixafor in subjects with mild, moderate, and severe renal impairment was 7%, 32%, and 39% higher, respectively, than that in subjects with normal renal function. Renal impairment had no effect on maximal plasma concentrations. The safety profile was similar among subjects with renal impairment and controls. No renal impairment-related trends in the incidence of adverse events (AEs) were apparent. A Plerixafor dose reduction to 160 μg/kg in patients with a creatinine clearance value <or= 50 mL/min is expected to result in exposure similar to that in patients with normal to mildly impaired renal function, and became the basis for this dose recommendation in the FDA approved indication in NHL and MM, when added to G-CSF for mobilization.

In a phase I trial evaluating single dose IV Plerixafor in healthy donors for stem cell harvest and use in allogeneic transplant, pharmacokinetic evaluation demonstrated that C_{max} following the 320 μg/kg IV dose remained below 1.0 μg/mL whereas 400 μg/kg (N = 3) and 480 μg/kg (N = 3) doses resulted in C_{max} levels of 1.8-2.2 μg/mL.

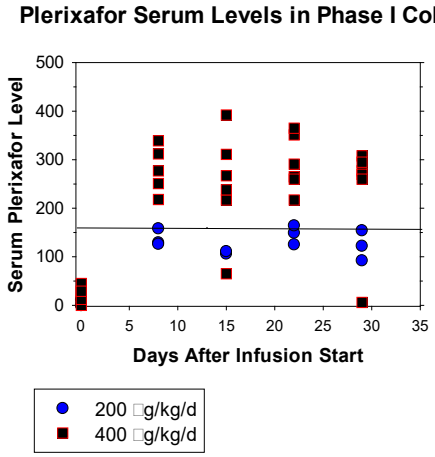


Figure 3.

Finally, chronic administration of Plerixafor has also been evaluated by continuous infusion for 10 days in HIV patients in an open-label dose escalation study with doses ranging from 2.5 to 160 µg/kg/h [25]. In this study, a 10-day infusion was administered via infusion pump at 40 mL/hour (daily solutions prepared by dilution of a 10 mg/mL solution of Plerixafor in 0.9% saline). **Note that the total dose infused in this study was much higher than those doses proposed here (i.e., the highest dose over 4 weeks for our protocol will be 11,200 mcg/kg compared to 38,400 mcg/kg total for the highest cohort in the cited study).** The median terminal elimination half-life was 8.6 hours (range: 8.1-11.1 hours). Cardiac toxicities were observed in the HIV+ patients dosed to achieve C_{max} levels above 2 µg/mL.

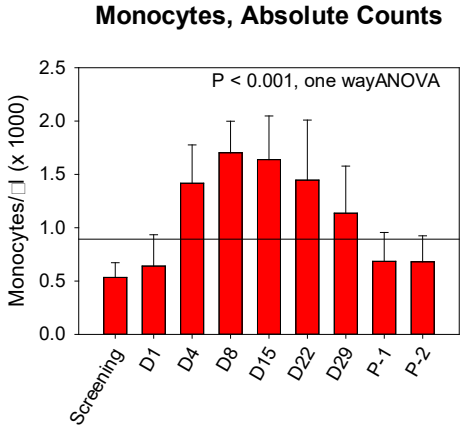


Figure 4. Sustained elevation of monocyte counts during Plerixafor infusion.

Our own studies in the Phase I component of this study's predecessor indicate that a four week infusion of Plerixafor at 400 µg/kg/d is well tolerated and achieves the target value necessary to ensure adequate bone marrow mobilization and prevent tumor entry (**Figure 3**). Furthermore, we noted a sustained elevation of monocytes that was sustained through the entire infusion, consistent with their being maintained in the circulation (**Figure 4**).

<u>Plerixafor for Stem Cell Mobilization</u>

Plerixafor is a bicyclam small molecule that selectively and reversibly inhibits CXCR4. In preclinical and clinical studies, it was found to lead to a rapid increase in circulating hematopoietic progenitor cells and mature lymphocytes.

In a phase I clinical trial conducted in healthy volunteers, a single dose of Plerixafor by SC injection (160 or 240 µg/kg) given alone or added to a mobilization regimen of daily G-CSF (10 µg/kg) for four days was shown to be generally safe and well-tolerated, as compared to a mobilization regimen consisting of G-CSF alone [26]. The most frequently reported AEs were injection site reactions, GI effects, paresthesias, and headaches. Plerixafor augmented CD34+ cell mobilization by G-CSF on average 3.8 fold. More recently, the safety of Plerixafor administered as a single agent by injection was further explored in healthy volunteers at doses up to 480 µg/kg [27]. No dose limiting toxicity was observed, and common adverse events were diarrhea, injection site erythema, perioral numbness, sinus tachycardia, headache, nausea, abdominal distention and injection site pain.

Similar to the experience in healthy volunteers, phase I evaluation of a single injection of Plerixafor (160 or 240 µg/kg) given to 13 cancer patients (MM, n=7; NHL, n=6) was well tolerated and only grade 1 toxicities were observed [28]. A rapid and statistically significant increase in the total WBC and PB CD34+ counts at both 4 and 6 hours following a single injection were noticed. The absolute CD34+ cell count increased from a baseline of 2.6 +/- 0.7/

μL (mean +/- SE) to 15.6 +/- 3.9/ μL and 16.2 +/- 4.3/ μL at 4 hours (P=.002) and 6 hours after injection (P =.003), respectively. The absolute CD34+ cell counts observed at 4 and 6 hours following Plerixafor were higher in the 240 μg/kg group (19.3 +/- 6.9/ μL and 20.4 +/- 7.6/ μL, respectively) compared with the 160 μg/kg group (11.3 +/- 2.7/ μL and 11.3 +/- 2.5/ μL, respectively).

Plerixafor was then studied for hematopoietic stem cell mobilization coupled with G-CSF for autologous stem cell transplantation. In a phase II, open label, crossover study in 25 patients with NHL and MM, patients received 3 days of G-CSF run-in, and then underwent mobilization with one regimen of either: (A) up to 4 days of 10 μg/kg of G-CSF or (B) up to 4 days of 10 μg/kg of G-CSF plus 160 μg/kg of Plerixafor [29]. Patients were apheresed one hour after the dose of G-CSF alone or 6 hours after the morning G-CSF plus Plerixafor dose for up to 4 days to achieve a target of 5 x 10^6 cells/kg. After a rest period, patients received 3 days of G-CSF run-in, followed by the opposite regimen (A after B or B after A) and were apheresed in the same manner. The purpose was to determine safety, apheresis yields, and transplantation success. After the initial 8 patients were dosed at 160 μg/kg, the protocol was amended to increase the G-CSF run-in from 3 to 4 days, and the Plerixafor dose to 240 μg/kg. Later, the protocol was further amended such that the G-CSF alone regimen was always used first. There was no drug-related SAE or unexpected AE. More patients achieved ≥5 × 10^6 CD34+ cells/kg after mobilization with Plerixafor plus G-CSF compared to G-CSF alone. Nine patients (8 NHL and 1 MM patient) who mobilized CD34+ cells poorly with G-CSF alone (<1.6 × 106 CD34+ cells/kg) improved when mobilized with Plerixafor plus G-CSF, with all patients achieving >2 × 106 CD34+ cells/kg (range: 2.78 to 13.6 CD34+ cells/kg). The median day of polymorphonuclear leukocyte (PMN) engraftment was Day 10 and Day 17 for platelets, when using cells collected by Plerixafor plus G-CSF. Durability of engraftment has been measured up to one year.

Two phase III, multi-center, randomized, double-blind, placebo-controlled, comparative trials examined the ability of Plerixafor (240 μg/kg) plus G-CSF (10 μg/kg) vs. placebo plus G-CSF (10 μg/kg) to mobilize CD34+ stem cells for autologous hematopoietic stem cell transplantation in patients with NHL (protocol 3101) and MM (protocol 3102), respectively. Patients were excluded if they previously attempted stem cell mobilization or received a prior stem cell transplant.

In protocol 3101, the addition of Plerixafor to a G-CSF regimen significantly increased the proportion of patients with NHL who were able to mobilize minimum (2 x 10^6 cells/kg) and target (5 x 10^6 cells/kg) numbers of CD34+ cells for autologous transplant and allowed both targets to be reached in significantly fewer apheresis days [30]. In 3102, the addition of Plerixafor to a G-CSF regimen, compared with G-CSF alone, significantly increased the proportion of patients with MM who were able to mobilize the target (6 x 10^6 cells/kg) number of CD34+ cells needed for autologous transplant and allowed this target to be reached in significantly fewer apheresis days [31]. In both trials, hematopoietic stem cells mobilized with Plerixafor + G-CSF were equally capable of prompt and durable PMN and PLT engraftment, compared to cells mobilized with G-CSF alone.

In the controlled Phase III studies in patients with NHL and MM (3101 and 3102), a total of 301 patients were treated in the G-CSF plus Plerixafor 240 μg/kg SC group and 292 patients were treated in the G-CSF plus placebo group. The safety profile of Plerixafor was consistent with that observed in previous mobilization studies and adverse events that occurred more frequently with Plerixafor than placebo were: insomnia, headache, dizziness, diarrhea, nausea, flatulence, abdominal pain, vomiting, abdominal distention, dry mouth, stomach discomfort,

constipation, dyspepsia, hypoesthesia oral, arthralgia, musculoskeletal pain, hyperhidrosis, erythema, injection site reactions, fatigue, and malaise [30, 31]. Overall, the AE data, combined with the laboratory and vital sign findings, indicate that Plerixafor 240 μg/kg, in conjunction with G-CSF for the mobilization and collection of CD34+ cells, is well-tolerated in patients with NHL or MM undergoing autologous stem cell transplant. No notable differences in the incidence of AEs were observed across treatment groups from chemotherapy/ablative treatment through 12 months post-transplantation.

Plerixafor has been studied in over 2000 human subjects in over 78 clinical trials, which have encompassed healthy volunteers, HIV infected patients, multiple myeloma patients, lymphoma patients, and patients with a variety of other malignancies. Mozobil® (Plerixafor injection) has been approved by the FDA in combination with G-CSF to mobilize hematopoietic stem and progenitor cells (HSPCs) for collection and subsequent autologous transplantation in patients with non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) based on phase III studies. Several reports have recently indicated that Plerixafor can also be safely administered with G-CSF in the context of a chemotherapy-based mobilization regimen [32-36]. Finally, based on favorable pharmacokinetic observations in healthy volunteers, the impact of intravenous Plerixafor in stem cell mobilization for cancer patients is now under investigation. In a phase I/II study, escalating doses of intravenous Plerixafor (up to 400 μg/kg) alone or added to G-CSF were administered to 25 patients with NHL (n=15) or HL (n=10). In the phase I portion of the study, one dose-limiting toxicity (grade 2 chest pain) was observed at 320 μg/kg and no grade 3/4 toxicities occurred at 400 μg/kg. A total of 24 of 25 patients (96%) met the goal collection of ≥ 2.0 x 106 CD34+ cells/kg and 21 of 25 patients (84%) collected ≥ 5.0 x 10^6 CD34+ cells/kg in a median 1 day of pheresis, including 6 of 6 patients in the 400 μg/kg cohort.

Impact on Graft Composition and Transplant Outcomes

Although collectively referred to as CD34+ progenitor or stem cells, the full repertoire and relative abundance of each cell type collected during PBSC harvest is thought to be governed by selection of the mobilization regimen. The relative importance of blood graft composition on hematopoietic reconstitution following autologous transplant has been recently reviewed [37]. A number of small studies indicate that cells mobilized with Plerixafor are phenotypically distinct from those derived through other mobilization approaches. In macaque, for instance, gene and micro-RNA expression profiling of Plerixafor-mobilized CD34(+) cells include more B-, T-, and mast cell precursors, whereas G-CSF-mobilized cells have more neutrophil and mononuclear phagocyte precursors [38]. When evaluated in both healthy donors and lymphoma patients, Plerixafor alone mobilizes more precursors of the plasmacytoid dendritic cell (pDC) lineage, relative to mobilization with G-CSF + Plerixafor or G-CSF alone . Authors hypothesize that stem cell products enriched in pDCs may lead to improved immunity in the recipient after transplant and reduced incidence of CMV. The impact of Plerixafor on DC graft composition was corroborated in MM and NHL patients mobilized according to the approved indication. In terms of DC subsets, grafts mobilized with P+G contained similar % of myeloid (MDC, Lin-CD11c+HLA-DR+CD123-) and BDCA3+ DCs. The percentage of plasmacytoid DCs (PDC; CD123+BDCA2+HLADR+) was significantly increased in the P+G grafts (median, 0.87% vs. 0.30%; p=0.002), leading to a significantly higher PDC/MDC ratio in the P+G group, 2.08 vs. 1.01, p<0.0001). It was also found that there were significantly more CD8+ IFN-gamma and TNF-alpha secreting T cells in the P+G group as compared to the G group (median, 12.3% vs. 5.3%, p=0.01; and 5.9% vs. 2.8%, p=0.02, respectively). Again, more

pDC cells, as well as CD34+CD45RA-CD123hi cells of unknown function, were also noted when Plerixafor was given intravenously at high doses with G-CSF, relative to G-CSF alone. Lastly, the addition of Plerixafor to G-CSF not only potentiates CD34+ peripheral stem cell yields, but also significantly increases the proportion of more primitive CD34+ CD38- subsets relative to G-CSF alone mobilization [39-41], the latter speculated to potentially promote superior engraftment after high-dose chemotherapy [42].

The functional consequence of qualitative differences in graft composition emerging from inclusion of Plerixafor in stem cell mobilization is not known. Human progenitor cells mobilized with Plerixafor were shown to more robustly repopulate NOD/SCID recipient mice, relative to cells derived through G-CSF mobilization in the same donors [43]. In the clinic, a higher median of absolute lymphocyte counts harvested through addition of Plerixafor to G-CSF mobilization compared with a control group mobilized with G-CSF alone (4.16×10^9 lymphocytes/kg vs. 0.288×10^9 lymphocytes/kg; $P < 0.0001$) correlated with better outcomes in progression-free survival after autologous transplant in NHL patients [44]. With a median follow-up of 20 months (range, 4-24 months), no relapses were reported in the AMD3100 (Plerixafor) group compared with 15 of 29 in the control group ($P < 0.02$). Despite various studies pointing to Plerixafor impacting the graft composition, meaningful differences in engraftment from cells mobilized with Plerixafor + G-CSF *vs* placebo + G-CSF were not apparent in either the NHL [30] or MM [31] phase III trials. In fact, absolute CD34+ cell dose transplanted, rather than qualitative differences that may have resulted from either mobilization approach, was associated with better long-term platelet recovery after ASCT in those trials [45].

Leukemia Stromal Interactions

Similar to normal hematopoietic stem cells, leukemic blasts express many of the same adhesion molecules such as CXCR4, VLA-4, VLA-5, and CD44 which allow them to interact with the marrow stroma [46-48]. The interaction of leukemic blasts with the marrow microenvironment is postulated to be important in mediating disease resistance, a process commonly referred to as cell-adhesion mediated drug resistance (CAM-DR) [49] which eventually promotes relapse. CAM-DR can provide protection from cell cycle-dependent chemotherapy through induction of quiescence of tumor cells, as well as through exposure to SDF-1α which activates the prosurvival PI3K/Akt and MAPK pathway, preventing apoptosis in cancer cells. The role of the SDF-1α/CXCR4 axis in mediating CAM-DR in malignancies is currently under investigation and provides a rationale for evaluating the potential activity of Plerixafor in chemo sensitization.

Pre-Clinical Studies of Chemo sensitization with Plerixafor

In preclinical models of leukemia, targeting the microenvironment with CXCR4 antagonists was sufficient to overcome resistance to cytarabine [50] and also provided responsiveness to antibody-mediated cytotoxicity [51]. Similarly, the addition of Plerixafor in a mouse model of APL was able to enhance the efficacy of cytarabine therapy compared with mice leukemic treated with cytarabine alone, which resulted in reduced tumor burden and improved survival [52]. The median overall survival for the untreated control, Plerixafor alone, cytarabine alone, and cytarabine + Plerixafor cohorts were 18, 19, 23 and 30 days, respectively (cytarabine *vs* cytarabine + Plerixafor cohorts: $p < 0.0006$). A survival advantage was also noted in two xenograft models of ALL exposed to a CXCR4 antagonist followed by chemotherapy

(vincristine or nilotinib), compared to chemotherapy alone [53]. Plerixafor alone had no detectable anti-tumor effect in these experiments.

In BCR-ABL(+) leukemia (CML), Plerixafor was able to inhibit tumor cell chemotaxis and confer added sensitivity to the tyrosine kinase inhibitors Imatinib and Nilotinib [54]. Using a functional mouse model of progressive and residual disease of CML, Plerixafor was also able to mobilize leukemic cells *in vivo*, such that when added to nilotinib, the leukemia burden in mice was significantly reduced below the baseline level suppression achieved by nilotinib alone [55]. Overall, these results support the notion that CXCR4 inhibition in conjunction with targeted tyrosine kinase therapy may overcome drug resistance in CML and potentially suppress or eradicate residual disease.

Disrupting the interaction of tumor cells to bone marrow niches also confers added sensitivity to therapy in multiple myeloma (MM). In a xenograft model, bortezomib-treated mice showed reduction in tumor progression compared with control (P = .041), and the mice treated with the combination of Plerixafor and bortezomib showed significant tumor reduction compared with control (P = .001) and bortezomib alone (P = .021) [56]. Tumor involvement in different organs was also evaluated in the treated groups. The Plerixafor alone group was similar to that of the control group in the BM, liver and spleen, indicating that mobilization of MM cells by Plerixafor does not lead to engraftment of MM cells into extra medullary sites. However, there was a significant decrease of tumor cells present in BM, liver and spleen in the bortezomib-treated group, and a significant decrease was further obtained in the group treated with the combination of Plerixafor and bortezomib [56].

Collectively these data suggest a pivotal role for the CXCR4/SDF-1 axis in sustaining viability of hematologic malignancies through interaction with the marrow microenvironment and provide a basis for evaluating Plerixafor as sensitization agent in the clinic.

<u>Clinical Experience with Plerixafor for Sensitization to Leukemia Treatment</u>

Elevated levels of CXCR4 expression on leukemic cells are associated with worse outcomes including shorter overall survival in AML [57-59]. Plerixafor has been shown to mobilize leukemic cells in humans [60] and was first reported to be used for sensitization in combination with reinduction chemotherapy in an AML patient who had relapsed from prior allogeneic transplant [61].

Formal clinical trial evaluation of Plerixafor given prior to salvage chemotherapy in relapsed or refractory AML patients has been evaluated in a phase I/II study [62]. A test dose of Plerixafor was administered SC followed by a 24 hour observation period to analyze its effects on AML blasts in the absence of chemotherapy. Plerixafor was then given 4 hours prior to MEC chemotherapy (mitoxantrone 8 mg/m^2/d, etoposide 100 mg/m^2/d and cytarabine 1,000 mg/m^2/d) daily for 5 days.

Forty patients have been enrolled in the study with median age of 49 yrs. (range 19-71). Baseline characteristics include 6 patients (15%) with secondary AML, 4 (10%) with prior transplant, 24 (60%) with intermediate and 10 (25%) with poor risk cytogenetics. Thirty-six patients (90%) received Plerixafor + MEC as their 1st salvage regimen for relapsed disease with 21 (53%) having a CR1 duration of < 12 months and 9 patients (6%) for primary refractory disease. The remaining four patients (10%) received the regimen as their 2nd salvage regimen. Three dose levels of Plerixafor: 80 , 160 and 240 µg/kg were tested in the phase I dose escalation. In the phase II, a total of 34 patients have been treated at the 240 µg/kg dose level. Common grade ≥3 adverse events consisted primarily of cytopenias and infections. No evidence

of hyper leukocytosis or significant delays in neutrophil recovery (ANC >500/mm3, median 27d, range 21-37) or platelet recovery (plt >50k/mm3, median 26d, range 20-40d) were observed. Of the 32 patients evaluable for response at the 240 µg/kg dose level, a complete remission (CR+CRi) has been achieved in 50% of patients (CR=13, CRi=3) which compares favorably to historical CR rates of 25-35%. Treatment failure was due to persistent disease in 14 patients (44%) and early death due to complications from infection in 2 patients (6%). One year KM estimate of overall survival is currently 56%.

Correlative studies demonstrated that Plerixafor mobilizes AML blasts (mean 2.5-fold increase, range 0.9-7.3 fold) into the peripheral circulation peaking at 6-8 hours after administration. FISH performed in patients with informative cytogenetic abnormalities indicates that mobilization occurs equally in both non-leukemic and leukemic populations. Higher baseline surface CXCR4 expression correlated with increased mobilization of AML blasts (Pearson's r=0.53, p=0.023) into the PB at 6 hrs. post-Plerixafor. It was concluded that Plerixafor can be safely administered in combination with cytotoxic chemotherapy in patients with AML [62].

A phase I study is also being conducted to determine the MTD and safety of Plerixafor when combined with cytarabine and daunorubicin (7+3 regimen) for newly diagnosed adult AML [63]. Plerixafor was given as a 30-min IV infusion, 4–5 hours before daunorubicin beginning on day 2 and repeated every day until day 7. Dose levels were from 240, 320, and 400 to 480 µg/kg. Three to 12 evaluable patients were enrolled in each cohort in a modified 3+3 design. Twenty-three patients (median age 57 years) have been enrolled in 4 cohorts. Plerixafor infusion on day 2 caused a rise in PB AML blasts (mean 3.01-fold increase) peaking at 2–4 hours after administration. On day 7, there was a mean 1.51-fold increase in PB AML blasts but far fewer total cells were detected.

Eighteen (86%) patients experienced adverse events (AEs) that were reported as at least possibly related to Plerixafor. The majority was grade 1/2 in severity and mainly included gastrointestinal disorders. Four (19%) patients experienced Grade 3 Plerixafor-related AEs including febrile neutropenia (n=3), neutropenia (n=1), nausea (n=1), infections (n=2) and decreased appetite (n=1) commonly observed with 7+3 regimen. One (5%) patient (480 µg/kg cohort) experienced Grade 4 related AEs of thrombocytopenia and asymptomatic pulmonary embolism (while receiving medroxyprogesterone); the latter was the only possibly-related SAE reported. The median time to neutrophil ($\geq 0.5 \times 10^9$/L) and platelet ($\geq 100 \times 10^9$/L) recovery for responders was 19.5 (range 13–35) and 21 (range 17–37) days, respectively. There were 4 (17%) Plerixafor unrelated deaths (240 µg/kg): 1 within 30 days post induction due to an AE of acute respiratory distress syndrome and 3 due to disease progression > 3 months post induction. No DLTs have been reported.

Of 21 patients with available data, 14 (67%) had complete response (CR), 2 had CR with incomplete count recovery (CRi), 2 had residual leukemia (RL), 2 had treatment failure (TF) due to resistant disease and 1 was not evaluable (NE) due to early death. Sixteen of 21 patients, majority of who had intermediate or poor risk cytogenetics, achieved a CR or CRi, with responses observed across all Plerixafor doses. Twice daily Plerixafor dosing and addition of G-CSF to augment mobilization are being currently explored.

Plerixafor is also being investigated as sensitization agent to conditioning chemotherapy in AML and MDS patients undergoing allogeneic transplantation [48]. In this Phase I/II study, G-CSF is administered at a standard dose beginning on day -9 daily for 6 days, and Plerixafor from day -7 at one of the 4 dose levels 0 (control), 80, 160, or 240 µg/kg, 8 hours prior of each

four daily doses of a standard preparative regimen consisting of 40 mg/m^2 Fludarabine and 130mg/m^2 IV Busulfan, days -6 through -3.

To date, twenty seven patients have been enrolled in the study to date with a median age of 48 years (range 25-65). Baseline characteristics include 13 patients (48%) with de novo AML, 6 (22%) with secondary AML, 5 with MDS and 3 with CML. Among the 24 AML/MDS patients, 14 (58%) had intermediate and 10 (42%) poor risk cytogenetics. Twelve patients (50%) had primary refractory AML, 5 were in 1st or 2nd relapse, 2 were untreated, and 3 were in CR1 and 2 in CR2. The source of stem cells was sibling donor in 16 and unrelated donor in 11. After phase I Plerixafor dose escalation in 16 patients, 11 patients received 240 µg/kg in Phase II. Common grade ≥ 3 adverse events which consisted primarily of neutropenic fever, infections, or rash were seen in 24/27 (89%) patients. There were no toxicities ascribed to the G-CSF/Plerixafor component of the regimen. No evidence of significant delays in neutrophil (ANC >500/mm3, median 12.5d, range 10-19) or platelet recovery (plt >20k/mm3, median 12d, range 9-74d) were observed. Grade I-II GVHD was seen in 10/27 patients (37%), with no occurrences of Grade III-IV GVHD. Of the 19 patients with active disease at study entry, 18 achieved a CR. Treatment failure was due to persistent disease in 1 pt (4%), relapsed disease in 10 patients (37%) and early death due to complications from intracranial hemorrhage in 1 patient (4%). Median progression-free survival (PFS) for all patients was 26.6 wks (95%CI: 18.1-33.9 wks) and 15.7 wks (95% CI: 12.1-26.6 wks) in relapsed patients. Median follow-up for all study patients was 19.14 wks (range: 0.7-54.6 wks).

Correlative studies analyzed from 16 patients enrolled in the Phase I portion of the trial demonstrate that G-CSF/Plerixafor mobilizes CD34+ cells, with the mean fold increase of 5.9-fold at 80 µg/kg Plerixafor; at 160 µg/kg, 13-fold; and at 240 µg/kg, 14.2-fold. Over time, the relative increase of FISH+ cells was significantly higher than that of FISH- cells, indicating preferential mobilization of cytogenetically abnormal leukemic over normal cells (p=0.005). The objective of the ongoing Phase II study is to determine if the combination of G-CSF/Plerixafor with busulfan/fludarabine improves PFS compared to historical controls receiving busulfan/fludarabine alone.

Another study is aiming to establish the maximum tolerated dose (MTD) of Plerixafor in combination with bortezomib in patients who have active relapse/refractory MM [64]. Patients with active disease received Plerixafor at the recommended dose SC on days 1-6 of every cycle. Planned dose levels include 160, 240, 320, 400, and 480 µg/kg. Bortezomib was given at the recommended dose twice a week on days 3, 6, 10, and 13 every 21 days. Dose levels include 1.0 and 1.3 mg/m2, 60-90 minutes after Plerixafor. Patients who had response or stable disease went on to receive a total of 8 cycles without planned maintenance therapy. The median number of cycles on therapy was 3 (1–11). Dose limiting toxicities including insomnia, restlessness, and psychosis were observed in two patients at dose level 6 (Plerixafor 400 µg/kg and bortezomib 1.3 mg/m^2). To further explore the safety of maximum tolerated dose, three additional patients were enrolled at dose level 5b (Plerixafor 320 µg/kg and bortezomib 1.3 mg/m^2). Overall, the combination proved to be well tolerated. There were no grade 4 toxicities. Grade 3 toxicities included lymphopenia (40%), hypophosphatemia (20%), anemia (10%), hyponatremia (10%), hypercalcemia (10%), and bone fracture due to myeloma bone disease (10%). One patient came off treatment due to grade 2 painful neuropathy at cycle 5. Twenty-three patients were evaluable for response, including 1 (4%) complete response (CR), 1 (4%) very good partial response (VGPR) and 3 (13%) MR, with an overall response rate (including MR) of 5 (22%) in this relapsed and refractory population. In addition, 15 (65%) patients achieved stable disease (SD),

with just 3 (13%) having progressive disease (PD) as their best response. The combination of Plerixafor and bortezomib is generally well tolerated with minimal neuropathy or other toxicities seen to date. The responses observed are encouraging in this relapsed and refractory population. Plerixafor was able promote transient de-adhesion of MM cells and accessory cells *in vivo* in most of the patients, indicating that chemo sensitization can potentially be achieved in patients with MM using this approach.

The toxicities and pharmacokinetics of the combination of Plerixafor and rituximab in previously treated patients with chronic lymphocytic leukemia (CLL) are being investigated in a phase I dose escalation study (Andritsos et al. 2010). Rituximab was administered three times a week as a 100 mg dose on day 1, followed by 375 mg/m^2 IV for 12 total doses. Plerixafor was administered beginning with the 4th dose of Rituximab, 4 hours prior to the rituximab, in 4 cohorts of patients receiving various doses: (1) 80 µg/kg, (2) 160 µg/kg, (3) 240 µg/kg, and (4) 320 µg/kg. Preliminary results from the study demonstrated that CLL cells were mobilized to the peripheral blood in a dose-dependent fashion by Plerixafor. The combination of Plerixafor + rituximab in CLL patients with WBC < 50×109/L was well tolerated, and no dose limiting toxicities were reported. The most common adverse events that were reported were nausea, fatigue, chills, and diarrhea. CLL cells were mobilized following Plerixafor, and partial remissions were seen in a proportion of patients. In some cases, maximum responses were seen several months after completion of rituximab, consistent with single agent therapy. Higher Plerixafor doses and IV administration are now being investigated in an amendment to the ongoing clinical trial.

Plerixafor in Gliomas

Prior to our study, the presence and activity of the CXCR4 was also found to be critical for the growth of both malignant neuronal and glial tumors. In an intracranial xenograft of U87 glioma, antagonism of CXCR4 alone resulted in inhibition of tumor growth and increased apoptosis, compared to saline [65]. The anti-tumor effect of AMD3100 on glioma cells was associated with the drug's ability to attenuate the AKT and MAPK pathways downstream of CXCR4 signaling. In another orthotopic model of glioblastoma multiforme, inhibition of CXCR4 was found to synergize with BCNU by inducing tumor regression *in vivo*, as a result of both increased apoptosis and decreased proliferation, and despite sub therapeutic doses of chemotherapy [66].

In addition to directly conferring tumor cell responsiveness to therapy, a new mechanism of action for Plerixafor in tumor abrogation has recently emerged. A common pathway for tumor invasion or metastasis, as well as disease recurrence, is the appearance of new blood vessels forming as a consequence of hypoxic conditions in the tumor microenvironment.

Revascularization at sites of hypoxia results from the recruitment and stimulation of CXCR4-positive bone marrow-derived progenitor cells through local upregulation of SDF1-α, which is in turn under positive regulation by HIF-1α [9, 67]. Of relevance, the process of vasculogenesis initiated after radiation therapy in an intracranial xenograft model of glioblastoma was recently shown to be a potential mechanism for disease recurrence [8]. In this model, Plerixafor was given chronically over a period that slightly overlapped with and extended beyond radiotherapy until hypoxia-induced SDF-1α levels resolved to baseline. In treated animals, disease recurrence was prevented, likely due to the mitigating effect of Plerixafor on the influx of bone marrow-derived cells to the brain. Effective abrogation of revascularization through CXCR4 antagonism was also recently demonstrated in xenograft models of lung and

breast, overcoming both the concomitant stimulation of angiogenesis by paclitaxel and G-CSF [68]. Taken together, these observations suggest a potential role for Plerixafor in directly sensitizing gliomas, and perhaps other solid cancers, to therapy or promote tumor cell apoptosis through deprivation of essential new vasculature.

Dose Selection in Combination with Chemotherapy

In preclinical animal toxicology studies, DLTs were primarily adverse neurologic events, including severe dyspnea, tremors, ventral recumbency, which, at higher dosages, progressed to convulsions. The MTD for these effects was approximately 70 mg/m^2, which correlates with a dose of approximately 1800 µg/kg in humans. Early evidence of adverse neurologic effects, including diarrhea, muscle twitches, tremor, and tachycardia, were seen at doses of 17.5 to 35 mg/m^2. This is thought to scale to an approximate human equivalent dose of 470 to 940 µg/kg. The effects tended to resolve within hours of the dose and appeared to be related to Cmax.

Plerixafor has been given at doses of up to 480 µg/kg SC and IV in healthy volunteers and in cancer patients. A maximum tolerated dose has not been established. Higher doses of Plerixafor injection were evaluated in healthy volunteers in three cohorts of six subjects who each received two different doses of Plerixafor separated by at least 2 weeks to allow for adequate pharmacodynamic wash-out [27]. The dosing cohorts evaluated were: 240 and 320 µg/kg (cohort 1); 320 and 400 µg/kg; (cohort 2); and 400 and 480 µg/kg (cohort 3). Plerixafor was considered reasonably safe with no dose-limiting toxicity and common adverse events that consisted of diarrhea, injection site erythema, perioral numbness, sinus tachycardia, headache, nausea, abdominal distention and injection site pain. No dose limiting toxicities occurred. Sinus tachycardia (all Grade 1) was observed in most subjects treated with 400 and 480 µg/kg doses of Plerixafor, which were usually associated with activity and resolved quickly following rest. Since these events occurred soon after Plerixafor administration, they may be related to the 400 and 480 µg/kg doses of Plerixafor [27], which are higher than the 240 µg/kg dose used in the majority of other mobilization trials.

Intravenous administration of Plerixafor has been evaluated in cancer patients to minimize discomfort, optimize normal and leukemia stem cell mobilization, and ease logistical problems in the timing of administration. The timing and magnitude of the peak leukemia cell mobilization in relation to the time of administration of the chemotherapeutic agents may be critical in the success of leukemia control. Giving the Plerixafor IV may reduce the variability of the PK parameters and the range of peak leukemia cell mobilization, thus allowing better timing of chemotherapy administration.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

Refer to the Participant Eligibility Checklist in Appendix A. Patients who do not meet our definition for the analysis population will be replaced.

3.1 Inclusion Criteria

3.1.1 Patients must have tissue confirmation of high grade (WHO Grade IV) glioma including but not limited to glioblastoma, gliosarcoma, glioblastoma with oligodendroglial features, and glioblastoma with PNET features.

3.1.2 The patient must have post-operative contrast enhanced imaging (CT or MRI) unless only biopsy performed. For patients having biopsy alone, post-operative imaging is not routinely obtained and therefore the preoperative study will serve as baseline.

3.1.3 Patient should have surgery (biopsy, partial resection or gross total resection) and no additional anti-cancer therapy except the Chemo-radiation as specified in the protocol.

3.1.4 Patients must be between the ages of 18 and 75 years old (inclusive).

3.1.5 Patients must have Karnofsky Performance score ≥ 60.

3.1.6 Adequate organ function is needed at time of screening visit including:

1. ANC ≥ 1500
2. Platelets ≥ 100,000 ml
3. Serum Creatinine ≤ 1.5mg/dl; Cr clearance should be > 50 mL/min
4. AST and ALT ≤ 3 times the upper limit of normal
5. If female of childbearing potential, negative pregnancy test

3.1.7 The patient or his/her legal representative must have the ability to understand and willingness to sign a written informed consent document.

3.1.8 Patient agrees to use an effective method of contraception (hormonal or two barrier methods) while on study and for at least 3 months following the Plerixafor infusion

3.2 Exclusion Criteria

Patients who meet any of the following criteria must not be permitted entry to the study

3.2.1 Prior or concurrent treatment with Avastin (bevacizumab).

3.2.2 Prior exposure to Plerixafor.

3.2.3 Prior use of other investigational agents to treat the brain tumor.

3.2.4 Recent history of myocardial infarct (less than 3 months) or history of active angina.

3.2.5 Prior malignancy except for non-melanoma skin cancer and carcinoma in situ (of the cervix or bladder), unless diagnosed and definitively treated more than 3 years prior to 1st dose of investigational drug.

3.2.6 Prior sensitivity to Plerixafor.

3.2.7 Pregnant or patients who are breastfeeding.

3.3 Informed Consent Process

All participants will be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB approved informed consent prior to participation in any study specific procedure. In the event that a patient cannot sign due to writing impairment, the patient will make their mark and the person obtaining consent will print the patient's name and date. All participants will receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

3.4 Randomization Procedures

There is no randomization procedure.

3.5 Study Timeline

Primary Completion:
We estimate that the study will reach primary completion 36-42 months from the time the study opens to accrual.

Study Completion:
We estimate that the study, including the up to 5 year long term follow up, will reach study completion 8 years after the time the study opens to accrual.

4. TREATMENT PLAN

4.0.1 Screening

The following procedures will be performed for all potential subjects at the Screening visit to be conducted **within 10 days prior to the start of WBRT component (Fraction 16 of RT):**

- Written informed consent must be obtained from the patient prior to performance of any study-specific tests or procedures within 35 days of the start of Fraction 16 of RT.
- Confirm Eligibility Criteria
- Demographics: birth date, race/ethnicity and gender at birth
- Medical history
- Cancer history
- Physical Exam
- KPS
- Concomitant medications
- Vital Signs (including height and weight)
- Laboratory tests: CBC with differential, Comprehensive Metabolic Panel (including: sodium, potassium, chloride, CO2, glucose, creatinine, urea nitrogen, calcium, protein, albumin, total bilirubin, ALK Phosphatase, AST, ALT), Pregnancy Test (only for women of child bearing potential) Provided a patient's platelets meet screening requirements and day 1 requirements for the Plerixafor infusion, it is not necessary for them to be taking

the concurrent TMZ at that time, only necessary that they were given TMZ as intention to treat when they began RT/TMZ

Fraction 16 of RT

- Institution of whole brain irradiation (at Fraction 16, day 21 of radiation component)
- Start tracking Adverse events

Prior to start of Plerixafor infusion:

- Placement of the PICC line and infusion pump
- Neuro-cognitive assessment (HVLT-R, COWA, and Trail making Tests); quality of life surveys (MDASI-BT and EQ-5D-5L). If patient is non-English speaking, this will be deferred on an ongoing basis.

Day 1 must occur 7 days (+/- 3 days) prior to the completion of XRT:

- Physical Exam
- KPS
- Vitals
- Laboratory tests: CBC with differential, Complete Metabolic Panel (including: sodium, potassium, chloride, CO2, glucose, creatinine, urea nitrogen, calcium, protein, albumin, total bilirubin, ALK Phosphatase, AST, ALT)
    - Patients may proceed with the Plerixafor infusion even if the Day 1 ANC and platelets no longer meet inclusion criteria 3.1.7 due to the concurrent temozolomide and radiation and at the investigator's discretion; Thrombocytopenia should be ≤ grade 2
- Begin Plerixafor infusion in outpatient unit.

Weekly following the start of the Plerixafor infusion through the end of infusion (+/- 3 days) (Day 8, Day 15, Day 22):

- Physical Exam
- KPS
- Vitals
- Laboratory tests: CBC with differential, Complete Metabolic Panel (including: sodium, potassium, chloride, CO2, glucose, creatinine, urea nitrogen, calcium, protein, albumin, total bilirubin, ALK Phosphatase, AST, ALT)
- Change infusion bag of Plerixafor
- Review concomitant medications and adverse events

Day 29 (+/- 3 days):

- Physical Exam
- KPS
- Vitals
- Laboratory tests: CBC with differential, Complete Metabolic Panel (including: sodium, potassium, chloride, CO2, glucose, creatinine, urea nitrogen, calcium, protein, albumin, total bilirubin, ALK Phosphatase, AST, ALT)
- Review concomitant medications and adverse events

Day 35 (+/- 7 days):

- MRI brain with and without contrast
- Start monthly TMZ

Concomitant medications and adverse events will be followed up until 30 days after the end of the Plerixafor infusion.

Investigator will manage monthly TMZ per their standard practice. Follow up MRIs will be done per standard of care (approximately every 8-12 weeks).

6 months after the start of XRT (+/- 2 weeks):

- MRI brain with and without contrast
- Neuro-cognitive assessment; quality of life assessment if patients are being followed at Stanford in Palo Alto

Long Term Follow-Up:
Unless a patient has specifically withdrawn consent to be followed for survival, he or she will be contacted (by phone or clinic visit) every 12 weeks (+/- 2 weeks) after completion of the Plerixafor infusion to collect data regarding survival status and subsequent anticancer therapy. Follow up will continue either for 5 years or until death, withdrawal, lost to follow up, or study termination.

Neuro-cognitive and quality of life assessments will be administered at 12 months, twenty-four months after start of radiation (+/- 3 months) and then yearly (+/- 3 months) until progression as long as patient is in follow-up if the patient is being followed at Stanford in Palo Alto. If a patient has definitive progression at any point during the long term follow up period, subsequent questionnaires will not be administered.

4.1 General Concomitant Medication and Supportive Care Guidelines

The use of all standard supportive medication, including appropriate antimicrobial prophylaxis for chemotherapy, is permitted, although concurrent treatment with immunosuppressive or immunomodulatory agents is discouraged. Concomitant systemic corticosteroids are to be avoided if at all possible. If used, doses of steroids should be the minimum necessary for appropriate clinical management.

The following are prohibited while on the infusion part of study:

- Other investigational agents
- Any concurrent chemotherapy, radiotherapy, hormonal therapy, immunotherapy, or other systemic therapy for cancer

4.2 Criteria for Removal from Study

The investigator has the right to discontinue a patient from study drug or withdraw a patient from the study at any time. In addition, patients have the right to voluntarily discontinue study drug or withdraw from the study at any time, for any reason. Reasons for discontinuation or withdrawal include, but are not limited to:

- Patient withdrawal of consent
- Progression of disease by RANO criteria, as determined by the investigator
- Investigator decision (e.g., symptomatic/clinical deterioration or not in the patient's best interest to continue in the study)
- Patient is lost to follow up
- Death
- Non-compliance of the patient with protocol mandated procedures
- Any unacceptable toxicity

4.3 Alternatives

The study participant would be eligible for standard of care treatment protocols or any additional investigational trials (per eligibility requirements) should withdrawal from our study be warranted.

5. INVESTIGATIONAL AGENT/DEVICE/PROCEDURE INFORMATION

5.1 Investigational Agent/Device/Procedure

The drug Plerixafor (AMD3100) will be supplied in infusion ready vials containing 10 ml of 20 mg/ml solution (which is stable for several months at room temperature) by Sanofi. There are no known incompatibilities of the agent with commonly used intravenous solutions. There is no need to administer the agent with food and there are no pre-medications necessary. There are no restrictions against any medications and pre-medications may be used on an as needed basis.

The dose will be 400 µg/kg per day for 4 weeks (+/-3 days)

Dose will be based on weight at screening and will not be modified. Actual body weight will be used to calculate dose except in patients who are overweight (in which case adjusted body weight will be used). The dose of Plerixafor will be adjusted for patients who weigh > 30% over their Ideal body weight. IBW is calculated as follows:

Males: IBW (kg) = 50.0 + [(2.3) (Height in inches – 60 inches)]
= 50.0 + [(2.3) (Height in cm) (0.39370079) - 60)]

Females: IBW (kg) = 45.5 + [(2.3) (Height in inches – 60 inches)]
= 45.5 + [(2.3) (Height in cm)(0.39370079) – 60)]

The ABW is calculated as follows:
ABW (kg) = IBW + 0.4(actual weight - IBW)

Plerixafor will be prepared weekly by the investigational pharmacy, with each dose being prepared no more than 4 hours prior to its use. Per Stanford Investigational Pharmacy policy, the pharmacy will prepare sterile products in an environment that complies with USP 797 parameters. The IV admixture area is kept clean and orderly and a demarcation line identifies the separation of the anteroom from the buffer area. Corrugated boxes are not allowed in the anteroom. A clean cart/dirty cart system is used to transfer inventory and compounding

supplies into and out of the cleanroom

Infusion bags will be changed every week while the patient is coming for follow up. Plerixafor will be infused using a 0.2 micron in-line filter that will be changed with each new dose for all administrations. The volume prepared by the investigational pharmacy will be determined to allow a fixed infusion rate of 1.5 mL/hour.

As per previously published technique, Plerixafor will be suspended in normal saline to the final solution [30]. The minimum rate will need to be 0.5 mL/hour to keep the Picc line patent however, the rate of infusion will be modified accordingly per the volume used in the diluent.

Please see "Investigator's Brochure" and section 2.5 above

5.2 Availability

Plerixafor is supplied in open-label 2 mL glass vials containing 1.7 mL of a 20 mg/mL sterile solution. Each Plerixafor vial will be used to provide a single dose only. Remaining drug solution in each vial must not be used.

The investigational product is shipped in cartons containing five (5) Plerixafor vials each. Each carton will also be affixed with a label describing the protocol number, contents of each carton, lot number, required cautionary statements or regulations, storage conditions, and the Sponsor's name and address.

The investigational product will be stored at room temperature (15-30^{o}C) in a secure location accessible only by authorized personnel. All drug supplies are to be used only for this protocol and not for any other purpose.

5.3 Agent Ordering

Plerixafor will be ordered using the Investigator Sponsored Trial (IST) portal at http://www.saists.com.

5.4 Agent Accountability

All Plerixafor sent to the site will be accounted for. In addition, the volume of Plerixafor dispensed for each patient will be recorded on an Investigational Product Accountability Log and the volume administered documented on the case report form (CRF). An accurate record of the date and amount of Plerixafor dispensed to each patient will be available for inspection at any time. Partially used vials may be destroyed per institutional guidelines and documented. All unopened and unused vials of Plerixafor will be destroyed upon completion of the study protocol or if drug expires unless otherwise directed by the Sponsor. The study site will document all receipt, complete destruction, and return (if applicable) of Plerixafor.

5.5 Radiation Therapy Planning

Radiotherapy will consist of two sequential components. The first 30 Gy will be standard

conformal IMRT to the tumor/tumor bed. The second 30 Gy (total 60 Gy in 30 fractions) will be whole brain radiotherapy.

Positioning, Immobilization and Simulation:

1. The patient will be simulated in the supine position in most situations, immobilized typically with a thermoplastic mask. The treatment planning simulation CT scan will be acquired in the treatment position. The treatment volumes will be defined by fusion with the pre- and post-resection MRI scans.
2. CT contrast may be omitted if medically indicated. The MRI sequences should include a T1-weighted post-contrast sequence (preferably stereotactic, thin slice, contiguous). Additionally, a T2/FLAIR sequence is helpful to identify non-enhancing tumor.

Equipment and Technique:

1. Radiotherapy will be delivered with megavoltage equipment, typically of 4MV or greater energy.
2. Intensity modulated radiotherapy (IMRT) via any method (e.g., VMAT, static field IMRT, tomotherapy) is preferred. 3D planning may be considered for the WBRT component.
3. Daily image-guidance is recommended to allow smaller planning target volume (PTV) margins, but is not required. The PTV margin should reflect if image-guided radiotherapy (IGRT) is used.

Target Volume Delineation:

Conformal RT Component– Treated from 2 to 30 Gy to the local tumor

1. A gross tumor volume (GTV) will be defined using the CT and MRI images. The GTV includes any enhancing tumor, if present following resection, as well as the post-operative resection cavity. The GTV also includes any non-enhancing tumor as identified on T2/FLAIR. T2/FLAIR signal consistent with edema is not specifically included in the GTV. Therefore, a distinction is made between T2 edema (typically without mass effect, sparing the cortical ribbon, obeying the grey/white junction, etc.) and T2 tumor (mass effect with sulcal effacement, involvement of the grey/white junction, obliteration of the cortical ribbon). Fusion of the pre-operative MRI to determine initial extent of the tumor is helpful.
2. The clinical target volume (CTV) is created by anatomically expanding the GTV by 15 to 20 mm, per physician preference. The CTV should be trimmed at anatomic boundaries to rational tumor spread, such as the tentorium, falx if not near the corpus callosum, and skull. At these boundaries, the CTV may be 0 mm.
3. A geometric PTV expansion of 3 to 5 mm will be applied to the CTV that is justified based on image guidance and immobilization. For treatment utilizing IGRT techniques, the PTV may be as small as 3mm. For non-IGRT approaches, a 5 mm expansion is recommended.

Whole Brain Radiotherapy Component – Treated from 32 to 60 Gy to the whole brain

1. The CTV will consist of the intracranial compartment, including the brain, cisterns, and meninges.
    a. Note: Algorithms which 'auto-contour' the brain are not adequate, as this would not typically include the pre-pontine cistern and all meningeal surfaces
2. The inferior extent of contouring will be the foramen magnum

Organs at Risk (OAR) and OAR Constraints:

1. OARs shall be contoured, including: optic nerves, optic chiasm, brainstem, lacrimal glands, retina, and cochlea and skin.
2. A 3 mm PRV expansion shall be applied to the above.
3. OAR Constraints to PRVs:
    1. Brainstem <55 Gy (except when the GTV involves the brainstem; Dmax may be 60.5 Gy in these circumstances)
    2. Chiasm and Optic nerves <55 Gy
    3. Retina < 45 Gy
    4. Cochlea < 40 Gy. This may be exceed to cover tumor per physician preference
    5. Lacrimal Gland <40 Gy
    6. Skin (defined as 'Body' – 4 mm) < 40 Gy if possible

Dose:

1. An initial dose of 30 Gy in 15 fractions of 2 Gy per day shall be delivered to the cover $\geq$ 95% of the tumor PTV to $\geq$ 30 Gy. Under coverage below 95% is acceptable to meet OAR constraints.
2. A second plan of 30 Gy in 15 fractions of 2 Gy per day shall be delivered to cover >90% of the whole brain. Under coverage below 95% is acceptable to meet OAR constraints.
    1. A VMAT WBRT plan is preferable to allow modulation of dose to meet OAR constraints.
    2. Preferably, the VMAT WBRT plan is planned first, so that it may be used as the base plan for the VMAT conformal RT component, to meet OAR constraints
    3. A 3D opposed lateral WBRT is acceptable, but not preferred. The 3D plan should be used as the base plan upon which to design the initial 30 Gy of IMRT.
3. IMRT plan heterogeneity should ideally be 110% (66 Gy Dmax) or less and preferably under 105% (63 Gy Dmax). A Dmax of 114% (68.4 Gy) is an acceptable variation. It is preferable that the initial IMRT plan and the WBRT plan are planned concurrently, to account for heterogeneity between plans.
4. Treatment shall be given daily.

6. DOSE MODIFICATIONS

There will be no dose modifications or delays for an individual patient's dose except in the circumstance of moderate to severe renal impairment. If a patient has a Creatinine clearance less than or equal to 50 mL/min, the dose of Plerixafor will be reduced by one-third. Plerixafor dose interruption is allowed for up to 3 business days to deal with possible adverse events, pump

malfunctions, abnormal clinically significant chemistries and or as per the physician's discretion. If the interruption is more than the mentioned time frame, the participant will be withdrawn from the study. Subject who are not able to finish the 4-week (+/- 3 days) infusion will be replaced.

6.1 Infusion Reactions

All patients will be provided the contact information for the 24 hour on call pharmacist and neurology physician.

For Grade 1 and 2 allergic reactions to Plerixafor infusion: oral steroids or antihistamine. Patients may be discontinued at investigator's discretion

For Grade 3 and higher allergic reactions: Standard allergy treatment could include intravenous solumedrol, intravenous Benadryl, and possibly epinephrine at the discretion of the treating physician. Plerixafor infusion will be discontinued.

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

Safety will be assessed by monitoring clinical and laboratory evaluations and AEs.

Definitions

Adverse Event

An AE is any untoward medical occurrence associated with the use of the investigational product (active or placebo drug, biologic, or device) in a clinical investigation patient, which does not necessarily have a causal relationship with the investigational product. An AE can, therefore, be any unfavorable and unintended symptom, sign, disease or condition, or test abnormality whether or not considered related to the investigational product.

AEs may include, but are not limited to:

Subjective or objective symptoms spontaneously offered by the patient and/or observed by the investigator or medical staff
Clinically significant laboratory abnormalities
A significant worsening of the patient's condition from study entry
Disease signs and symptoms and/or laboratory abnormalities existing prior to the use of the study treatment that resolve but then recur after treatment
Disease signs and symptoms and/or laboratory abnormalities existing prior to the use of the study treatment which increase in frequency, intensity, or a change in quality after treatment

Serious Adverse Events (SAEs)

A SAE is any adverse event that results in any of the following outcomes:

- Death
- A life-threatening experience

- Requires inpatient hospitalization or prolongs existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Important medical events that may jeopardize the patient and may require medical or surgical intervention to prevent 1 of the outcomes listed above

Hospitalizations that occur under the following circumstances are not considered to be SAEs:

- were planned before entry into the clinical study;
- are for elective treatment of a condition unrelated to the studied indication or its treatment;
- Occur on an emergency or outpatient basis and do not result in admission (unless fulfilling the criteria above), are part of the normal treatment or monitoring of the studied indication and not associated with any deterioration in condition.

Severity

The investigator will grade AEs using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) (version 5.0, 27 Nov 2017). Grades refer to the severity of the AE. The CTCAE v 5.0 displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade	Description
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)
3	Severe or medically significant but not immediately life-threatening hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self-care ADL
4	Life-threatening consequences; urgent intervention indicated
5	Death related to AE

Action taken

The investigator should record what action, if any, was taken to the planned administration of the investigational product due to the AE (i.e., discontinuation, modification, or interruption of the treatment).

Relationship to the investigational product

When recording and reporting an AE or SAE, the investigator will provide an assessment of the relationship between the AE or SAE and the study drug(s) and/or study procedure. Related AEs or SAEs are those that are judged to be possibly or definitely related by the investigator. Unrelated AEs or SAEs are those that are judged to be unlikely or not related to the study drug(s) by the investigator. Definitions of relationship criteria are as follows:

Related

Definitely related: There is strong evidence that there is a causal relationship between exposure and AE

Possibly related: There is some evidence supporting the possibility of a causal relationship between exposure and AE

Unrelated
Remote/Unlikely related: There is no evidence of a causal relationship between exposure and AE; however, such a relationship cannot be ruled out

Unrelated: There is no suspicion of a causal relationship between exposure and AE

Describe all known or potential risks associated with this Investigational Drug/Device/Procedure. Include Adverse Event description, grade, expectedness, and attribution to the study treatment.

The most common adverse reactions (>= 10%) reported in patients who received Mozobil in conjunction with G-CSF regardless of causality and more frequent with Mozobil than placebo during hematopoietic stem cell mobilization and apheresis were diarrhea, nausea, fatigue, injections site reactions, headache, arthralgia, dizziness and vomiting. Per label (USPI) serious hypersensitivity reactions, including anaphylactic-type reactions, some of which have been life-threatening with clinically significant hypotension and shock, have occurred in patients receiving Plerixafor. In randomized studies, 34% of patients with non-Hodgkin's lymphoma and multiple myeloma had mild to moderate injection site reactions at the site of subcutaneous administration of Plerixafor. These included erythema, hematoma, hemorrhage, induration, inflammation, irritation, pain, paresthesia, pruritus, rash, swelling, and urticaria. Mild to moderate systemic reactions were observed in less than 1% of patients approximately 30 min after Plerixafor administration. Events included one or more of the following: urticaria (n = 2), periorbital swelling (n = 2), dyspnea (n = 1) or hypoxia (n = 1). Symptoms generally responded to treatments (e.g., antihistamines, corticosteroids, hydration or supplemental oxygen) or resolved spontaneously. Vasovagal reactions, orthostatic hypotension, and/or syncope can occur following subcutaneous injections. In Plerixafor oncology and healthy volunteer clinical studies, less than 1% of subjects experienced vasovagal reactions following subcutaneous administration of Plerixafor doses ≤ 240 mg/kg. The majority of these events occurred within 1 hour of Plerixafor administration. Other adverse reactions that occurred in < 5% of patients but were reported as related to Plerixafor during mobilization and apheresis included abdominal pain, hyperhidrosis, abdominal distention, dry mouth, erythema, stomach discomfort, malaise, constipation, dyspepsia, and musculoskeletal pain.

In the study that assessed continuous infusion of Plerixafor over 10 days, four SAE's were noted: thrombocytopenia, infection of a PICC line and arrhythmia (> 25 PVC's/min) and panic attack associated with paresthesias [18].

7.2 Adverse Event Reporting

Adverse events will be graded according to CTCAE v5.0. This is an off-label indication for Plerixafor. Investigators will reference safety information to assess expectedness: IB. All adverse events except those clearly attributable to the underlying disease will be reported, including definitely, probably and possibly related. Both Serious and Non-Serious Adverse Events will be

clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will assess each Adverse Event (AE) to determine whether it is unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochure, and related to the investigation. All Serious Adverse Events (SAEs) will be tracked until resolution or until 30 after the last dose of the study treatment.

SAEs CTCAE Grade 3 and above, and all subsequent follow-up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) using the study specific CRF regardless of the event's relatedness to the investigation. Following review by the DSMC, events meeting the IRB definition of 'Unanticipated Problem' will be reported to the IRB using eProtocol within 10 working days of DSMC review, or within 5 working days for deaths or life-threatening experiences.

In addition, within 24 hours (US) or one business day (EU) of first knowledge of such serious and related adverse event, we will notify Sanofi via fax, attention Sanofi Pharmacovigilance (PV), 908-203-7783 (US) or +1-908-203-7783 or via email at: USPVmailbox@sanofi-aventis.com. Additionally, the Investigator will transmit to Sanofi PV an information copy of any such report sent to the governing regulatory authority, prior to or at the time of authority filing. The Investigator will make available to Sanofi promptly such records as may be necessary and pertinent to investigate any such expedited adverse event, if specifically requested by Sanofi.

Furthermore, the Investigator will inform Sanofi of the following:

- Any events that result in protocol amendments for safety reasons, as well as any safety related regulatory action such as a clinical hold of the Research;
- Any pregnancies occurring in patients who are exposed to the Product in connection with the Research. Please see section 10.2.1.3 for additional reporting guidance;
- In addition, the Investigator will notify Sanofi within 24 hours (US) or one business day (EU) of first knowledge of any Product complaints (communication of dissatisfaction that alleges deficiencies related to the identity, quality, durability, effectiveness, safety, labeling, purity, stability, and appearance) by fax to 508-661-8771 (US) or Sanofi Customer Services Europe, +31 (0)35 699 1222.
- The Investigator will also inform Sanofi within 1 business day of becoming aware of any actions from any authority that may affect the performance of the Research

Safety reporting rules are to be complied with, according to current PV specifications (QGSD-007589). Sponsor is to provide Sanofi with: results relevant to final diagnosis of any SAE; routine transmission of any overdose with Plerixafor; periodic reports; study report must contain section with safety review and conclusion –to be reviewed by Sanofi before finalization.

Pregnancy reporting

All patients must agree to an effective method of contraception while on study treatment and for at least 3 months following Plerixafor treatment (including both female patients of child-bearing potential and male patients with partners of child-bearing potential). Effective birth control includes: a) birth control pills, depot progesterone, or an intrauterine device plus one barrier method, or b) two barrier methods. Effective barrier methods are: male and female condoms, diaphragms, and spermicides (creams or gels that contain a chemical to kill sperm).

For patients using a hormonal contraceptive method, information about any interaction of Plerixafor with hormonal contraceptives is not known.

The Investigator will inform Sanofi PV within 24 hours of the Investigator's first knowledge of pregnancy in a female patient or the female partner of a male patient at any time after the first dose of Plerixafor. Pregnant female patient(s) must not receive additional study treatment. The pregnancy will be followed until the outcome is known (i.e., delivery, elective termination, spontaneous abortion). The Investigator will obtain follow-up information no later than two months after the gestational period to obtain maternal, fetal, and neonatal outcome and any other relevant information. If the pregnancy results in the birth of a child, additional follow-up information may be requested. The Investigator must complete as much information as possible on the relevant Pregnancy Notification Forms (PNF) A and B, and fax the forms to the Sanofi PV.

8. CORRELATIVE/SPECIAL STUDIES

During their pre-, post- and 6 month MRI's, patients undergo rCBV analysis using standard sequencing. This will require no extra risk or time since these sequences are part of the standard clinical brain tumor protocols.

8.1 Laboratory Correlative Studies

None are planned during this Phase II study.

9. STUDY CALENDAR

	Screening (- 10 days from fraction 16 of RT)	Fraction 16 of RT	Pre-Plerixafor	Day 1 (- 7 days from end of RT +/- 3)	Day 8 (+/- 3)	Day 15 (+/- 3)	Day 22 (+/- 3)	Day 29 (+/-3)	Day 35 (+/-7)	30 days post Plerixafor	6 months after start of XRT (+/- 2 wks.)	Every 12 weeks after Plerixafor (+/- 2 weeks)
Consent [e]	X											
Eligibility	X											
Demographics	X											
Physical Exam	X			X	X	X	X	X				
Medical/Cancer History	X											
Vitals	X			X	X	X	X	X				
Height/Weight [c]	X			X	X	X	X	X				
KPS	X			X	X	X	X	X				
Hematology	X			X	X	X	X	X				
Chemistry	X			X	X	X	X	X				
Pregnancy [a]	X											
Neurocognitive Assessments [d]			X								X	X[b]
Quality of Life [d]			X								X	X[b]
PICC Placement			X									
Continuous Plerixafor 400 mcg/kg/day				X----------|								
Whole Brain RT		X----------|										
MRI									X		X	
AEs		X----------|										
Con Meds	X----------|											
Survival Status												X
Subsequent anti-cancer therapy												X

[a.] females of childbearing potential only

[b.] At 12 months, 24 months after start of radiation (+/- 3 months) and then yearly (+/- 3 months) until progression if patient is being followed at Stanford Palo Alto

[c.] height only required at screening

[d.] only for English speaking patients
[e]. Consent: - 35 days from fraction 16 of RT

10. MEASUREMENTS

For clinicaltrials.gov and Stanford Clinical Trials Directory compliance

Primary Outcome Measure Definition: Six month progression free survival from the start of Chemoradiation

Title: Rate of Six month PFS

Time Frame: Six month progression free survival after the start of Chemoradiation

Safety Issue: Is this outcome measure assessing a safety issue? No

10.1 Primary measure: Progression Free Survival

Our primary objective in this Phase II study is to ascertain the six month progression free survival (PFS) rate in GBM patients treated with a 4 week (+/-3 days) infusion of Plerixafor at 400 micrograms per kilogram per day layered onto a modified radiation therapy framework that includes a whole brain component.

10.1.1 Relevant Subset

- All patients who have completed the 28 day Plerixafor infusion will be included in the primary analysis.

10.1.2 Measurement Definition

- The primary outcome is progression free survival as determined by MR and clinical status at six months from start of the Chemoradiation..

10.1.3 Measurement Methods

- MRI, which will be performed prior to RT onset, one month after RT (which will correlate to one week after Plerixafor completion) and six months after the start of Chemoradiation.

Positive Response: Requires all of the following:

- Stable or improved non-enhancing (T2/FLAIR) lesions
- No new lesions
- Stable or improved clinical status

Progressive Disease (PD): Requires any of the following

- ≥ 25% increase in the SPD of measurable enhancing target lesions plus >5 mm absolute increase in the sum of the longest diameters (SLD) of target lesions compared to the best response after initiation of therapy
- Clear progression of enhancing non-target disease
- Significant increase in T2/FLAIR non-enhancing disease not caused by co-morbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, post-operative changes or other treatment effects)

- Any new lesions
- Clear clinical deterioration not attributable to other causes apart from the tumor (e.g., seizures, medication adverse effects, complications of therapy, cerebrovascular events, infections etc.)
- Increasing steroid doses alone do not constitute PD.

10.1.4 Measurement Time Points

- An MRI will be done 35 +/-3 days after the completion of XRT. Follow up MRIs will be done per standard of care (approximately every 8-12 weeks), with one occurring 6 months post the start of radiation, until progression or withdrawal of consent.

10.1.5 Response Review

- A futility analysis will be performed after the first 16 patients have been followed for 6 months (see Section 12.2 for details).

10.2 Secondary Outcome: Median Survival

10.2.1 Relevant Subset

- All patients who have completed the 28 day Plerixafor infusion.

10.2.2 Measurement Definition

- Survival at date of last follow-up.

10.2.3 Measurement Methods

- Patients will be followed through course so status can be assessed.

10.2.4 Measurement Time Points

- Status at time of last follow-up will be recorded. The start of irradiation will be considered Day 0.

10.3 Secondary Outcome: Toxicities

10.3.1 Relevant Subset

- All patients who have completed WBRT component, whether or not they receive Plerixafor infusion.

10.3.2 Measurement Definition

- All toxicities will be recorded and graded according to CTCAE.

10.3.3 Measurement Methods

- The CTCAE version 5.0 will be used to grade toxicities.

10.3.4 Measurement Time Points

- Adverse events/toxicities will be recorded as soon as they are reported. Patients in follow up will be queried for possible toxicities.

10.3.5 Response Review

- Identification of adverse events will be made by the clinical coordinator and the primary treating doctor. In instances where the event is not clear, the Protocol Director will have the ultimate responsibility for calling and grading an adverse event.
- All Gr.3 and above toxicities will be reviewed by the Protocol Director and Co-Director.

10.4 Secondary Outcome: Pattern of Failure

10.4.1 Relevant Subset

- All patients who have completed WBRT component, whether or not they receive Plerixafor infusion, in adherence to the intent to treat principle.

10.4.2 Measurement Definition

- The 95% isodense field will be considered the maximal treatment field
- When progressive disease defined, MR images will be co-registered with treatment planning imaging to determine whether the first progression occurs within the maximal treatment field, in the brain outside of the maximal treatment field, in the spine and outside the CNS.

10.4.3 Measurement Methods

- MR images from the MRI demonstrating progression will be co-registered with treatment planning imaging.

10.4.4 Measurement Time Points

- MRI's are part of the standard of care and are done generally every two months or sooner if necessary.

10.4.5 Response Review

- The determination of failure pattern will be a consensus decision between Drs. Soltys, Thomas, Iv, Nagpal, and Recht. In cases where there is disagreement, the opinion of another Stanford neuroradiologist will be sought.

10.5 Secondary Outcome: Neuro-Cognitive and Quality of Life Outcomes

10.5.1 Relevant Subset

- All patients who are competent in English and who have completed WBRT component, whether or not they receive Plerixafor infusion, in adherence to the intent to treat principle.

10.5.2 Measurement Definition

- A series of neurocognitive assessments in which it is possible to develop a score will be used to assess for cognitive effects and quality of life at various points in the clinical course.

10.5.3 Measurement Methods

- Patients will undergo neurocognitive assessment at the time they are receiving WBRT and then at 6, 12 and 24 months after start of radiation.
- The neurocognitive assessments will be administered by the study coordinator.

10.5.4 Measurement Time Points

- Patients will undergo testing Pre Plerixafor and then 6, 12 and 24 months after the initiation of radiation.

10.5.5 Response Review

- At the end of the study, the collected data will be organized and first analyzed by the study team to assess changes in neurocognitive performance and QOL over time.

11. REGULATORY CONSIDERATIONS

11.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

11.2 Data and Safety Monitoring Plan

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. The DSMC will audit study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of the following types of documents participating in the study: regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

11.3 Data Management Plan

The Protocol Director, or his designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document treatment outcomes for data analysis. Case report forms will be developed using the On Core database system and will be maintained by Sophie Bertrand. CRFs will be kept in a locked office, only accessible to the research team.

12. STATISTICAL CONSIDERATIONS

A Senior Biostatistician in the Quantitative Sciences Unit (QSU) will oversee all analyses and interpret the results. All analyses will be completed in the R statistical computing environment or SAS™ version 9.

12.1 Statistical Design

A single arm (non-randomized trial).

12.1.1 Randomization
Not applicable. This is a single arm clinical trial

12.2 Interim analyses

We employ a Simon 2-stage design where the first 16 patients who have completed the 4 week(+/-3 days) infusion are enrolled and assessed before completing our targeted enrollment of 20 patients. Thus, the interim analysis for futility will be performed on the first 16 patients. The trial will be stopped for futility if 6 or fewer subjects of the 16 achieve PFS by 6 months. The probability of stopping the trial for futility is 0.82 if the response rate is as low as 0.30.

12.3 Descriptive Statistics and Exploratory Data Analysis

Patient characteristics will be summarized using proportions for categorical variables, means and standard deviations for continuous variables (median and inter-quartile ranges where appropriate).

12.4 Primary Analysis

For the Phase II component, the primary endpoint is 6-month progression free survival (PFS), as defined in Section 10.1.

12.4.1 Analysis Population
Our analysis population for assessing treatment efficacy consists of GBM patients treated with a 4-week (+/-3 days) infusion of Plerixafor at 400 micrograms per kilogram per day layered onto a modified radiation therapy framework that includes a whole brain component, where the targeted enrollment for our study is 20 patients. Our safety population consists of all patients (at least 20 GBM patients) enrolled in the trial and exposed to any study drug.

12.4.2. Analysis Plan
Our Primary objective in the Phase II component is to assess progression free survival at 6 months. We consider the experimental treatment to be promising if it yields a response (ability to achieve PFS by 6 months) in 50% of subjects, and we consider the treatment to not be promising if the response rate is as low as 30%. To assess whether the regimen is promising, we utilize a Simon 2-stage design. In the first stage we evaluate the first 16 patients. If 6 or fewer subjects achieve a response, the trial will be stopped for futility. If more than 6 achieve PFS at 6 months, four more patients will be enrolled in the study. If a total of 8 or fewer achieve a response, the therapy will not be considered promising and will not warrant further study. If a total of 9 or more of the 20 achieve a response, the therapy will be a candidate for further study. Our design has adequate operating characteristics. Specifically, we have less than 0.10 probability of incorrectly concluding the therapy is promising if it is not (assuming a response rate of only 0.30). Further we have over 0.70 probability of correctly concluding the therapy warrants further study if the true response rate is 0.5. The probability of stopping the trial early for futility is 0.82 if the response rate is as low as 0.30.

Our secondary objective is to characterize median survival time, with the expectation that median survival will exceed 20 months, the best GBM benchmark based on recent publications [69]. We will report and interpret the median survival and accompanying 90% lower confidence interval around the

observed median.

Although unexpected, it is possible that some patients drop out of the study prior to completing the treatment regimen. Although the analysis population for the primary outcome includes only patients that completed the Plerixafor infusion treatment, all patients will be included in descriptive statistics and Kaplan-Meier estimates (see Section 12.3). It should be noted that based on the ongoing trial, we expect at most 1 patient could fail to complete the treatment regimen.

We will assess reported toxicities, pattern of failure (out-of-field occurrence or occurrence outside of the brain), and changes in neurocognitive measures and quality of life over time. Adverse events and qualifying DLT will be tabulated by cohort, site and severity. Proportions of these patients in each response category will be tabulated; the combined proportion in categories CR (complete response), Cri (CR with incomplete count recovery), PR (partial response), SD (stable disease) will be computed along with an exact 90% confidence intervals. Duration of response progression-free survival and median survival time will be computed from start of induction therapy and summarized with Kaplan-Meier estimates. Other graphical tools will include a swimmer's plot to view progression by patient, spaghetti plot for changes in neurocognitive assessments over time, and boxplots and histograms for continuous characteristics. Changes in neurocognitive outcomes will be analyzed with a linear mixed-effect model, with a random effect for patient, to account for correlation over time within patient.

12.5 Secondary Analysis

Included in the section Analysis Plan 12.4.2

12.6 Sample Size

12.6.1 Accrual estimates

We expect to accrue the total number of 20 patients to complete the 4 weeks (+/-3 days) within the 24-month timeline proposed.

12.6.2 Effect size justification

Based on positive results (> 80% who achieve a 6 month PFS) from an ongoing Phase I/II study using Plerixafor infusion, we hypothesize a true response rate of 50% or more.

12.6.3 Sample size justification

Our design that includes 20 subjects has adequate operating characteristics. Specifically, with our decision rule applied in the two stages as described above, we have less than 0.10 probability of incorrectly concluding the therapy is promising if it is not (assuming a response rate of only 0.30). Further we have over 0.70 probability of correctly concluding the therapy warrants further study if the true response rate is 0.5. The probability of stopping the trial early for futility is 0.82 if the response rate is as low as 0.30.

12.7 Criteria for future studies

This Phase II study will provide additional data that should supplement that obtained with our prior Phase I/II protocol using Plerixafor as a continuous infusion. It is possible if our secondary endpoint

of 32 month median survival is achieved, that this will justify incorporating this into the standard treatment framework. More likely, however, is that it will provide compelling data for a larger randomized study assessing this strategy with standard of care.

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APPENDICES

APPENDIX A: Participant Eligibility Checklist

Protocol Title:	A follow-up study to add whole brain radiotherapy (WBRT) to standard temozolomide chemo-radiotherapy in newly diagnosed glioblastoma (GBM) treated with 4 weeks continuous infusion Plerixafor
Protocol Number:	BRN0037
Principal Investigator:	Lawrence Recht, MD

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB approved protocol)	Yes	No	Supporting Documentation*
1. Patients must have tissue confirmation of high grade (WHO Grade IV) glioma including but not limited to glioblastoma, gliosarcoma, glioblastoma with oligodendroglial features, glioblastoma with PNET features.	☐	☐	
2. The patient must have post-operative contrast enhanced imaging (CT or MRI) unless only biopsy performed (in which case post-operative imaging is not routinely obtained. In these patients, the preoperative study will serve as baseline).	☐	☐	
3. Patient should have surgery (biopsy, partial resection or gross total resection) and no additional anti-cancer therapy except the Chemoradiation as specified in the protocol.	☐	☐	

4. Patients must be between the ages of 18 and 75 years old (inclusive)	☐	☐	
5. Patients must have Karnofsky Performance score ≥ 60.	☐	☐	
6. Adequate organ function is needed at time of screening visit including: 1. ANC ≥ 1500 2. Platelets ≥ 100,000 ml 3. Creatinine ≤ 1.5mg/dl; Cr clearance should be > 50 mL/min 4. AST and ALT ≤ 3 times the upper limit of normal 5. If female of childbearing potential, negative pregnancy test	☐	☐	
7. The patient or his/her legal representative must have the ability to understand and willingness to sign a written informed consent document.	☐	☐	
8. Patient agrees to use an effective method of contraception (hormonal or two barrier methods) while on study and for at least 3 months following the Plerixafor infusion	☐	☐	
Exclusion Criteria (From IRB approved protocol)			
1. Prior or concurrent treatment with Avastin (bevacizumab).	☐	☐	
2. Prior exposure to Plerixafor.	☐	☐	
3. Prior use of other investigational agents to treat the brain tumor.	☐	☐	
4. Recent history of myocardial infarct (less than 3 months) or history of active angina.	☐	☐	
5. Prior malignancy except for non-melanoma skin cancer and carcinoma in situ (of the cervix or bladder), unless diagnosed and definitively treated more than 3 years priorto 1st dose of investigational drug	☐	☐	
5. Prior sensitivity to Plerixafor.	☐	☐	

6. Pregnant or patients who are breastfeeding	☐	☐	

*All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

This subject is [☐ eligible / ☐ ineligible] for participation in the study.

Treating Physician Signature:	Date:
Printed Name:	

Secondary Reviewer Signature:	Date:
Printed Name:	

Study Coordinator Signature:	Date:
Printed Name:	