Phase I/II study of local field irradiation and temozolomide followed by continuous infusion Plerixafor as an upfront therapy for newly diagnosed glioblastoma GBM.

NCT01977677

29-Apr-2015

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Version #11 / Version Date: (04-29-2015)

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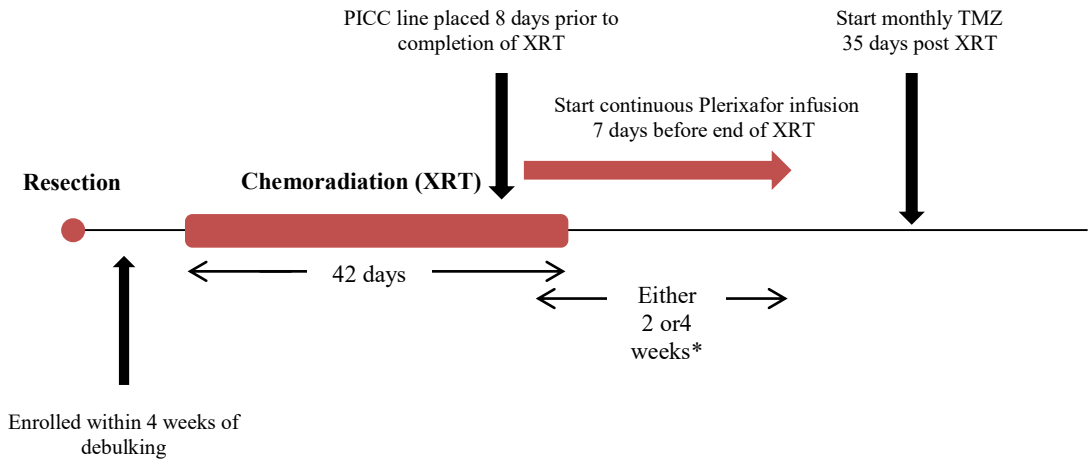
PROTOCOL SYNOPSIS

TITLE	A Phase I/II study of local field irradiation and temozolomide followed by continuous infusion Plerixafor as an upfront therapy for newly diagnosed glioblastoma GBM.
STUDY PHASE	I/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
SECONDARY OBJECTIVE(S)	Safety of the continuous infusion of Plerixafor subsequent to irradiation
TREATMENT SUMMARY	200 micrograms per kilogram per day for four weeks, in second patient will increase to target dose of 400 micrograms per kilogram per day for four weeks, and continue treatment of subsequent patients at the target dose if tolerated.
SAMPLE SIZE	29 patients
STATISTICAL CONSIDERATIONS	Dose-escalation study with planned dose levels using the modified toxicity probability interval (mTPI). For efficacy, a greater than 50% PFS at 6 mos. PFS will be considered a positive signal and warrant further study.

SCHEMA



*Infusion will last for either two or four weeks with Phase I dose determined per the adjustment rules described in section 12.1; Phase II dose and duration determined in the Phase I

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
ECG	Electrocardiogram
GBM	Glioblastoma
GI	Gastrointestinal
HIV	Human Immunodeficiency Virus
IBW	Ideal Body Weight
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
RANO	Revised Assessment in Neuro-oncology
RR	Response rate
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
XRT	Chemoradiation

1. OBJECTIVES

1.1. Primary Objectives

- *To assess the safety of using continuous infusion Plerixafor beginning one week prior to the end of concurrent chemotherapy with Temozolomide and radiation therapy in patients with newly diagnosed GBM.*

- *To assess the efficacy of Plerixafor as measured by progression free survival at 6 months (PFS6) from the start of irradiation.*

2. BACKGROUND

2.1 Glioblastoma

Although radiotherapy delays the recurrence of most glioblastomas (GBMs), these tumors invariably regrow and prove fatal for more than 75% of the patients by two years after diagnosis. Importantly, most recurrent tumors recur within the field of high dose radiation [1-4]. Thus, any method of reducing local recurrences will improve the survival of GBM patients. Following irradiation, tumor recurrence requires the formation of new blood vessels. Inhibiting blood vessel growth will delay or prevent regrowth of GBM within an irradiated field and thus improve patient outcome.

Though it is often unrecognized by investigators in the field, tumors have two main ways to grow blood vessels: By angiogenesis, the sprouting of endothelial cells from nearby blood vessels, and vasculogenesis, the formation of blood vessels by circulating cells, primarily of bone marrow origin. The presence of circulating proangiogenic cells was first demonstrated by Asahara and colleagues [5], and is now recognized as a way in which blood vessels can be formed in damaged normal tissues and tumors, particularly following therapy [6, 7]. We have shown that local tumor irradiation, by killing the endothelial cells in and surrounding the tumor, abrogates local angiogenesis [8, 9]. The tumor must therefore rely on the vasculogenesis pathway for regrowth after irradiation.

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 [9, 10]. Perixafor is a reversible inhibitor of binding and would prevent or markedly delay 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

- State the primary purpose for the protocol:
  - **Treatment**
- State the interventional model from these choices:
  - **Single Group**
- State the number of intervention arms.
  - **One**
- State whether the study will be masked (at least one party is unaware of the treatment)
  - **Open**: no masking is used
- State whether the study is randomized.
  - **No**
- State type of primary outcome or outcome that the protocol is designed to evaluate:
  Safety (Phase I)
  Efficacy (Phase II)

Phase I

The Phase I is designed to evaluate safety. A total of 9 newly diagnosed patients will be enrolled within four weeks of surgery (biopsy, partial resection or gross total resection). In order to minimize the chances for local angiogenic migration into the irradiated field, irradiation will be administered to the area encompassed by T2 FLAIR imaging plus a 2 cm margin to 46 Gy with a focal boost to the enhancing area to 60 Gy over a six-week period. Temozolomide 75 mg/M^2/d starting the day of radiotherapy will be administered for 42 days concurrent with irradiation. Eight days prior to irradiation completion, patients will have a PICC line placed. Seven days prior to irradiation completion, the continuous infusion of Plerixafor will be initiated using a step-wise dose escalation based on the modified toxicity probability interval (mTPI) method. All treatments will start one week prior to completion of radiotherapy with concurrent temozolomide for patients with newly diagnosed glioblastoma. This dose escalation study begins with an intermediate dose of 200 µg/kg per day for four weeks. The first 3 patients will be enrolled at this starting dose. Enrollment within a dose level will be staggered so that there is at least 1 week between patients. If tolerability and safety is established after the 4 week treatment period, then the fourth patient will be enrolled at the higher target dose of 400 µg/kg per day for four weeks. We will wait one week after the last infusion at one dose level before proceeding to the next dosage level.

If a patient experiences a DLT, then s/he will be removed from study and the next patient treated at the lower dose level as dictated by the mTPI table.

Once the infusion phase is completed, patients will then receive adjuvant temozolomide in standard monthly dosages (i.e., 150 mg/m^2 x 5 days), beginning at day 35 after finishing XRT (to ensure no overlap with Plerixafor).

Phase II
If in the Phase I, patients achieve the highest AUC level (i.e., 400 μg/kg per day for 4 weeks) without significant toxicity, we will then conduct a lead-in Phase II study wherein an additional 20 patients will be enrolled so that efficacy (i.e., PFS 6) can be measured. There will be no delay between enrolling patients in this group and multiple patients can be enrolled and treated at the same time.

2.5 Correlative Studies Background

We have recently developed and validated with several transplanted tumors in mice (including intracranially implanted human GBM) a new paradigm for the treatment by radiotherapy of solid tumors: 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 [9, 10]. The significance of our findings is illustrated by the fact that our strategy to prevent GBM recurrence has been highlighted in recent commentaries in prominent biomedical journals [11]. Furthermore, our findings of the importance of CD11b+ monocytes and/or macrophages to tumor response to irradiation have now been confirmed by others [12].

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 CD11b+ monocytes (which are highly proangiogenic) [13] both inhibits the radiation-induced influx of the CD11b+ monocytes and prevents tumor recurrence following single or fractionated doses of irradiation **(Figs 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 **(Fig 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 [10].

• 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 **(Figs 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 transactivates SDF-1, the key chemokine responsible both for the mobilization of bone marrow derived proangiogenic cells and their retention in the irradiated tumor. [9, 10].

• 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 **(Fig 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.

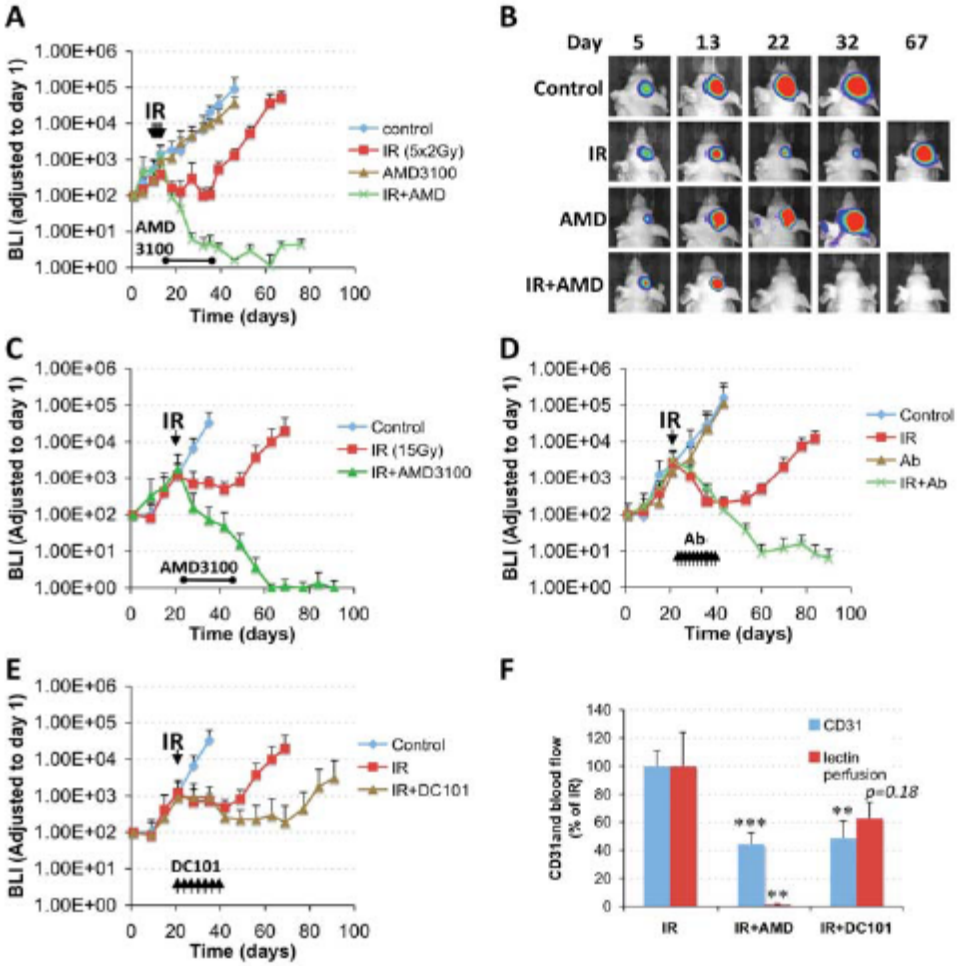


Figure 1. Inhibition of the interaction of SDF-1 with CXCR4 with AMD3100 prevents tumor recurrence post irradiation. A Growth curves of i.c. U251 by bioluminescent imaging (BLI) following whole brain fractionated irradiation (5 daily doses of 2 Gy starting on day 11 after transplantation) with AMD3100 (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]

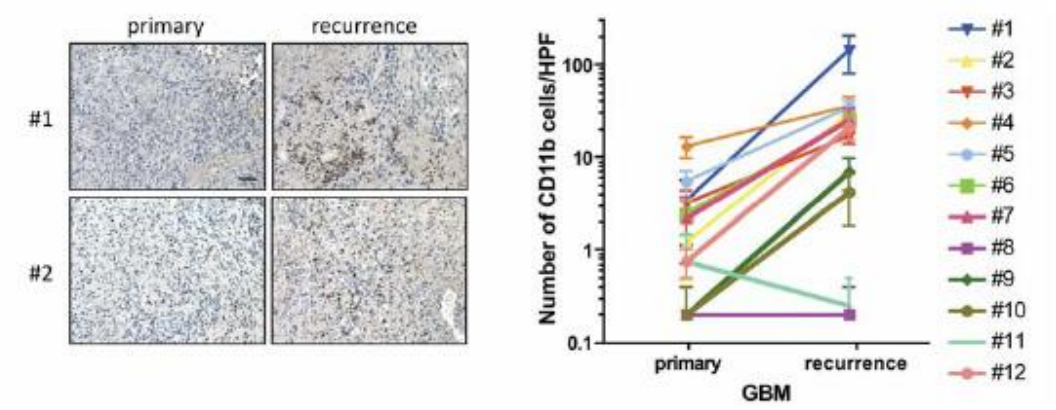


Figure 2. CD11b+ cells are increased with GBM tumor recurrences in patients., Left: IHC staining for CD11b of GBM clinical samples both before treatment (primary) and after recurrence **Right** Significant increased levels of CD11b+ cells in the recurrent human GBMs compared to the untreated tumors. 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) [17], initial Plerixafor clinical trials were conducted for the treatment of HIV-1 infection [18]. In follow-up to the observation that Plerixafor given to healthy volunteers and HIV-1 infected patients elicited increases in white blood cell (WBC) counts, studies have been done in healthy volunteers to assess the effect on circulating peripheral blood stem cells (PBSCs) [19]. 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 [20]. In fact, disruption of the SDF-1α/CXCR4 through G-CSF exposure [21] 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 [22]. 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) [23, 24].

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) [25]. 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 [26] and from 40–320 μg/kg in 29 additional healthy volunteers [27]. 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 [28]. 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 [29]. 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 postdose 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 320μg/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.

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 [30]. In this study, a 10 day infusion was administered via infusion pump at 40mL/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.

Plerixafor for Stem Cell Mobilization

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 [31]. 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 [32]. 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 [33]. 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 [34]. 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 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 [35]. 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 [36]. 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, hypoaesthesia oral, arthralgia, musculoskeletal pain, hyperhidrosis, erythema, injection site reactions, fatigue, and malaise [35, 36]. 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 [37-41]. 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. 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 [42]. 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 [43]. 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 [44-46], the latter speculated to potentially promote superior engraftment after high-dose chemotherapy [47].

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 [48]. 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 x 10^9 lymphocytes/kg vs. 0.288 x 10^9 lymphocytes/kg; $P < 0.0001$) correlated with better outcomes in progression-free survival after autologous transplant in NHL patients [49]. 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 [35] or MM [36] 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 [50].

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 [51-53]. 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) [54] 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 chemosensitization.

Pre-Clinical Studies of Chemosensitization with Plerixafor

In preclinical models of leukemia, targeting the microenvironment with CXCR4 antagonists was sufficient to overcome resistance to cytarabine [55] and also provided responsiveness to antibody-mediated cytotoxicity [56]. 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 [57]. 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 [58]. 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 [59]. 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 [60]. 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) [61]. 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 extramedullary 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 [61].

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.

Clinical Experience with Plerixafor for Sensitization to Leukemia Treatment

Elevated levels of CXCR4 expression on leukemic cells are associated with worse outcomes including shorter overall survival in AML [62-64]. Plerixafor has been shown to mobilize leukemic cells in humans [65] 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 [66].

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 [67]. 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 hyperleukocytosis 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 [67].

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 [68]. 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 x 10^9/L) and platelet ($\geq$100 x 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 [53]. 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 $\geq$ 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 [69]. 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 chemosensitization 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

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 [70]. 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 subtherapeutic doses of chemotherapy [71].

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 [11, 72]. 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 [10]. 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 [73]. 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 [32]. 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 [32], 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.

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, glioblastoma with PNET features.

3.1.2 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.

3.1.3 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.

3.1.4 For those patients in which steroids are clinically indicated, there must be a stable or decreasing dose of steroid medication for ≥ one week prior to the start of infusion.

3.1.5 Patients must be between the ages of 18 and 75 years old.

3.1.6 Patients must have Karnofsky Performance score ≥ 60.

3.1.7 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. Serum potassium, magnesium and calcium within normal limits (supplementation to maintain normal electrolyte levels is acceptable)
6. If female of childbearing potential, negative pregnancy test

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

3.1.9 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 or arrhythmia.

3.2.5 Prior malignancy except previously diagnosed and definitively treated more than 3 years prior to trial or whose prognosis is deemed good enough to not warrant surveillance.

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. The participant 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 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 yearsthe 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 **7 days prior to the start of Plerixafor infusion (+/- 3 days):**

- Written informed consent must be obtained from the patient prior to performance of any study-specific tests or procedures
- Confirm Eligibility Criteria
- Post-operative, contrast-enhancing imaging
- Demographics: birth date, race/ethnicity and gender at birth
- Medical history
- Cancer history
- Physical Exam
- KPS
- Concomitant medications
- Vital Signs (including height and weight)
- ECG
- 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 and magnesium), Pregnancy Test (only for women of child bearing potential), INR, PTT, and Urinalysis with microscopic

Day 0 (within 3 days of beginning of Plerixafor infusion) :

- Placement of the PICC line and infusion pump

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

- Physical Exam
- KPS
- Vitals: pre-dose, 15 min (+/- 5 min), 30 min (+/- 5 min), 1 hr (+/- 15 min), 2 hrs (+/- 15 min) 3 hrs (+/- 30 min) and 6 hrs (+/-30 min) after the start of Plerixifor infusion
- 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 and magnesium), Troponin
    - 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 temodar and radiation and at the investigator's discretion; Thrombocytopenia should be ≤ grade 2
- Phase I only: SDF-1 levels (within 24 hours prior to the start of infusion)
- Phase I only: Samples for PK analysis will be drawn prior to and 1 hour (+/- 10 min) after start of the infusion of Plerixafor

- ECGs: prior to the start of the Plerixafor infusion and 6 hours (+/- 30 min) after
- Phase I only: ECG 3 hours (+/- 30 min) after start of Plerixafor infusion
- Begin Plerixafor infusion in outpatient unit.
- Observe patients for 6 hours after the start of the Plerixafor infusion
- Patient will be discharged home after at least 6 hours with infusion pump.

Day 4 (+/- 2 days) for Phase I patients only

- 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 and magnesium),
- ECG

Weekly following the start of the Plerixafor infusion through the end of infusion (+/- 3 days):

- Physical Exam
- KPS
- Vitals
- ECG
- 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 and magnesium)
- Phase I only: PKs
- Change infusion bag of Plerixafor
- Review concomitant medications and adverse events
- On the last day of infusion, an SDF level will be checked (+/-1 week)

- **Biweekly following the start of Plerixafor infusion through the end of infusion (+/- 3 days):**Laboratory tests: Troponin

4 weeks after the end of XRT (+/- 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

Post-Progression 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) following disease progression to collect data regarding survival status and subsequent anticancer therapy. Follow up will continue for either 5 years or until death, withdrawal, lost to follow up, or study termination.

4.1 General Concomitant Medication and Supportive Care Guidelines

The use of all standard supportive medication, including appropriate antimicrobrial 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 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
- DLT (any AEs with suspected causal relationship with Plerixafor >= grade 3 including ECG changes indicative of ischemia, ventricular tachycardia)
- Investigator decision (eg, 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 four possible dose levels are:

- 200 µg/kg per day for 2 weeks
- 200 µg/kg per day for 4 weeks
- 400 µg/kg per day for 4 weeks
- 100 µg/kg per day for 4 weeks

The starting dose in the Phase I is 200 µg/kg per day for 4 weeks

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. 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 dilutent

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. Each vial will be affixed with a label describing the protocol number, content of each vial, lot number, required cautionary statements or regulations, storage conditions, and the Sponsor's name and address.

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.

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.

6.1 Dose Limiting Toxicities

DLT is defined as any hematologic or non-hematologic AE grade ≥3 with a suspected causal relationship to Plerixafor (including including ECG changes indicative of ischemia, ventricular tachycardia).

- Patients will be monitored for DLTs for 5 weeks from the start of the Plerixafor infusion.
- DLT's in the first cohort may occur before or after 14 days. If a DLT occurs in weeks 3 or 4 of treatment, we will treat next patient with a dose of 200 µg/kg/d x2 weeks. If a DLT occurs from the start of treatment through the second week of treatment, we will

treat next patients at a dose of 100 µg/kg/d x 4 weeks.If a patient experiences a DLT, the infusion will be discontinued. The next patient treated at the lower dose level as dictated by the mTPI table. If an early dose limiting toxicity occurs at lowest dose level (200 µg/kg/d x 2 wk or 100µg/kg x 4wks) then the protocol will be stopped at that point.

6.2 Infusion Reactions

For the start of the Plerixafor infusion, all patients will be closely monitored on site for at least 6 hours. All patients will be evaluated weekly by a physician for the duration of the infusion.

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

Since prior studies using this medication have included premature ventricular contractions (PVCs), there will be a temporary interruption of plerixafor for treatment for abnormalities in serum magnesium, calcium, or potassium at a Grade 2 or more severe. The study medication may be resumed following correction of these electrolyte abnormalities as confirmed by repeat serum level testing. Supplementation to include magnesium sulfate, calcium carbonate, or potassium chloride may be used as clinically indicated.

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 4.03, May 2009). Grades refer to the severity of the AE. The CTCAE v 4.03 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-Hodgkins'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 v4.03. 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), [redacted] (US) or [redacted] or via email at: [redacted] 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 [redacted] (US) or Sanofi Customer Services Europe, [redacted]
- 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 Genzyme PV.

8. CORRELATIVE/SPECIAL STUDIES

Patients will undergo pharmacokinetic analysis

8.1 Laboratory Correlative Studies

8.1.1 Pharmacokinetic analysis of Plerixafor

8.1.1.1 Collection of Specimen(s)

Pharmacokinetics

Patients enrolled in the Phase I will undergo pharmacokinetic analysis. 2.5 mL of blood will be collected in Na-Heparin tubes. Samples will be drawn prior to and 1 hour after start of the infusion of Plerixafor and weekly afterwards while infusions continue, the last sample being drawn at the time the infusion is discontinued. Samples will be processed at Stanford and then sent to Tandem Laboratories who will measure plasma levels of Plerixafor.

SDF-1

Blood samples will be collected in two 8 mL green top tubes at within 24 hours to the start of Plerixafor infusion and at time of infusion discontinuation (+/- 1 week)

8.1.1.2 Handling of Specimens(s)

Pharmacokinetics:
Minimum of 0.5 mL in the primary and 0.5 mL in the back up

SDF-1

1. Peripheral blood will be drawn into 2 green top tubes and **placed immediately on ice**.
2. Peripheral blood mononuclear cells will be isolated by Ficoll centrifugation technique under sterile conditions:
3. 2 green top tubes (16 mL) will be placed into one 50mL conical tube
4. Volume will be brought to 50mL with RPMI per conical.
5. One 50mL sample will be divided into two 50mL tubes and underlayed with 13mL of Ficoll (now total of 2 conicals).
6. Centrifuge at 400G for 30min with NO break at room temperature (24C).
7. Plasma layer will be removed and frozen in four aliquots at -80C for future assessment of SDF-1 level by ELISA.
8. Buffycoat will be removed and washed 3 times with 30mL of RPMI in a 50mL tube pelleting by centrifuge for 15 minutes at 1200G (1800 RPM) with break.
9. Sample will be resuspended in 1mL of cRPMI and cell count performed.
10. Aliquots of live cells ($20x10^6$ per aliquot) will be frozen in FCS + 10% DMSO. Samples will be placed in -80 for 3 days then in liquid nitrogen for storage

8.1.1.3 Shipping of Specimen(s)
PKs will be batch shipped to Tandem Laboratories at the completion of the Phase I.

SDF-1 will be picked up by a representative of [redacted] lab at Stanford University

8.1.1.4 Site(s) Performing Correlative Study
Pharmacokinetics
Samples will be processed at Stanford and then sent to Genzyme/Sanofi who will measure plasma levels of Plerixafor.

SDF-1
Samples will be processed at Stanford and picked up by a representative of [redacted] lab at Stanford University

8.1.1.5 Coding of specimens for privacy protection
All PK and SDF-1 samples will be labeled the patient's study specific study number and initials.

9. STUDY CALENDAR

	Day -7 (+/- 3)	Day 0 (+/-3)	Day 1	Day 4 [b] (+/-2)	Day 8 (+/- 3)	Day 15 (+/- 3)	Day 22 (+/- 3)	Day 29 (+/-3)	Day 35 (+/-7)	30 days post Plerixafor	MRI Eval (every 8-12 wks per SOC)	6 months after start of XRT (+/- 2 wks)	Every 3 months after progress-ion
Continuous Plerixafor Infusion			X----------|----------|[a]										
PICC Placement		X											
Informed Consent	X												
Demographics	X												
Medical History	X												
Con Meds	X----------|											
Physical Exam	X		X		X	X	X [a]	X[a]					
Vitals	X		X	X [b]	X	X	X [a]	X[a]					
Height	X												
Weight	X												
KPS	X		X		X	X	X [a]	X[a]					
CBC with diff	X		X	X [b]	X	X	X [a]	X[a]					
Serum Chemistry	X		X	X [b]	X	X	X [a]	X[a]					
Troponin			X			X		X[a]					
Urinalysis	X												
Coag Panel (INR/PTT)	X												
SDF-1[b]			X			X[c]		X[ac]					
PK[b]			X		X	X	X [a]	X[a]					
ECG	X		X	X [b]	X	X	X [a]	X[a]					
Adverse Events			X----------|										
MRI									X		X	X	
Pregnancy Test	X												
TMZ (per SOC)									X----------|			
Survival Status													X

a. Not applicable for patients enrolled in the 200 µg/kg/d x 2 wks (continuous infusion) dose level
b. Phase I patients only
c. At discontinuation of Plerixafor infusion (+/- 1 week)

10. MEASUREMENTS

For clinicaltrials.gov and Stanford Clinical Trials Directory compliance

Primary Outcome Measure Definition: Safety and tolerability of the continuous infusion of Plerixafor subsequent to irradiation.

Title: A Phase I/II study of local field irradiation and temozolomide followed by continuous infusion Plerixafor as an upfront therapy for newly diagnosed glioblastoma GBM.

Time Frame: Six month progression free survival from first day of radiation.

Safety Issue: Is this outcome measure assessing a safety issue? Yes

> **Note: Each outcome measure listed within the protocol will necessitate legally required results reporting to clinicaltrials.gov within one year after the completion of the primary outcome measure.**

10.1 Primary and Secondary Outcome measures

Our primary objective in the Phase I component is to ascertain whether the highest dose is tolerated with acceptable side effects (400 micrograms per kilogram per day for four weeks). Our Primary objective in the Phase II component is to assess progression free survival at 6 months.

10.1.1 Relevant Subset

The first 9 patients will be treated accordingly to the dosing algorithm depicted in Table 12.1. The starting dose will be 200 micrograms per kilogram per day for four weeks. We will wait one week after the completion of the infusion at one dose level before proceeding. Once the final dose is determined at the Phase I portion of this study, then another 20 patients will be treated at the dose determined maximally tolerable.

10.1.2 Measurement Definition

- The primary outcome is dose-limiting toxicity, which is defined as the absence of cardiac arrhythmia or grade III or IV adverse events.

10.1.3 Measurement Methods
ECG will be performed twice weekly basis during treatment, a baseline troponin at the initiation of the study, and physical examination weekly during treatment. Follow up will take place with physical examination and a clinic visit monthly after treatment.

10.1.4 Measurement Time Points
ECG will be assessed on a twice weekly basis during treatment, a baseline troponin at the initiation of the study, and physical examination weekly during treatment. Follow up will take place with

physical examination and a clinic visit monthly after treatment.

10.1.5 Response Review
Not applicable.

10.2 Secondary Outcome: Efficacy

10.2.1 Relevant Subset
Patients in the Phase II study will be evaluated for PFS 6 months after completion of chemoradiation

10.2.2 Measurement Definition
This endpoint of PFS6 months post start of chemoradiation will be assessed using RANO criteria.

10.2.3 Measurement Methods
Endpoint will be measured based on the RANO criteria, using both clinical examinations and MRIs with and without contrast.

Complete Response (CR): Requires all of the following:

- Complete disappearance of all enhancing measurable and non-measurable disease
- Stable or improved non-enhancing (T2/FLAIR) lesions
- No new lesions
- Off corticosteroids (or on physiologic replacement dose)
- Stable or improved clinical status

Partial Response (PR): Requires all of the following:

- ≥ 50% decrease compared with baseline in the sum of the products of the diameter (SPD) of measurable enhancing target lesions
- No progression of non-target disease (enhancing and non-enhancing (T2/FLAIR) lesions)
- No new lesions
- Corticosteroid dose stable or decreased
- Stable or improved clinical status

Stable Disease (SD): Requires all of the following:

- Does not qualify for CR, PR or PD
- Stable non-enhancing (T2/FLAIR) lesions
- Corticosteroid dose not increased ≥ 50%
- 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 (eg 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 (eg, seizures, medication adverse effects, complications of therapy, cerebrovascular events, infections etc)
- Increasing steroid doses alone do not constitute PD.

10.2.4 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.

10.2.5 Response Review
The Investigators delegated to do so, will evaluate the MRIs for response

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 OnCore database system and will be maintained by [redacted] CRFs will be kept in a locked office, only accessible to the research team.

12. STATISTICAL CONSIDERATIONS

[redacted] in the department of Biostatistics, Health Research and Policy will serve as the primary statistician.

12.1 Statistical Design

This is a dose-escalation study with three planned dose levels using the modified toxicity probability interval (mTPI). The mTPI method is a competitor to the usual 3+3 dose escalation scheme and to the Continuous Reevaluation Method (CRM), mTPI has the advantage of having been peer-reviewed in both the statistical literature and in the oncology literature [74]. The design has the further advantage of allowing the investigators to conduct the study without constant reference to a biostatistician (as required by the CRM approach). It also allows for planned de-escalation as in this protocol

Planned dose levels are 200 µg/kg/d x 2 wks (continuous infusion), 100 µg/kg/d x 4 wks, 200 µg/kg/d x 4 wks, and 400µg/kg/d x 4 wks. The starting total dose will be the middle dose 200 µg/kg/d x 4 wks.

If a DLT occurs from Day 15-28, we will treat next patient with a dose of 200 µg/kg/d x 14 days. If a DLT occurs from day 0-14, we will treat next patients at a dose of 100 µg/kg/d x 28 days. If a patient experiences a DLT, the infusion will be discontinued. The next patient treated at the lower dose level as dictated by the mTPI table. If an early dose limiting toxicity occurs at lowest dose level,200 µg/kg/d x 2 wk or 100µg/kg x 4wks, then the protocol will be stopped at that point.

The design is based on a Bayesian calculation of the posterior probability of toxicity at each dose. The following settings have been used:
* The target toxicity level. In this study we use the same level of 30% as in [74], which also corresponds informally to the target toxicity of the 3+3 design.
* The precision for that target toxicity (here plus or minus 5 percentage points).
* The maximum sample size across all dose (here 9).
* Planned dose levels 200 µg/kg/d x 2 wks (continuous infusion), 100 µg/kg/d x 4 wks, 200 µg/kg/d x 4 wks and 400µg/kg/d x 4 wks. The starting total dose will be the middle dose 200 µg/kg/d x 4 wks.

The prior is a beta with parameters 1 and 1 (roughly equivalent to two observations, one of these a DLT). Briefly, with the above settings, when the posterior probability falls below 0.25 (0.30-0.05), escalation to the next higher dose level is indicated; when the posterior probability falls above 0.35 (0.30+0.05), de-escalation is recommended; otherwise the next patient is to be treated at the same dose level. The rules for escalation and de-escalation for this design are contained in Table 12.1; they do not need to be modified during the course of the study. At the end of the study the toxicity data at all doses is combined using the "pool adjacent violators" algorithm to make toxicity probabilities non-decreasing; the maximum tolerated dose is calculated as the dose level that comes closest to the target toxicity level.

Table 12.1 show the action to be taken as each patient becomes evaluable for toxicity. It is used for all dose levels as follows:

- E means that the next patient is to be treated at the next higher dose. If the current dose is the highest planned 400 µg/kg/d x 28 d, the next patient is to be treated at the **same** dose level.
- S means that the next patient is to be treated at the same dose level. **Note that a minimum of 3 subjects must be studied at a dose before escalation is indicated.**
- D means that the next patient is to be treated at the next lower dose. If the current dose is the

lowest planned 200 µg/kg/d x 14 d, the next patient is to be treated at the **same** dose level.

- U means that the current dose level is too toxic. No further patients are to be treated at this or at any higher dose level. If the current dose level is the lowest planned (200 µg/kg/d x 2 wks total dose) no further patients will be treated. If the current dose level is not the lowest planned, the next patient should be studied at the next lowest dose (100 µg/kg/d x 4 wks).

These definitions are in accord with our reading of the code used to produce the simulations which validated the mTPI design.

Toxicity will be monitored in the expansion phase at each increment of 5 patients. If DLT is observed in one out of the first 5 patients, or 2 of the first 10, or 2 of the first 15, toxicity will be reviewed to assess whether the trial should continue. This stopping rule has a 5% chance of being triggered if the true DLT toxicity rate is 5%, it has a 62% chance of being triggered if the true DLT rate is 20% and an 88% chance of being triggered if the true DLT rate is 30%.

12.1.1 Randomization

Not applicable.

12.2 Interim analyses

Patient characteristics will be summarized using proportions for categorical variables, means and standard deviations for continuous variables (median and inter-quartile rages for variables that exhibit skewness). 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, CRi, PR, SD will be computed along with an exact 95% confidence interval. Duration of response progression-free survival and overall survival will be computed from start of induction therapy and summarized with Kaplan-Meier estimates.

12.3 Descriptive Statistics and Exploratory Data Analysis

Not applicable.

12.4 Primary Analysis

For the Phase II component, it is expected that no more than 50% of patients will have stable disease at 6 months (defined as stable MRI and clinical status, including being on a stable dose of steroids and not on bevacizumab). We will therefore consider this regimen of interest if significantly more than 50% of patients achieve this benchmark.

12.4.1 Analysis Population

A total of 9 patients will be treated in the Phase I component with the first at the starting dose of 200 µg/kg/d x 4 wks. If the regimen proves too toxic or if none of the dose levels appears to reach 30% DLT, fewer patients may be treated.

Table 12.1

		Cumulative Number of Patients Treated at This Dose								
		1	2	3	4	5	6	7	8	9
Cumulative Number of DLTs at This Dose	0	E	E	E	E	E	E	E	E	E
	1	D	S	S	S	S	E	E	E	E
	2		U	D	S	S	S	S	S	S
	3			U	U	D	S	S	S	S
	4				U	U	U	D	D	S
	5					U	U	U	U	U
	6						U	U	U	U
	7							U	U	U
	8								U	U
	9									U

12.4.2. Analysis Plan
Since our primary objective in the Phase I component is to ascertain whether the highest dose is tolerated with acceptable side effects, this will be completed using the schema described in Table 12.1.

12.4.3.
Patients will be followed clinically, by ECG, and laboratory measures to ensure that no serious adverse effects occur and that safety is established at the proposed treatment doses.

12.5 Secondary Analysis

Our Primary objective in the Phase II component is to assess progression free survival at 6 months.

12.5.1 Analysis Population

20 additional patients will be enrolled in the Phase II component of this study for determination of efficacy.

12.5.2 Analysis Plan

For the Phase II component, it is expected that no more than 50% of patients will have stable disease at 6 months (defined as stable MRI and clinical status, including being on a stable dose of steroids and not on bevacizumab). We will therefore consider this regimen of interest if significantly more than 50% of patients achieve this benchmark.

12.6 Sample Size

12.6.1 Accrual estimates

We expect to accrue the total number of 29 patients within the 36 month timeline proposed.

12.6.2 Sample size justification

In collaboration with our statistician, it was determined that our sample size of 29 total patients would substantiate enough power to reach our points of analyses.

12.6.3 Effect size justification

This is a single-arm (non-randomized) study, therefore the historical control rate has been used to

determine our effect size justification.

12.7 Criteria for future studies

This is a pilot study with the expectation that no more than 50% of patients will have stable disease at 6 months (defined as stable MRI and clinical status, including being on a stable dose of steroids and not on bevacizumab). We will therefore consider this regimen of interest if significantly more than 50% of patients achieve this benchmark and would move further to future studies should this benchmark be reached.

13. REFERENCES

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APPENDICES

APPENDIX A**: Participant Eligibility Checklist**

Protocol Title:	A Phase I/II study of local field irradiation and temozolomide followed by continuous infusion Plerixafor as an upfront therapy for newly diagnosed glioblastoma GBM.
Protocol Number:	
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. For those patients in which steroids are clinically indicated, there must be a stable or decreasing dose of	☐	☐	

steroid medication for ≥ one week prior to the start of infusion.			
5. Patients must be between the ages of 18 and 75 years old	☐	☐	
6. Patients must have Karnofsky Performance score ≥ 60.	☐	☐	
7. 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. Serum potassium, magnesium and calcium within normal limits (supplementation to maintain normal electrolyte levels is acceptable) 6. If female of childbearing potential, negative pregnancy test	☐	☐	
8. The patient or his/her legal representative must have the ability to understand and willingness to sign a written informed consent document.	☐	☐	
9. 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 or arrhythmia.	☐	☐	

5. Prior malignancy except previously diagnosed and definitively treated more than 3 years prior to trial or whose prognosis is deemed good enough to not warrant surveillance.	☐	☐	
6. Prior sensitivity to Plerixafor.	☐	☐	
7. 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.

Signature:	Date:
Printed Name:	

APPENDIX B**: Protocol Pre-review Checklist**

No	Description	Yes	No
1	Protocol is in the appropriate template for either Interventional or Non-interventional study		
2	Title page includes names and addresses of Principal Investigator, Co-investigators, Biostatistician and Coordinator as appropriate		
3	Sponsor information is included if appropriate		
4	Title page includes protocol version and date		
5	Protocol document has page numbers		
6	Schema is legible		
7	List of Abbreviations is relevant to this protocol		
8	Table of contents is complete		
9	Instructions in the template are deleted		
10	Section titles are appropriately designated and numbered		
11	All the sections in the template are complete		
12	Study calendar is complete		
13	Protocol contains adequate background/rationale information		
14	Objectives and measurements of outcome are clearly stated		
15	The sections on objectives, eligibility, outcome measurements, statistics and study calendar are consistent		
16	Monitoring plan refers to Stanford DSMC		
17	Statistical section is appropriate for this study		
18	Appropriate references are included		
19	Protocol includes eligibility checklist		
20	Protocol includes questionnaires as needed		
21	Protocol includes CRF statement		
22	Protocol document is well organized		

Note: Some of the requirements are applicable to only **Interventional** studies as specified in the template.