Stanford Cancer Institute

TITLE: Phase II Study of MGTA-145 in combination with plerixafor in the mobilization of hematopoietic stem cells for autologous transplantation in patients with multiple myeloma

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PROTOCOL SYNOPSIS

TITLE: Phase II Study of MGTA-145 in combination with plerixafor in the mobilization of hematopoietic stem cells (HSCs) for autologous transplantation in patients with multiple myeloma

STUDY PHASE: Phase 2, open label, proof of concept study

STUDY CENTER: Single Center, Stanford Cancer Institute

INVESTIGATIONAL PRODUCT: MGTA-145 (intravenous drug)

INDICATION: Hematopoietic stem cell (HSC) collection for autologous stem cell transplantation in multiple myeloma

PRIMARY OBJECTIVE(S)

1. To assess the efficacy of MGTA-145 in combination with plerixafor in mobilizing adequate number of hematopoietic stem cells ($\geq$ 2 x 10^6 CD34+ cells/kg) in patients with multiple myeloma (MM) in preparation for autologous stem cell transplantation (ASCT).

SECONDARY OBJECTIVE(S)

1. To assess the efficacy of MGTA-145 and plerixafor in mobilizing different HSCs target goals in patients with MM in preparation for ASCT.
2. To assess the safety and tolerability of MGTA-145 and plerixafor for mobilizing HSCs in patients with MM.
3. To assess the engraftment rate and time to engraftment following ASCT after HSC mobilization with MGTA-145 and plerixafor in patients with MM undergoing upfront ASCT.
4. To assess rate of ongoing engraftment at day 30 and 100 after stem cell infusion in patients with MM who are mobilized with MGTA-145 and plerixafor undergoing upfront ASCT.
5. To assess transplant and disease-related outcomes after mobilization of HSCs with MGTA-145 and plerixafor in patients with MM undergoing upfront ASCT.

PRIMARY ENDPOINT(S)

1. To evaluate the rate of successful collection in patients with MM of ≥2.0 x 10^6 $CD34^+$ cells/kg in up to two days of apheresis after MGTA-145 + plerixafor dosing.

SECONDARY ENDPOINT(S)

1. To assess the rate of successful collection of **(a)** ≥4.0 x 10^6 $CD34^+$ cells/kg in up to two apheresis sessions after MGTA-145 and plerixafor dosing in patients with MM **(Key Secondary Endpoint) (b)** ≥6.0 x 10^6 $CD34^+$ cells/kg with up to two apheresis sessions after MGTA-145 and plerixafor dosing in patients with MM **(c)** ≥2.0 x 10^6 $CD34^+$ and ≥4.0 x 10^6 $CD34^+$ cells/kg with one apheresis session after MGTA-145 and plerixafor dosing.
2. To assess infusion related toxicities with MGTA-145 and adverse events with mobilization of HSCs with MGTA-145 and plerixafor.

3. To assess time to neutrophil (defined as the first day of ANC ≥0.5 x 10^9/L for 3 days following stem cell infusion) and platelet engraftment (defined as first day of platelet count more than or equal to 20 x 10^9/L without transfusion in the last 7 days and with platelet count ≥20 x 10^9/L on 2 separate, subsequent days), and to also determine time to first day of platelet count ≥ 50 x 10^9/L, without need for platelet transfusion in 48 hours in patients undergoing upfront ASCT.
4. To assess rates of ongoing successful engraftment at 30- and 100-days post-transplant after successful primary engraftment in patients undergoing upfront ASCT.
5. To assess rate of transplant related mortality, non-relapse mortality, progression free survival and overall survival at 100 days in patients with undergoing upfront ASCT.

Exploratory Objectives

1. Assess the composition of the apheresis product with mobilization with MGTA-145 and plerixafor in patients with MM.
2. Assess proliferation and differentiation potential of HSCs collected with MGTA-145 and plerixafor via colony-forming unit assays.
3. To assess immunological recovery in patients with MM undergoing upfront transplantation after infusion of HSCs collected with MGTA-145 and plerixafor mobilization.
4. To assess patient reported symptoms with stem cell mobilization with MGTA-145 and plerixafor in patients with MM.

Exploratory Endpoints

1. To assess the composition of the apheresis product obtained after MGTA-145 and plerixafor mobilization through enumeration $CD34^+CD90^+CD45RA^-$ cells in the product and immunological characterization of the T cell subsets, B cell subsets, NK cells, neutrophils, and myeloid / monocytic cells in the product using flow cytometry and CyTOF (Cytometry by Time of Flight) and minimal residual disease (MRD) assessment using next generation flow cytometry (NGF).

2. Assess proliferation and differentiation potential of HSCs collected with MGTA-145 and plerixafor via colony-forming unit assays from the apheresis autograft.

3. To assess immunological characterization of white cell population subsets (T cell subsets, B cell subsets, NK cells, neutrophils, and myeloid / monocytic cells) in patient's peripheral blood at day 28 using flow cytometry and at day 100 using flow cytometry and mass cytometry/CyTOF and assess immunological characterization of white cell population subsets in the bone marrow microenvironment at day 100 following transplant.

4. To assess patient reported symptoms, using items for infusion reaction and pain adapted from the patient reported outcomes common terminology criteria for adverse events (PROCTCAE) as well as the brief pain inventory tool following plerixafor and MGTA-145 infusion.

BACKGROUND AND RATIONALE:

Autologous stem cell transplant is an effective therapy for multiple myeloma and remains a standard of care for eligible patients.[1] While most patients undergo upfront transplant soon after stem cell collection, some patients may opt to delay transplant until relapse. Collection of CD34+ hematopoietic stem cells (HSCs) to rescue the bone marrow from effects of high-dose chemotherapy

in ASCT is most commonly done through mobilization and collection of HSCs from the peripheral blood. The two common strategies to achieve this are chemotherapy-based mobilization along with granulocyte colony stimulating factor (G-CSF) or G-CSF mobilization with or without plerixafor. While these methods are efficacious, the process can be long and is associated with some adverse effects. For example, chemotherapy-based mobilization can take two to three weeks and result in complications like neutropenic fever and significant cytopenias.[2-4] G-CSF injections can result in significant bone pain. Rare serious adverse effects with G-CSF include splenic rupture and pulmonary toxicity. Even with G-CSF and plerixafor use, at least 4 days of GCSF injections are needed even before mobilization can begin, requiring 5-8 days for collection.[5]

The ideal stem cell mobilization and collection method should result in rapid and reliable collection of adequate CD34+ HSCs, with minimal adverse effects. A minimum of 2.0 x 10^6 $CD34^+$ cells/kg are recommended to proceed with transplant per Stanford BMT Division institutional protocol and International Myeloma Working Group guidelines. Collection of an additional 2.0 x 10^6 $CD34^+$ cells/kg is preferred as a back-up stem cell infusion per institutional protocol.

Innovation in the stem cell mobilization method to reduce the time taken to collect stem cells and decrease adverse effects will be beneficial from both patients and health care resource utilization. MGTA-145 is a CXCR2 agonist, which has shown promising activity for rapid mobilization of CD34+ HSCs in pre-clinical models.[6] Its activity was synergistic with plerixafor, a CXCR4 antagonist, in animal models and in a phase I study of healthy volunteers, with rapid mobilization of stem cells.[7]

Given the promising results seen in pre-clinical models and a phase 1 study of healthy volunteers, we expect this agent to result in fast, effective and safe stem cell mobilization in patients with multiple myeloma, in combination with plerixafor. This **phase II proof of concept study** will evaluate stem cell mobilization in patients with myeloma with a combination of MGTA-145 and plerixafor. The dose of MGTA-145 (0.03 mg/kg) in combination with plerixafor has been identified in a phase I study of healthy volunteers.[7] There were minimal additional side effects observed with MGTA-145 in that study, with most patients reporting transient back pain lasting less than 20 minutes in the MGTA-145 vs. placebo arm. Additional expected side effects, including diarrhea, were noted in the MGTA-145 + plerixafor arm.[7] With this regimen, patients will undergo same day mobilization after administration of MGTA-145 and plerixafor for a maximum of 2 apheresis sessions.

TREATMENT SUMMARY:

This is a phase 2 study to evaluate safety and efficacy of stem cell mobilization (for high-dose therapy and autologous stem cell rescue) with a combination of MGTA-145 and plerixafor, followed by same day apheresis in patients with multiple myeloma. The dose of MGTA-145 in combination with plerixafor has been determined in a phase 1 study of healthy volunteers. Patients will undergo up to 2 days of mobilization followed by same day apheresis sessions.

The primary endpoint is successful collection of at least ≥2.0 x 10^6 $CD34^+$ cells/kg with up to two days of apheresis sessions after MGTA-145 and plerixafor dosing. Key secondary endpoint is the collection of ≥4.0 x 10^6 $CD34^+$ cells/kg in up to two days of apheresis. Other secondary endpoints include collection of ≥2.0 x 10^6 $CD34^+$ cells/kg and ≥4.0 x 10^6 $CD34^+$ cells/kg on day one of apheresis and of ≥4.0 x 10^6 $CD34^+$ cells/kg and ≥6.0 x 10^6 $CD34^+$ cells/kg in up to two days of apheresis after MGTA-145 and plerixafor dosing, assessment of adverse events and successful engraftment as described above.

The study is divided into three phases:

1. Pre-mobilization phase:

Patients will undergo screening procedures after signing informed consent. They will undergo baseline evaluation during the pre-mobilization phase up to 30 days before mobilization and be enrolled if they meet eligibility criteria.

2. Stem cell mobilization/apheresis phase:

Patients will undergo sequential administration of plerixafor 0.24 mg/kg (dose 0.16 mg/kg for creatinine clearance $\leq$ 50 ml/minute) subcutaneously followed 2 hours later by MGTA-145 at a dose of 0.03 mg/kg intravenously. This will be followed by apheresis. Apheresis should begin as soon as feasible after MGTA-145 infusion is complete.

Each session of apheresis will be standardized to **process 3 blood volumes, with a margin of +/- 10% or 4.5 hours, whichever is longer.**

A second day of mobilization and apheresis will be pursued in patients who have not collected 6.0 x 10^6 $CD34^+$ cells/kg in one session.

A minimum of 2.0 x 10^6 $CD34^+$ cells/kg are recommended to proceed with transplant per Stanford BMT Division institutional protocol and International Myeloma Working Group guidelines. Collection of an additional 2.0 x 10^6 $CD34^+$ cells/kg is preferred as a back-up stem cell infusion per institutional protocol.

If the minimum goal of 2.0 x 10^6 $CD34^+$ cells/kg is not met after 2 days of collection, patients may proceed with washout protocol and alternative stem cell mobilization strategy at the discretion of the treating physician.

For patients collecting between 2.0 and 4.0 x 10^6 $CD34^+$ cells/kg, patients may proceed to transplant or collect additional stem cells with alternative strategies to meet the total goal of 4.0 x 10^6 $CD34^+$ cells/kg per treating physician discretion. If an additional collection is sought at the discretion of the treating physician, it is recommended not to combine the standard of care mobilized graft and the MGTA-145 + plerixafor mobilized graft for one transplant.

3. Event Monitoring/Follow-up:

   a. A patient will be considered to have failed mobilization and will discontinue therapy on trial and go to Event Monitoring if they fail to collect the minimum of 2.0 million CD34 cells/kg after two apheresis sessions.

   b. Patients who are not proceeding with upfront ASCT (i.e patients who collect and store stem cells and opt for delayed transplant) will be followed in Event Monitoring until 30 days after completion of treatment. After that no, further follow-up is required.

   c. If patients are proceeding with upfront transplant, they will be followed until day 100 for secondary outcomes of engraftment, disease progression and survival. After that, no further follow-up is required. Patients will be offered participation in an institutional transplant registry study for longer term follow up.

d. Adverse Events occurring after the patient completes study intervention (MGTA-145 and plerixafor) will be collected on the Event Monitoring Form if they are >/= grade 3, at least possibly related to study treatment and have not been previously reported.

e. For patients proceeding with upfront transplant (defined up to 60 days after HSC mobilization), myeloablative melphalan conditioning (melphalan 140- 200 mg/m^2) on day -2 followed by infusion of collected HSCs cells on day 0 per institutional standard of practice protocol. The maximum interval between stem cell collection and transplant on this study is 60 days. Within 60 days, the timing of transplant is at the discretion of the treating physician.

f. If these patients undergo rescue mobilization for any reason (including physician discretion to collect more than 2 million CD34+ cells/kg) and proceed to upfront transplant with MGTA-145 and plerixafor mobilized graft, they will be followed for secondary transplant related outcomes. On the other hand, if they undergo upfront transplant with the stem cell graft obtained from rescue mobilization only, they will not be followed for secondary outcomes. It is recommended not to combine grafts from different mobilization protocols

Eligibility Criteria:

Inclusion Criteria

1. Diagnosis of multiple myeloma per the International Myeloma Working Group (IMWG) criteria
2. Age: 18 to 70 years
3. Eligible for ASCT per institutional guidelines
4. Within one year of start of myeloma therapy
5. Cardiac and pulmonary status sufficient to undergo apheresis and transplantation per institutional transplant guidelines.
6. Calculated creatinine clearance > 30 mL/min according to the Modification of Diet in Renal Disease (MDRD) formula.
7. Absolute neutrophil count $\geq$ 1500 x10^6/L and platelets $\geq$ 100,000 x10^6/L
8. Ability to understand and the willingness to sign a written informed consent document.
9. Agreement to use an approved form of contraception for male patients or female patients of childbearing potential.

Exclusion Criteria

1. History of prior stem cell transplant for multiple myeloma or other indications
2. Planned tandem stem cell transplant
3. Prior history of failure to collect HSCs.
4. Liver function tests: Total bilirubin >1.5x upper limit of normal (ULN) in the absence of a documented history of Gilbert's syndrome and/or AST/ALT > 3x ULN.
5. Known allergy to MGTA-145 or plerixafor.
6. Lifetime exposure to lenalidomide or another immunomodulatory drug greater than 6 cumulative months of treatment i.e more than six 28-day cycles or more than eight 21-day cycles.

7. Pregnant or lactating women.

Safety Monitoring of the Research: The PI will review the study periodically to review the progress of this protocol and be kept aware of efficacy and toxicity issues. This study will be monitored according to the Stanford Cancer Institute Data Safety Monitoring Plan that is currently in place for investigator-initiated clinical trials and will report to the Stanford Cancer Institute Data Safety Monitoring Committee (DSMC).

Toxicity will be captured as per NCI CTCAE version 5.0, wherein the term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. Non-hematologic toxicities will be evaluated via the ordinal CTC standard toxicity grading. Duration of toxicity events can be incorporated into assessments of toxicity grading. Hematologic toxicity measures of thrombocytopenia, neutropenia, and leukopenia will be assessed using continuous variables as the outcome measures as well as categorization via CTC standard toxicity grading. Overall toxicity incidence will be explored and summarized. Frequency distributions, graphical techniques and other descriptive measures will form the basis of these analyses.

DRUG AVAILABILITY

COMMERCIAL AGENT: Plerixafor

PHARMACEUTICAL COMPANY SUPPLIED: MGTA-145

SAMPLE SIZE: 25 patients

STATISTICAL CONSIDERATIONS

Sample Size: For this single-arm trial with a binomial primary endpoint, the maximum sample size is 25 evaluable patients.

Based on historical data with HSC mobilization with plerixafor and G-CSF[5,8-10], we hypothesize that at least 95% of subjects who undergo mobilization with this novel regimen of MGTA-145 and plerixafor will meet the primary end-point of collecting $\geq 2.0 \times 10^6$ $CD34^+$ cells/kg in two days of apheresis. We model the primary endpoint as a binomial random variable.

The trial will be conducted in two sequential segments: the first for evaluation of safety and feasibility, and then if successful, the number will be expanded to further evaluate safety and feasibility. In the first segment, 15 patients will be evaluated for the primary endpoint. In the second segment, which will be contingent upon achieving success in the first segment, 10 patients will be enrolled to gain additional safety and feasibility experience with this regimen and to evaluate the key secondary endpoint. Accounting for both segments, the total sample size is 25 patients.

A responder is defined as a patient meeting the primary endpoint. The statistical design for the primary endpoint is based on a binomial exact test for the null hypothesis of a responder rate being 70% or less and the alternative hypothesis of a responder of 95% or more. The criterion for success of the trial is at least 13 out of 15 patients are responders. The type I error rate is 5% and statistical power is 85%.

This design features an **implicit futility stopping rule**. Patients will be enrolled continuously to the first segment after initial safety run-in. The criterion for success is that at least 13 out of 15 patients are responders. **This implies that if 3 or more patients are non-responders at any point, then the trial will be stopped for futility.**

The collection of ≥4.0 x 10^6 CD34+ within 2 days a **key secondary endpoint** of this trial. We hypothesize that at least 75% of the 25 patients will meet this secondary endpoint. The statistical design for the secondary endpoint is based on enrolling enough patients in the second segment such that the total sample size will produce a precision estimate with a lower binomial confidence limit that excludes the response rate of the historical controls (i.e., 50%). For this secondary endpoint, we compute a precision estimate of our hypothesized response rate for the scenario that both segments are completed. We model the secondary endpoint as a binomial random variable. We define precision to be the length of the binomial confidence interval for the hypothesized response rate of 75%. We compute precision using the method of Wilson at the significance level of 90% which is appropriate for an early-phase trial. Based on these specifications, the precision is 26.8%.

Early Safety Run-in: The first six patients accrued to this study will be patients who are planned for upfront transplant following collection. Additional patients will not begin mobilization until these six patients have completed stem cell mobilization and are at least 21 days following infusion of stem cells after myeloablative melphalan conditioning. Safety review will be done to evaluate study drug-related adverse events, in particular any unexpected grade 3 or higher treatment related adverse events during this period. Engraftment data will also be reviewed. If any unexpected grade 3 or higher adverse events possibly, probably or definitely related to the study drug are noted in the first three patients, enrollment will pause while the PI reviews the study with the FDA to determine whether study can continue.

IND Number: 151874

STUDY SCHEMA

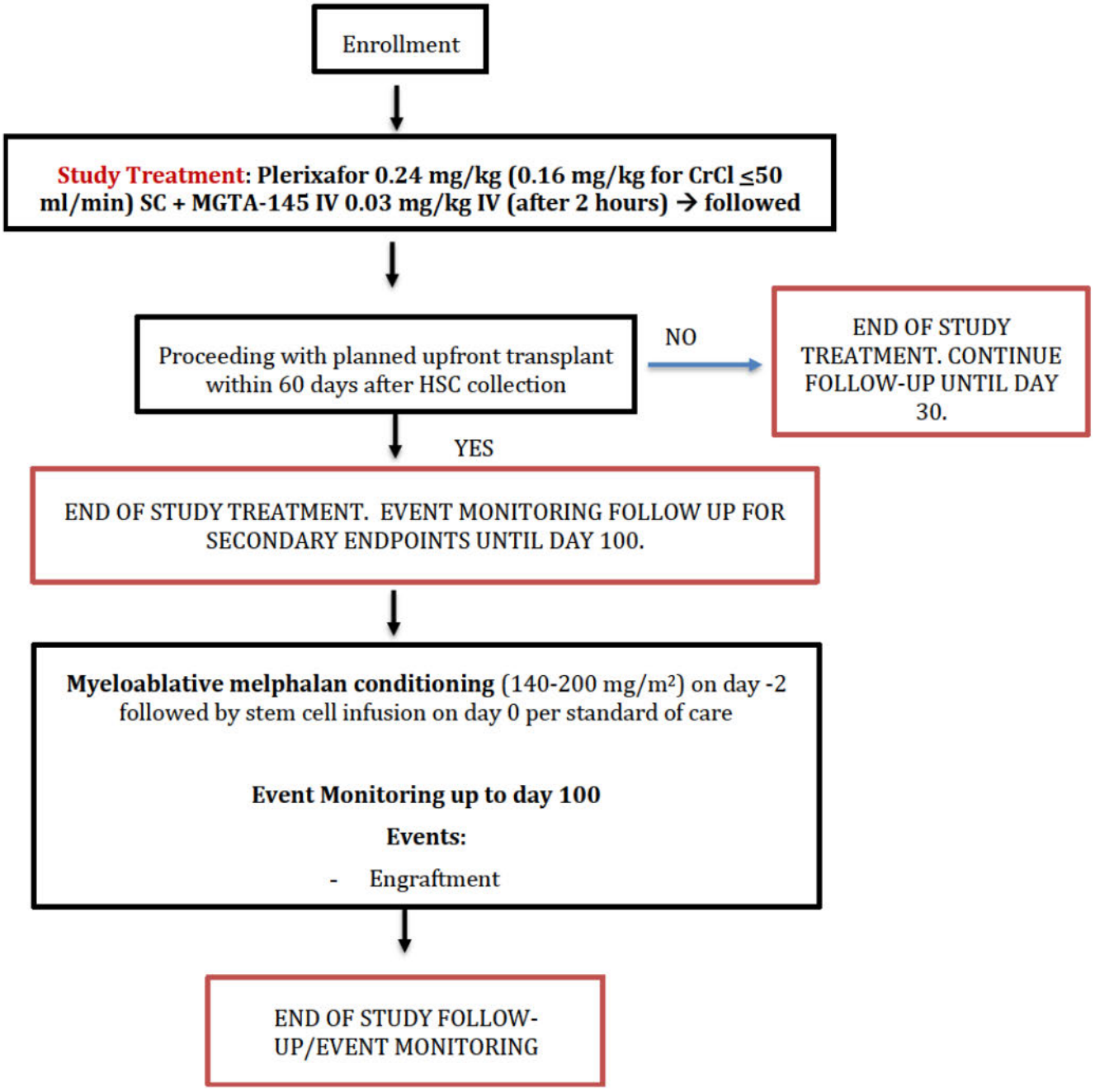


TABLE OF ABBREVIATIONS

AE	Adverse event
ALT	Alanine aminotransferase
ASCT	Autologous stem cell transplant
AST	Aspartate aminotransferase
CXCR2	Chemokine receptor 2
CXCR4	Chemokine receptor 4
CXCL2	Chemokine (C-X-C motif) ligand 2
CBC	Complete blood count
CD34	Cluster of differentiation 34
CFU	Colony forming unit
CRF	Case report form
CR	Complete response
CTCAE	Common terminology criteria for adverse events
CyTOF	Cytometry by time of flight
DLT	Dose limiting toxicity
DSMB	Data safety monitoring board
ECG	Electrocardiogram
ECOG	Eastern cooperative oncology group
EDC	Electronic data capture
EOS	End of Study
G-CSF	Granulocyte–colony-stimulating factor
GCP	Good clinical practice
HSCs	Hematopoietic stem cells
IB	Investigator's brochure
ICF	Informed consent form
IRB	Institutional review board
IV	Intravenous
MDRD	Modification of diet in renal disease
MM	Multiple myeloma
MRD	Minimal residual disease
NHL	Non-Hodgkin lymphoma

NK cells	Natural killer cells
OS	Overall survival
PB	Peripheral blood
PFS	Progression free survival
PK	pharmacokinetics
QoL	Quality of life
SAE	Serious adverse event
SC	Subcutaneous
ULN	Upper limit of the normal range

1 OBJECTIVES/ENDPOINTS

1.1 Primary Objective

1. To assess the efficacy of MGTA-145 in combination with plerixafor in mobilizing adequate number of hematopoietic stem cells (HSCs) in patients with multiple myeloma (MM) in preparation for autologous stem cell transplantation (ASCT).

1.2 Primary Endpoint:

1. To assess the rate of successful collection of ≥2.0 x 10^6 $CD34^+$ cells/kg in up to two days of apheresis after MGTA-145 + plerixafor dosing in patients with MM.

1.3 Secondary Objectives

1. To assess the efficacy of MGTA-145 in combination with plerixafor in mobilizing different HSCs target goals in patients with MM in preparation for ASCT.

2. To assess the safety and tolerability of MGTA-145 and plerixafor for mobilizing HSCs in patients with MM.

3. To assess the rate and kinetics of engraftment following ASCT after HSC mobilization with MGTA-145 + plerixafor in patients with MM undergoing upfront ASCT.

4. To assess rate of ongoing engraftment at day 28 and 100 after ASCT with HSCs with MGTA-145 and plerixafor in patients with MM undergoing upfront ASCT.

5. To assess transplant and disease-related outcomes after mobilization of HSCs with MGTA-145 and plerixafor in patients with MM undergoing upfront ASCT.

1.4 Secondary Endpoints

1. To assess the rate of successful collection of **(a)** ≥4.0 x 10^6 $CD34^+$ cells/kg in up to two apheresis sessions after MGTA-145 and plerixafor dosing in patients with MM. **(Key Secondary Endpoint) (b)** ≥6.0 x 10^6 $CD34^+$ cells/kg with up to two apheresis sessions after MGTA-145 and plerixafor dosing in patients with MM. **(c)** ≥2.0 x 10^6 $CD34^+$ and ≥4.0 x 10^6 $CD34^+$ cells/kg with one apheresis session after MGTA-145 and plerixafor dosing.

2. To assess infusion related toxicities with MGTA-145 and adverse events with mobilization of HSCs with MGTA-145 and plerixafor.

3. To assess time to neutrophil (defined as the first day of ANC ≥0.5 x 10^9/L for 3 days following stem cell infusion) and platelet engraftment (defined as first day of platelet count more than or equal to 20 x 10^9/L without transfusion in the last 7 days and with platelet

count ≥20 x 10^9/L on 2 separate, subsequent days and to also determine time to first day of platelet count ≥ 50 x 10^9/L (without transfusion in 48 hours) in patients undergoing upfront ASCT.

4. To assess rates of ongoing successful engraftment at 30- and 100-days post-transplant after successful primary engraftment in patients undergoing upfront ASCT.

5. To assess rate of transplant related mortality, non-relapse mortality, progression free survival and overall survival at 100 days in patients undergoing upfront ASCT.

1.5 Exploratory Objectives

1. Assess the composition of the apheresis product with mobilization with MGTA-145 and plerixafor in patients with MM.

2. Assess proliferation and differentiation potential of HSCs collected with MGTA-145 and plerixafor via colony-forming unit assays.

3. To assess immunological recovery in patients with MM undergoing upfront transplantation after infusion of HSCs collected with MGTA-145 and plerixafor mobilization.

4. To assess patient reported symptoms with stem cell mobilization with MGTA-145 and plerixafor in patients with MM.

1.6 Exploratory Endpoints

1. To assess the composition of the apheresis product obtained after MGTA-145 and plerixafor mobilization through enumeration $CD34^+CD90^+CD45RA^-$ cells in the product and immunological characterization of the T cell subsets, B cell subsets, NK cells, neutrophils, and myeloid / monocytic cells in the product using flow cytometry and CyTOF (Cytometry by Time of Flight).

2. Assess proliferation and differentiation potential of HSCs collected with MGTA-145 and plerixafor via colony-forming unit assays from the apheresis autograft.

3. To assess immunological characterization of white cell population subsets (T cell subsets, B cell subsets, NK cells, neutrophils, and myeloid / monocytic cells) in patient's peripheral blood at day 28 using flow cytometry and at day 100 using flow cytometry and mass cytometry/CyTOF and assess immunological characterization of white cell population subsets in the bone marrow microenvironment at day 100 following transplant.

4. To assess patient reported symptoms, using items for infusion reaction and pain adapted from the patient reported outcomes common terminology criteria for adverse events (PROCTCAE) as well as the brief pain inventory tool following plerixafor and MGTA-145 infusion. (Questionnaire in **Appendix B**)

2 BACKGROUND

2.1 Autologous Transplant and Stem Cell Mobilization in Multiple Myeloma

2.1.1 Multiple Myeloma and Role of Transplant:

Annually, over 30,000 patients are diagnosed with multiple myeloma in the United States *(NCI SEER program)*.[11] Autologous stem cell transplant (ASCT) for the treatment of myeloma was first described in 1980s.[12] Subsequent studies, including large randomized clinical trials demonstrated clear evidence of survival benefit of transplant and it became a standard of care treatment for patients with myeloma. ASCT is typically pursued after a few cycles of induction therapy. It involves administration of high-dose melphalan chemotherapy followed by rescue of the bone marrow with HSCs collected from the patient prior to chemotherapy administration.[1] Over the past two decades, several new drugs have been approved for the treatment of multiple myeloma and have resulted in dramatic improvement of patient outcomes.[13,14] Even though treatment of myeloma has evolved significantly with the availability of novel agents in the past two decades, ASCT still remains a cornerstone of treatment in eligible patients.[15]

The IFM-2009 randomized clinical trial evaluated outcomes of patients with early vs. delayed transplant following induction chemotherapy with bortezomib, lenalidomide and dexamethasone. Early transplant was associated with improved progression free survival (PFS) and higher minimal residual disease negativity compared with delayed ASCT at the time of first relapse. [1,16] Overall survival outcomes were similar in patients proceeding with early transplant vs. patients delaying transplant until the time of first relapse. Similarly, the FORTE trial compared patients proceeding with early ASCT vs. not after induction with carfilzomib, lenalidomide and dexamethasone. Interim results show that patients with high-risk myeloma undergoing transplant had higher persistent MRD negativity rates and lower risk of relapse.[17] Follow-up for survival outcomes is still ongoing. A meta-analysis of randomized clinical trials from 2000-2017 showed that high-dose therapy/ASCT was associated with superior PFS and minimal toxic effects compared to standard dose therapy.[18] Therefore, all eligible patients with multiple myeloma should be considered for transplant. Early transplant is preferred, especially in intermediate or high-risk patients. Standard risk patients may choose to delay transplant until first-relapse.[15] In such patients, stem cell collection and storage is recommended after a few cycles of induction therapy, as long-term lenalidomide maintenance therapy can impair future stem cell collection.[15,19]

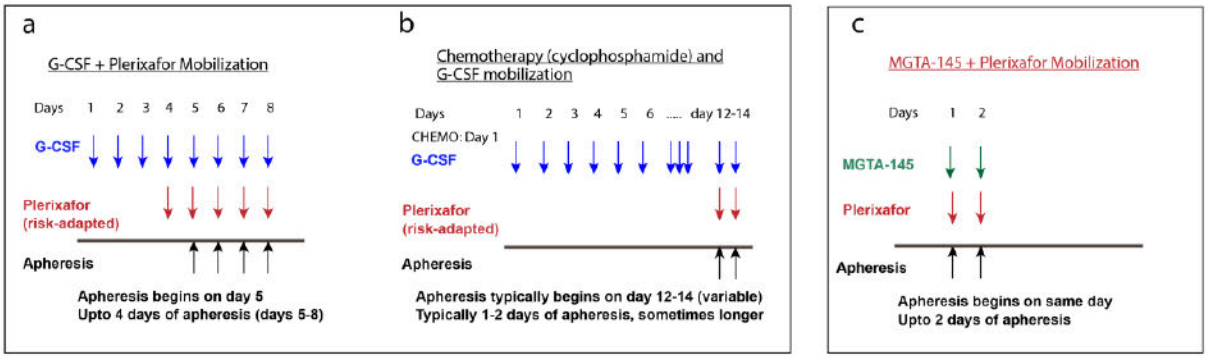
2.1.2 Stem Cell Mobilization for ASCT:

Collection of CD34+ HSCs to rescue the bone marrow from effects of high-dose chemotherapy in ASCT is most commonly done through mobilization and collection of stem cells from the peripheral blood. A minimum of 2×10^6 $CD34^+$ cells/kg are recommended for transplant, and many institutional protocols collect up to 4×10^6 $CD34^+$ cells/kg. [20] At baseline, only a small number of HSCs, identified by presence of CD34 positivity on surface, are circulating in peripheral blood. Before stem cells can be collected, stimulation and mobilization of stem cells from the bone marrow is required. It was initially achieved with myelosuppressive chemotherapy like cyclophosphamide and etoposide, where rebound of the marrow from the myelosuppressive effects of chemotherapy was associated with increased HSCs in the peripheral blood.[20] The introduction of G-CSF further increased the HSCs in the circulation after myelosuppressive therapy and shortened the time needed for achieving adequate stem cell counts in the blood. G-CSF was also explored as a single agent for HSC collection.

However, failure rates with G-CSF alone were as high as 40-50%.[21] Plerixafor, a CXCR-4 inhibitor was studied in addition to G-CSF, with excellent outcomes for mobilization.[5] It has now become a standard of care regimen.[3,5,20] Protocols with G-CSF and plerixafor may include use of plerixafor on day 4 of G-CSF in all patients or use of plerixafor in a risk-adapted manner based on peripheral blood CD34 count after 4 days of G-CSF.[22,23] A limitation in the use of plerixafor is the need to combine it with several days of G-CSF injections. Plerixafor alone has been studied for stem cell mobilization in healthy volunteers and by itself has limited feasibility for a rapid collection.[24] Given the lack of robust response with single agent plerixafor in healthy donors, it has not been evaluated in large studies for stem cell mobilization in patients with hematologic malignancies undergoing ASCT.

Therefore, the two common strategies to achieve HSC collection in the current era are chemotherapy-based mobilization (typically cyclophosphamide) along with G-CSF or G-CSF mobilization with plerixafor, in all patients or in a risk-adaptive algorithm.[20] While these methods are efficacious, the process can be long as shown in **Figure 1** and can be associated with adverse effects. Cyclophosphamide mobilization can take two to three weeks and result in complications like neutropenic fever and significant cytopenias.[2-4] G-CSF and plerixafor mobilization requires up to 4 days of G-CSF injections before mobilization can begin **(Figure 1)**. Injections are continued through collection, which can be as long as 4 sessions, therefore requiring 5-8 days for collection.[5] G-CSF may result in significant bone pain, fatigue, headache, and fever. Rare serious adverse effects with G-CSF include splenic rupture and pulmonary toxicity.

Figure 1: Stem cell mobilization: A comparison of the standard of care processes (panel a and b) vs. novel proposed mobilization method of MGTA-145 and plerixafor (panel c)



Standard of care stem cell mobilization methods

Novel Method to be evaluated in clinical trial

2.1.3 Rationale for proposed study:

The optimal stem cell mobilization and collection method should result in rapid and reliable collection of adequate CD34+ HSCs with minimal adverse effects. There is a need for innovation to reduce the time taken for stem cell mobilization and further decrease the adverse effects associated with stem cell collection in patients with multiple myeloma. This will be beneficial to patients and also improve healthcare resource utilization. **We are proposing a study of a novel agent for stem cell mobilization, MGTA-145 in combination with plerixafor for rapid, same day stem cell mobilization in patients with multiple myeloma (Figure 1).**

2.2 MGTA-145

MGTA-145 (also known as Gro-beta truncate; GROβT) is a synthetically manufactured naturally occurring four amino acid truncated protein variant of CXCL2, and has a 69 amino acid protein primary structure as listed below.
TELR**C**Q**C**LQT LQGIHLKNIQ SVKVKSPGPH **C**AQTEVIATL KNGQKA**C**LNP ASPMVKKIIE KMLKNGKSN

MGTA-145 has two disulfide bridges: C5 to C31 and C7 to C47, and is comprised of an alpha helix and a beta-sheet.

CXCL2 preferentially binds to the cell-surface chemokine receptor CXCR2, which is expressed predominantly on neutrophils and endothelial cells. GROβT binds to the CXCR2 receptor and with greater potency than full-length GROβ.[25]

Pharmaceutical Properties: MGTA-145 binds to the cell-surface chemokine receptor CXCR2, which is expressed predominantly on neutrophils and endothelial cells to induce mobilization of HSCs into the peripheral blood. HSC mobilization is dependent on the equilibrium of MMP-9 and tissue inhibitor of metalloproteinase-1 (TIMP-1) shifting towards MMP-9 following MMP-9 release by neutrophils upon CXCR2 binding. Studies showed that the addition of AMD3100 (plerixafor) inhibits cross-communication between CXCR4 and CXCR2 receptors which normally function to diminish the CXCR2 response. The addition of plerixafor helps enhance the release of MMP-9, leading to increased mobilization of hematopoietic cells.

MGTA-145 Physical and Chemical Properties

Molecular Formula (net)	C325H557N97O95S6
Molecular Mass (average)	7536.0
Appearance of Solution	Clear, colorless solution, essentially free of visible particles
Solubility	>100 mg/mL

Formulation: MGTA-145 is formulated at 20 mg/mL in 20 mM sodium acetate, 150 mM sodium chloride, 0.02% (w/v) Polysorbate 80 at pH 4.0. MGTA-145 is supplied in a single-dose 2 mL Type I amber glass vial. Each vial contains not less than 1 mL.

Storage Conditions

Vials of MGTA-145 should be stored at -20°C ± 5°C in the carton to protect from light.

Instructions for Use and Handling

MGTA-145 (injection) is a sterile solution for IV use supplied as a single-dose vial packaged in a carton.

Vials of MGTA-145 should be thawed overnight at 4°C prior to use and are stable at 4°C for up to 30 days. Once thawed, MGTA-145 should not be refrozen.

MGTA-145 should be diluted in 0.9% saline in accordance with the study protocol and pharmacy manual. Solutions of MGTA-145 (0.036 to 1.44 mg/mL) prepared in 0.9% saline may be stored for no more than 8 hours at room temperature prior to administration.

MGTA-145 should be administered as an IV infusion over 3-10 minutes, as clinically indicated.

Preclinical data: Preclinical studies demonstrated that GROβT (MGTA-145), when administered simultaneously with plerixafor, led to a synergistic increase in mobilization of highly engraftable HSCs in mice. Mobilization was rapid, occurring within minutes of administration of GROβT, and robust, mobilizing significantly more HSCs than either GROβT or plerixafor alone.[6] In addition to the evaluation of mice in this study, safety and pharmacokinetic studies of MGTA-145 have been done in rats and non-human primates as described in the accompanying **Investigator's Brochure**.

Clinical data: In a phase 1 study[6], adult healthy males received escalating doses of placebo injection followed by MGTA-145 four weeks later. Most common side effect was acute lumbar back pain, which lasted 10 minutes or less and did not require treatment. Back pain was seen is subjects who received infusions of 0.09 mg/kg or higher and was seen in 29.7% of subjects receiving MGTA-145 vs. 5.3% receiving placebo. Single agent MGTA-145 resulted in CD34 mobilization at higher doses, but it was not felt to be sufficient for adequate stem cell collection with MGTA-145 used as a single agent.

A phase 1 study of MGTA-145, with or without plerixafor was conducted in healthy volunteers to determine the safety and dose of MGTA-145.[7] MGTA-145 was administered to 79 healthy volunteers. The study had four parts. In Part A, subjects received single dose of MGTA-145 alone (0.0075 -0.3 mg/kg). In Part B, subjects received single dose of MGTA-145 (0.015-0.15 mg/kg) with plerixafor. In Part C, subjects received 2 daily doses of MGTA-145 (0.03 and 0.07 mg/kg) and plerixafor. In Part D, subjects received single dose of MGTA-145 (0.015 and 0.03 mg/kg) and plerixafor, followed by same day apheresis. MGTA-145 was found to have a good safety profile, with most common side effect of transient back pain lasting less than 20 minutes in most patients. In Part A, back pain was seen in 79% (19/24) of volunteers receiving single agent MGTA-145 vs. 0% (0/12) of volunteers receiving plerixafor. There were no other treatment emergent adverse effects (TEAE) in subjects in Part A receiving MGTA-145 (0.0075 to 0.3 mg/kg). When MGTA-145 (0.015 to 0.15 mg/kg) was combined with plerixafor in Part-B, TEAE were seen in 81% (26/32) of subjects compared to 58% (7/12) of patients receiving plerixafor alone. TEAE in the MGTA-145 and plerixafor arm included back pain/musculoskeletal pain in 62.5% (n=20), diarrhea, nausea and dizziness in 15.6% subjects each (n=5 each), respectively, abdominal pain in 12.5% (n=4), vomiting and headache in 9.4 % (n=3 each) of subjects each, respectively and paresthesia in 6.3% of subjects. This data is shown in the **Table 1** below (*source: DiPersio et al TCT 2019 oral presentation)*

Table 1: Adverse effects with MGTA and plerixafor in a phase 1 study of healthy volunteers

	Part A		Part B		Part C		Part D
	MGTA-145 (0.0075 - 0.3 mg/kg)	Placebo	MGTA-145 + plerixafor (0.015 - 0.15 mg/kg)	Plerixafor	MGTA-145 + plerixafor (0.03 - 0.07 mg/kg)	Plerixafor	MGTA-145 + plerixafor (0.015 - 0.03 mg/kg)
	n=24 n (%)	n=12 n (%)	n=38 n (%)	n=14 n (%)	n=8 n (%)	n=2 n (%)	n=8* n (%)
Subjects with any drug related TEAE	**19 (79.2)**	**-**	**31 (81.6)**	**8 (57.1)**	**6 (75.0)**	**-**	**8 (88.9)**
Diarrhea	-	-	6 (15.8)	5 (35.7)	1 (12.5)	-	1 (11.1)
Nausea	-	-	7 (18.4)	2 (14.3)	1 (12.5)	-	4 (44.4)
Abdominal discomfort/pain	-	-	5 (13.2)	4 (28.6)	-	-	3 (33.3)
Vomiting	-	-	3 (7.9)	1 (7.1)	-	-	1 (11.1)
Back pain / Musculoskeletal pain[2]	19 (79.2)	-	24 (63.2)	2 (14.3)	4 (50.0)	-	3 (33.3)
Dizziness / Lightheadedness	-	-	5 (15.6)	1 (7.1)	-	-	4 (44.4)
Headache	-	-	4 (10.5)	1 (7.1)	2 (25.0)	-	2 (22.2)
Dysgeusia	-	-	-	2 (14.3)	-	-	-
Paraesthesia	-	-	2 (5.3)	-	1 (12.5)	-	1 (11.1)

There was no dose response in AEs, so data are aggregated.
[1] All AEs are grade 1 except for grade 2 abdominal pain (1), nausea (1), and back pain (1) in the plerixafor + MGTA-145 0.075 mg/kg 2h stagger cohort (Part B) and grade 2 headache (1) in the plerixafor + MGTA-145 0.015 mg/kg cohort (Part D).
[2] Back pain was associated with MGTA-145 infusion, lasted <20 minutes in most cases and did not require medical therapy.
* A 9th subject enrolled in Part D but did not undergo leukapheresis.

Source: Dipersio et al. TCT 2020 oral presentation

At the 0.03 mg/kg dose the median CD34+ cell count in peripheral following MGTA-145 and plerixafor administration was 40 cells/microL (range 18-63), which was higher than that observed with plerixafor alone (median 24, range: 13-78). The dose of 0.03 mg/kg was determined to be safe and effective, with predictable, dose linear pharmacokinetics that are not affected by the co-administration of plerixafor. Administration of plerixafor followed by MGTA-145 after a 2-hour interval was associated with the best stem cell mobilization outcomes, as determined by peripheral blood CD34 counts and will be the regimen used in this trial.

A study of MGTA-145 in subjects with renal impairment is ongoing. The primary objective of that study is to establish the effect of renal impairment on MGTA-145 pharmacokinetics. A single, monotherapy dose 0.07 mg/kg was used for this study. Twenty-three adult subjects were enrolled into 1 of the following 3 groups based on eGFR, determined by the Modification of Diet in Renal Disease Study (MDRD) equation: 1. normal ≥ 90; 2. mild 60 to 89; and 3. moderate 30 to 59 mL/min/1.73 m2). Overall, the results demonstrate that MGTA-145 was well tolerated in subjects with normal kidney function and in subjects with renal impairment. Renal impairment had no impact on the PK of MGTA-145. MGTA-145 is not excreted in urine.

Non-clinical pharmacokinetics: Please refer to accompanying *Investigator's Brochure* for pharmacokinetic studies in mice, rats and non-human primates.

Clinical pharmacokinetics: In a phase 1 study[6], adult healthy males received escalating doses of placebo injection followed by MGTA-145 weeks later. The maximum tolerated dose was determined to be 0.2 mg/kg.
In the Magenta Therapeutics phase 1 study of healthy volunteers, pharmacokinetic studies of different doses of MGTA-145, with or without plerixafor were done. MGTA-145 plasma drug concentration increased proportionally with the infused dose and was not affected by plerixafor. The Cmax of MGTA-145 increased along with dose linearly and AUC increased in a supra proportional manner in the dosing range of 0.0075 mg/kg to 0.3 mg/kg. In Part A, the mean Cmax of MGTA-145 ranged from 35.12 µg/L to1461.55 µg/L, and the mean area under the concentration-time curve from time zero to time of last measurable concentration (AUClast) of MGTA-145 ranged from 20.1 h*µg/L

to 2149.9 h*µg/L. Mean half-life (t1/2) ranged from 0.51 to 1.97 hours. Co-administration of plerixafor of 10 minutes or 2 hours had no impact on the PK profile of MGTA-145. Following the administration of a second dose on Day 2 in Part C, minimal accumulation was observed. Accumulation was higher with the 0.07 mg/kg dose than with the 0.03 mg/kg dose. Urine excretion of MGTA-145 was assessed from 4 hours to 16 days after dosing, and MGTA-145 is not excreted in urine.

The infusion time in the study of healthy volunteers was set at 10 minutes. Subsequent pharmacokinetic modeling done by Magenta Therapeutics supports an infusion time of 1 to 10 minutes as deemed acceptable in future studies.

Please refer to the accompanying ***Investigator's Brochure*** and additional ***Population Pharmacokinetic Analysis of MGTA-145 (DR-20-002) Version 00 dated 14 July 2020*** for additional details.

Regulatory Compliance: Plerixafor is FDA approved for stem cell mobilization in combination with G-CSF in patients with multiple myeloma. MGTA-145 is not FDA approved. Therefore, an Investigational New Drug application (IND) is required and will be submitted.

2.3 Rationale

MGTA-145 (GROβT) activates the CXCR2 pathway in neutrophils. It has shown promising activity for rapid mobilization of CD34+ HSCs in pre-clinical evaluation in mice.[6] When used in combination with plerixafor, a CXCR4 inhibitor, MGTA-145 has the potential to rapidly and reliably mobilize adequate numbers of HSCs for stem cell transplantation. It has been evaluated with plerixafor in a phase I study of healthy volunteers, where it was found to be safe and resulted in rapid mobilization of stem cells on the same day.[7] These HSCs were transplanted into sublethally irradiated (200 cGy) NSG mice at limit dilution (3 cell doses). Engraftment of human $CD45^+$ ($hCD45^+$) in peripheral blood was measured at week 4 weeks post-transplant by flow cytometry. Engraftment was more robust in mice injected human HSCs obtained from MGTA-145 and plerixafor vs. those obtained with G-CSF alone.[7] Given the promising results seen in pre-clinical models and phase 1 data, we expect that the combination of MGTA-145 and plerixafor will result in fast, effective and safe stem cell mobilization in patients with multiple myeloma. MGTA-145 is a potential alternative to G-CSF when used in combination with plerixafor to achieve same day dosing and apheresis, resulting in patient convenience, improved health care resource utilization and potentially fewer adverse effects than associated with multi-day G-CSF based regimens.

Therefore, to evaluate the efficacy and safety of this novel mobilization regimen, we are proposing a **phase II, proof of concept study of MGTA-145 and plerixafor in patients with myeloma**. Patients will receive plerixafor at 0.24 mg/kg (0.16 mg/kg for creatinine clearance ≤ 50 ml/min) SC followed by MGTA-145 at 0.03 mg/kg IV after a two-hour interval. This will be followed by same day apheresis. Dosing may be repeated on day 2 based on CD34+ yield of apheresis product. This study will also include correlative studies to understand the graft composition, proliferation of HSCs *in vitro* with colony forming unit (CFU) assays, engraftment kinetics and immune reconstitution following infusion of stem cells mobilized with this novel regimen.

2.4 Study Design

- This is a phase II, open label, proof of concept study to evaluate the safety and efficacy of HSC mobilization with a novel drug, MGTA-145 at a fixed dose level in combination with plerixafor in patients with MM for stem cell mobilization in preparation of autologous stem cell transplant.
- Primary purpose of the study intervention: **Interventional** (HSC mobilization)
- Single cohort, single center, open label study with one intervention arm
- Randomization: Non-randomized
- Primary outcome: Efficacy
- Secondary Outcomes: Efficacy, safety, engraftment, survival

2.5 Correlative Studies Background

Correlative Study #1: Assessment of graft composition of stem cell populations and other white blood cell subsets by flow cytometry and CyTOF.

Aim: To assess the composition of the apheresis product obtained after MGTA-145 and plerixafor mobilization through enumeration $CD34^{+}CD90^{+}CD45RA^{-}$ cells in the product and immunological characterization of the T cell subsets, B cell subsets, NK cells, neutrophils, and myeloid / monocytic cells in the product using a combination of flow cytometry and CyTOF.

CD90+CD34+ stem cells have been shown to be true self-renewing pluripotent HSCs among the CD34+ cells, whereas CD90(-)CD34+ cells have been shown to be differentiated committed progenitor cells.[26] While data is limited, there is evidence that CD90+CD34+ cells in the graft drive the engraftment efficiency of stem cell grafts. MGTA-145 was associated with preferential mobilization of CD90+ CD34+ stem cells in pre-clinical models vs. grafts mobilized by G-CSF. [6,7] In the phase1 study of MGTA-145 with plerixafor, the median CD90+CD34+ stem cell proportion was 33%, higher than that observed in a small subset of patients (n=2) mobilized with G-CSF. This correlative study will provide data on the CD90+CD34+ cells fraction from apheresis products collected with this novel mobilization regimen (**Table 2**).

Secondly, we will evaluate graft composition of T, B and NK cells and their subsets using validated flow cytometry/CyTOF panels (**Table 3**). It has been shown that higher lymphocyte subset proportions in the stem cell graft are associated with better post-transplant immune reconstitution and survival.
Assessment of graft composition will be done by flow cytometry in the lab of Dr. Judith Shizuru at Stanford University and by CyTOF in the Human Immune Monitoring Center at Stanford University. Correlation of fraction of CD90+CD34+ cells with engraftment kinetics will be explored. This data will be compared with data from a contemporaneous cohort of patients with multiple myeloma undergoing HSC collection with currently approved regimens. Samples will be obtained under a separate IRB approved biorepository protocol.
Thirdly, we will evaluate graft contamination using next generation flow cytometry assay minimal residual disease negativity testing. MRD NGF will be based on the Euro-Flow standardized algorithm and will be done at Mayo Clinic.[27-29] The sensitivity of the assay is 1 in 1×10^5 to 1×10^6 cells. It is a 2 tube, 8 color assay and the following panels are used:

Tube #1: CD38/CD56/ CD45/CD19/CD117/ CD81/ CD138/ CD27

Tube #2: CD38/ CD56/ CD45/ CD19/Kappa/Lambda/ CD138/ CD27

Table 2: Flow Cytometry Panel with Antibodies for HSC and progenitor cell staining

Fluorochrome	Target	Clone	Vendor	Catalog No.	Final Dilution	µL Ab/ 100 µL cocktail
FITC	hCD45RA	HI100	eBiosciences	11-0458-42	1:100	1
PE	hCD90 (20 µg/mL)	PR13	Pacific GMP	lot# 102-14P/N 70-0031	1:10	10
APC-Cy7	hCD123	6H6	eBiosciences	47-1239-42	1:100	1
APC	hCD34	561	BioLegend	343608	1:100	1
PE-Cy7	hCD38	HIT2	eBiosciences	25-0389-42	1:100	1
BV510	hCD10	HI10a	BioLegend	312219	1:100	1
Pac Blue	hCD117	104D2	eBiosciences	48-1178-42	1:100	1
PerCP-Cy5.5	hCD19	HIB19	BD Biosciences	561295	1:100	1

Table 3: CyTOF Panel with Antibodies for peripheral blood mononuclear cell immunophenotyping

PBMC phenotyping			
Metal label	**Specificity**	**Clone**	**Conjugated by**
102-110Pd			
113In	CD57	HCD57, BioLegend	HIMC
115In	live/dead		
139La			
141Pr			

142Nd	CD19	SJ25C1, Southern BioTech	HIMC
143Nd	CD4	SK3, BioLegend	HIMC
144Nd	CD8	SK1, BioLegend	HIMC
145Nd	CD39B	BioLegend	HIMC
146Nd	IgD	IA6-2, BioLegend	HIMC
147Sm	CD85j	292319, R&D Systems	HIMC
148Nd	CD11c	Bu15, BioLegend	HIMC
149Sm	CD16	3G8, BioLegend	HIMC
150Nd	CD3	UCHT1, BD	HIMC
151Eu	CD38	HB-7, BD	HIMC
152Sm	CD27	L128, BD	HIMC
153Eu	CD11b	ICRF44, BioLegend	HIMC
154Sm	CD14	M5E2, BioLegend	HIMC
155Gd	CCR6	11A9, BD or G034E3, BioLegend	HIMC
156Gd	CD94	HP-3D9, BD	HIMC
157Gd	CD86	IT2.2, BioLegend	HIMC
158Gd	CXCR5	RF8B2, BD	HIMC
159Tb	CXCR3	G025H7, Biolegend	HIMC
160Gd	CCR7	150503, R&D Systems	HIMC
161Dy			
162Dy	CD45RA	HI100, BioLegend	HIMC
163Dy			
164Dy	CD20	2H7, BioLegend	HIMC
165Ho	CD127	A019D5, BioLegend	HIMC
166Er	CD33	P67.8, BD	HIMC

167Er	CD28	L293, BD	HIMC
168Er	CD24	ML5, BioLegend	HIMC
169Tm	ICOS	DX29, BD	HIMC
170Er	CD161	DX12, BD	HIMC
171Yb	TCRgd	B1, BioLegend	HIMC
172Yb	PD-1	EH12.1, BD	HIMC
173Yb	CD123	9F5, BD	HIMC
174Yb	CD56	NCAM16.2, BD	HIMC
175Lu	HLA-DR	G46-6, BD	HIMC
176Yb	CD25	M-A251, BD	HIMC

Correlative Study #2: Assess proliferation and differentiation potential of HSCs collected with MGTA-145 and plerixafor via colony-forming unit (CFU) assays from the apheresis autograft.

CFU assays are done to evaluate the proliferation and differentiation potential of HSCs.[30-32] They provide *in-vitro* functional data of collected HSCs, which complements the data obtained from flow cytometric evaluation of the apheresis product. The apheresis product containing the HSCs is plated on a culture medium. Cultures are grown for a fixed duration of time (10-14 days) and progenitor colonies growing in the culture media are then quantified. They include: colony forming unit erythrocytic (CFU-E), blast forming unit erythrocytic (BFU-E), colony forming unit granulocytic and monocytic (CFU-GM), colony forming unit granulocytic, erythrocytic, monocytic and megakaryocytic (CFU-GEMM), and highly proliferative progenitors (HPP).

CFU assays will be done per Stanford BMT Cellular Therapy Facility standard protocol, which is summarized below:

- Desired plating concentration for unmanipulated fresh or thawed peripherally collected autologous HSCs: Starting Product: 1-5x10^5 cells
- Required volume of fresh or thawed cell suspension and Iscove is added to CFU media tube
- Following the addition cells and Iscove are added to methyellulose tissue culture medium, the cells are vortexed
- Once the cell- methyellulose tissue culture solution settles, 2 wells of a 24-well plate are each seeded with 0.25mL of the solution
- The remaining wells are filled with ~0.5mL PBS to prevent evaporation
- Plate is incubated for 14 days, then read for CFUs.

Correlative Study #3: To assess immunological characterization of white cell population subsets (T cell subsets, B cell subsets, NK cells, neutrophils, and myeloid / monocytic cells) in patient's peripheral blood at day 28 using flow cytometry and at day 100 using flow cytometry and CyTOF

HSCs infused after high-dose chemotherapy repopulate the host hematopoiesis and lead to gradual immune reconstitution. The time taken for recovery of different immune cell subsets can vary. Immune reconstitution not only impacts infectious disease outcomes, but also has been shown to have an impact on survival outcomes. Early lymphocyte count recovery is associated with better survival after ASCT in patients with multiple myeloma and non-Hodgkin's lymphoma.[33,34] It has been shown that higher lymphocyte subset proportions in the stem cell graft are associated with post-transplant lymphocyte count, which in turn has a strong correlation with survival outcomes.[33,35]

White cell subsets will be evaluated by flow cytometry and (CyTOF (Cytometry by Time-Of-Flight) at day 28 and day 100 (**Table 4** and **Table 5**). Mass cytometry allows simultaneous testing of several antigens and is advantageous to provide a detailed overview of different cell populations and when studying small transitional immune populations. [36,37] The process is well-established at the human immune monitoring core laboratory facilities at Stanford University. [38-43]

Table 4: Flow Cytometry Panel with Antibodies for T and NK cell staining

Fluorochrome	Target	Clone	Vendor	Catalog No.	Final Dilution	µL Ab/ 100 µL cocktail
FITC	hCD103	Ber-ACT8	BD Biosciences	550259	1:100	1
PE	hCD62L	DREG-56	BD Biosciences	555544	1:100	1
PerCP-Cy5.5	hCD4	RPA-T4	BD Biosciences	560650	1:100	1
APC-H7	hCD8	SK1	BD Biosciences	560179	1:100	1
APC	hCD56	B159	BD Biosciences	555518	1:50	2
PE-Cy7	hCD45RA	HI100	BD Biosciences	560675	1:100	1
BV510	hCD31	WM59	BD Biosciences	563454	1:100	1
APC-R700	hCCR7	3D12	BD Biosciences	565867	1:100	1
BV421	hCD3	SK7	BD Biosciences	563798	1:100	1

Table 5: Flow Cytometry Panel with Antibodies for B cell staining

Fluorochrome	Target	Clone	Vendor	Catalog No.	Final Dilution	µL Ab/ 100 µL cocktail
FITC	hIgM	G20-127	BD Biosciences	555782	1:100	1
PE	hCD24	ML5	BD Biosciences	555428	1:100	1
PerCP-Cy5.5	hCD19	HIB18	BD Biosciences	561295	1:100	1
APC-H7	hCD20	2H7	eBiosciences	561172	1:100	1
APC	hCD38	HIT2	BD Biosciences	555462	1:100	1
PE-Cy7	hCD27	M-T271	BD Biosciences	560609	1:100	1
BV510	hIgD	IA6-2	BD Biosciences	563034	1:100	1
BV421	hCD3	SK7	BD Biosciences	563798	1:100	1

2.6 Patient Reported Outcome Measures: Background and Evaluation

A combined questionnaire incorporating items adapted for infusion site reaction from the Patient Reported Outcomes Common Terminology Criteria for Adverse Events (PRO-CTCAE) as well as the Brief Pain Inventory will be used to measure patient reported outcomes. Patients will be asked to complete questionnaires on each day of collection and within one week following stem collection.

PRO-CTCAE is a self-reporting tool, which has been previously validated in patients undergoing treatment for hematologic malignancies.[44,45] It covers various symptoms and assesses the presence, frequency and severity of these symptoms. We will assess the following **symptomatic AEs: 'pain and swelling at injection site'**

The **Brief Pain Inventory (BPI)** consists of 2 domains: pain severity and pain interference with a recall of 24 hours. [46] We will use the short-form BPI with 9 items, which requires about 5 minutes to complete, on average. Patients are asked if they have pain and the location of pain, Pain severity is scored on a scale of 0-10 with 0 = no pain and 10 = pain as bad as you can imagine. The BPI pain severity questions assess pain at its "worst," "least," "average," and "now" (current pain). A composite mean severity score of these four items may be calculated. Pain interference is estimated by 7 questions that ask patients how pain has interfered with seven daily activities: walking, work, mood, enjoyment of life, relations with others, and sleep. Pain interference is scored as a mean of the 7 interference items. Imputation for missing data may be used if at least 50% (4 of 7 items have been completed at a given time point). Questions about pain medications and their effectiveness are also included.

A copy of the instrument is provided in the **APPENDIX B**: PRO Questionnaire . It is also available in other languages including Spanish. It is validated for both acute and chronic pain. It can be self-reported or asked in an interview format. As described above, there is no scoring algorithm, but "worst pain" or the arithmetic mean of the four severity items can be used as measures of pain severity; the arithmetic mean of the seven interference items can be used as a measure of pain interference.

Internal consistency for BPI ranges from 0.80 to 0.87 for 4 items for pain severity as demonstrated in numerous studies, including cancer patients [47,48]. Test-retest (1 day to 1 week) reliability for ratings of pain as worst (0.93) and usual or average pain (0.78) in patients with metastatic cancer were acceptable [46]. Factor structure and the validity of BPI has been successfully tested [49]. The validity of BPI was assessed using known-group methods with mean scores compared across cancer patients taking different types of pain medication (narcotic versus non-narcotic pain relievers). The BPI mean scores significantly differed between the 2 groups, demonstrating strong evidence of validity [47]. For conditions such as bone metastasis and fibromyalgia, the minimum clinically important difference for BPI is approximately a 2-point change from baseline [50,51]. Another study on buprenorphine used in lower back pain used a minimal clinically important difference of 2 points for BPI [52].

PRO questionnaires will be designed in a paper format to be filled by the patient or study team member interviewing the patient. Data will be uploaded in the EDC tool.

3 PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

Patient eligibility will be reviewed. Inclusion and exclusion criteria are listed below. Eligible patients will be invited to enroll on to the study and will sign informed consent as described in **Section 3.3**. Refer to the Participant Eligibility Checklist in **APPENDIX B**: PRO Questionnaire .

3.1 Inclusion Criteria

3.1.1 Diagnosis of multiple myeloma per the International Myeloma Working Group (IMWG) criteria.

3.1.2 Age: 18 to 70 years

3.1.3 Eligible for ASCT per institutional guidelines

3.1.4 Within one year of start of myeloma therapy

3.1.5 Cardiac and pulmonary status sufficient to undergo apheresis and transplantation per institutional transplant guidelines.

3.1.6 Calculated creatinine clearance > 30 mL/min according to the Modification of Diet in Renal Disease (MDRD) formula

3.1.7 Absolute neutrophil count $\geq$ 1500 x10^6/L and platelets $\geq$ 100,000 x10^6/L

3.1.8 Ability to understand and the willingness to sign a written informed consent document.

3.1.9 Agreement to use an approved form of contraception for male patients or female patients of childbearing potential.

3.2 Exclusion Criteria

3.2.1 History of prior stem cell transplant for multiple myeloma or other indications

3.2.2 Planned tandem stem cell transplant

3.2.3 Prior history of failure to collect HSCs.

3.2.4 Liver function tests: Total bilirubin >1.5x upper limit of normal (ULN) in the absence of a documented history of Gilbert's syndrome and/or AST/ALT > 3x ULN

3.2.5 Known allergy to MGTA-145 or plerixafor

3.2.6 Lifetime exposure to lenalidomide greater than 6 cumulative months of treatment, i.e. more than six 28-day cycles or more than eight 21-day cycles

3.2.7 Pregnant or lactating women.

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 will 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 will be retained in the medical record or research file.

3.4 Study Timeline

Primary Completion: The study is anticipated to reach primary completion 15 months from the time the study opens to accrual. We anticipate accrual to be completed in 9-12 months and the remainder of the time is required to complete intervention and follow-up for primary endpoint.

Study Completion: The study is anticipated to reach study completion 18 months from the time the study opens to accrual.

4 TREATMENT PLAN

This is an open label, proof of concept, phase 2 study to evaluate safety and efficacy of stem cell mobilization (for high-dose therapy and autologous stem cell rescue) with a combination of MGTA-145 and plerixafor, followed by same day apheresis in patients with multiple myeloma.

The primary endpoint is successful collection ≥2.0 x 10^6 $CD34^+$ cells/kg with up to two apheresis sessions after MGTA-145/plerixafor dosing. Secondary endpoints include collection of ≥2.0 x 10^6 $CD34^+$ cells/kg and ≥4.0 x 10^6 $CD34^+$ cells/kg on day one of apheresis and ≥4.0 x 10^6 $CD34^+$ cells/kg and ≥6.0 x 10^6 $CD34^+$ cells/kg in up to two days of apheresis after MGTA-145/plerixafor dosing, assessment of adverse events and successful engraftment as described above.

4.1 Study Treatment and Follow-up

The study is divided into three phases:

4.1.1 Pre-mobilization phase:

a. Patients who are eligible will be invited to participate in the study. Patients will undergo screening and baseline evaluation.

b. If patients do not meet eligibility criteria after signing consent on the study, they will be deemed screen failures and may be replaced to meet accrual goal. Patients may be rescreened at the discretion of the principal investigator. Patients will be enrolled after they meet eligibility criteria.

c. Chemotherapy, including immunomodulatory drugs (IMiDs) to be stopped 14 days prior to start of mobilization.

4.1.2 Study Treatment: stem cell mobilization/apheresis:

All patients will receive MGTA-145 and plerixafor at single, weight-based doses. Dose of MGTA-145 has been determined from a phase 1 study. Patients will undergo sequential administration of plerixafor 0.24 mg/kg subcutaneously followed 2 hours later by MGTA-145 at 0.03 mg/kg intravenously (3 to10 minute infusion) as shown in **Table 6.** For patients with creatinine clearance < 50 ml/minute, plerixafor dose will be reduced to 0.16 mg/kg per package insert. This will be followed by apheresis.

Table 6: Treatment Plan

MGTA-145			Plerixafor		
Dose (mg/kg)	**Total Doses Administered**	**Route**	**Dose (mg/kg)**	**Total Doses Administered**	**Route**
0.03	1 per day for 1 to 2 days	IV	0.240 or 0.16*	1 per day for 1 to 2 days	SC

IV=intravenous; SC=subcutaneous. * Plerixafor dose reduced to 0.16 mg/kg per package insert for creatinine clearance ≤ 50 ml/minute

- **Drug discontinuation/dose modifications:**

There are no dose modifications for the investigational agent (MGTA-145). If any patient experience grade 3 or higher non-hematological toxicity, not appropriately controlled by appropriate interventions, and considered possibly, probably or definitely related to MGTA-145, the study drug will be discontinued and patient will go to event monitoring.

- **Infusion reaction:**

Patients should be carefully monitored during MGTA-145 infusion. If an infusion-related reaction develops, then the infusion should be temporarily interrupted or slowed down. Subjects who experience adverse events during the infusion must be treated according to the investigator's judgment and best clinical practice. As described above, if patient experiences a grade 3 or higher infusion reaction with MGTA-145 that is not adequately controlled by appropriate interventions, treatment with MGTA-145 will be discontinued.

- **Apheresis:**

Apheresis collection will begin as soon as feasible after MGTA-145 infusion. Apheresis should commence (i.e. inlet flow initiated) no later than 30 minutes after the start of MGTA-145 infusion. Apheresis procedure, including the machine, collection procedure and supportive care will be in accordance with standardized procedures of Stanford Blood and marrow transplantation (BMT)/Apheresis program. Each session of apheresis will be standardized to **process at least 3 blood volumes, with a margin of +/-10% or 4.5 hours, whichever is longer**. The processing and cryopreservation of the apheresis product will be in accordance with Stanford BMT/Cell Therapy Facility (CTF) standardized procedures.
A second day of mobilization and apheresis will be pursued in patients who have not collected 6.0×10^6 $CD34^+$ cells/kg in one session.

A minimum of 2.0×10^6 $CD34^+$ cells/kg should be collected in one to two apheresis sessions. If this goal is not met, patient may proceed with washout protocol and alternative stem cell mobilization strategy as routine clinical care at discretion of treating physician.

Patients who have collected between 2.0 to 3.9×10^6 $CD34^+$ cells/kg may proceed to transplant at the discretion of the treating physician per current institutional guidelines. Alternatively, they can proceed with washout protocol and alternative stem cell mobilization strategy as routine clinical care. This decision will be made by the treating physician in consultation with the PI. A minimum of 2.0×10^6 $CD34^+$ cells/kg are recommended for successful engraftment for transplant and while a back-up is preferred, it is not mandatory. This is in accordance with current institutional standard of practice.

If an additional collection is sought at the discretion of the treating physician, it is recommended not to combine the standard of care mobilized graft and the MGTA-145 + plerixafor mobilized graft for one transplant.

- **Patient Reported Outcomes Assessment**

Patient reported outcomes will be assessed as described in **Section 2.6** above using a patient reported questionnaire incorporating items from PRO-CTCAE, as well as the Brief Pain Inventory.

Electronic questionnaires will be designed that will be administered using a tablet. Paper copies will be available as back-up and may be used if unable to complete the electronic questionnaires. Electronic questionnaires will be directly uploaded into the Medrio database. If paper copies are used, they will be handed to the study coordinator and stored in a locked room at Stanford Health Care. Paper answers will be entered into electronic format by the study staff.

PRO questionnaire is designed to be self-administered by the patient (assistance can be provided by study/clinical staff, as needed) on each day of apheresis and at the post-apheresis clinic visit (within 7 days of apheresis). On the day of apheresis, the questionnaire should be given to the patient after at least 30 minutes following MGTA-145 infusion. It is recommended to give it to the patient towards the end of the apheresis session.

General Concomitant Medication and Supportive Care Guidelines During Study Treatment

- Other growth factor medications (e.g., G-CSF, granulocyte-macrophage colony-stimulating factor) are prohibited up to 7 days prior to and during the mobilization and apheresis phase of the study.
- Patients enrolled on this study will refrain from participation in any other investigational study drug in which they will receive an investigational study drug for at least four weeks before dosing of MGTA-145 and plerixafor.
- Immunomodulatory drugs (IMiDs) to be stopped 14 days prior to start of mobilization. Other cancer directed therapy to be stopped at least 10 days prior to start of mobilization.
- Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose of MGTA-145 or until start of transplant conditioning (whichever comes first) will be recorded in the medical records.

4.1.3. Event Monitoring/Follow-up:

a. A patient will be considered to have failed mobilization and will discontinue study drug and go to Event Monitoring if they fail to collect the minimum of 2.0 million CD34 cells/kg after two apheresis sessions.

b. If patients undergo rescue mobilization for any reason (including physician discretion to collect more than 2 million CD34+ cells/kg) and proceed to upfront transplant with MGTA-145 and plerixafor mobilized graft, they will be followed for secondary transplant related outcomes. On the other hand, if they undergo upfront transplant with the stem cell graft obtained from rescue mobilization only, they will not be followed for secondary outcomes. It is recommended not to combine grafts from different mobilization protocols

c. Patients who are not proceeding with upfront ASCT will be followed in Event Monitoring until 30 days after completion of study drug. After that no, further follow-up is required in person or via phone visits. Patients' electronic medical records may be periodically reviewed to follow-up on myeloma treatment, response and laboratory values for up to 5 years or when they undergo ASCT later in disease course, whichever is later.

d. If patients are proceeding with upfront transplant, they will be followed until day 100 for secondary outcomes of engraftment, disease progression and survival. After that, no further follow-up is required in person or via phone visits. Patients electronic medical records may be periodically reviewed to follow-up on myeloma treatment, response and laboratory values for up to 5 years

e. Adverse Events occurring after the patient completes study drug (MGTA-145 and plerixafor) will be collected on the Event Monitoring Form if they are >/= grade 3, at least possibly related to study drugs and have not been previously reported.

f. For patients proceeding with upfront transplant (defined up to 60 days after HSC mobilization), myeloablative melphalan conditioning (melphalan 140- 200 mg/m^2) on day -2 followed will by infusion of collected HSCs cells on day 0 per institutional standard of practice protocol. The maximum interval between start of stem cell collection on study and transplant on this study is 60 days. Within 60 days, the timing of transplant is at the discretion of the treating physician. If transplant is not planned or is planned after 60 days of collection, these patients will be considered in the delayed transplant group and transplant related outcomes will not be followed.

4.2 Criteria for Patient Withdrawal

Patients may be withdrawn from the study for the following reasons:

a. If a patient signs the consent form, but has not received the study drug, the patient will be regarded as non-evaluable and will be replaced. This includes patients unable to proceed with planned stem cell collection for any reason, including medical issues that preclude further participation or require a prohibited concomitant treatment, or a change of plan. Such patients will be withdrawn from the study. When such an event happens before the study drug is given, then patient will not be followed for events/outcomes. This patient will be deemed a 'cancel' and no further data submission is necessary. Patients may be replaced to meet accrual goal.
b. Patients who request to withdraw from the study at any point will be removed.

c. Patients who are lost to follow-up before end of study (defined as the inability to contact the patient on 3 separate documented occasions) will be withdrawn from the study.

d. Patients who proceed to rescue mobilization and undergo transplant during the study with a standard of care mobilized HSC graft.

4.3 Study Stopping Rules

The following are specific study stopping rules. Enrollment will be paused pending discussions with the FDA, agent manufacturer and Stanford IRB should any of the following study stopping rules occur:

(1) Any patient with a study agent related Grade 4 or Grade 5 event

(2) More than 1 (one) patient experiences failed/delayed engraftment due to neutrophil recovery beyond Day 42

(3) More than 1 (one) patient develops symptomatic hyperleukocytosis.

Symptomatic hyperleukocytosis is defined as a white blood cell (WBC) count of over 100 x 10^9/L (100,000/microL) associated with symptoms thought to be due to tissue hypoxia. most common symptoms include respiratory (such as dyspnea and hypoxia with or without diffuse interstitial or alveolar infiltrates on imaging studies) or neurological distress (such as visual changes, headache, dizziness, tinnitus, gait instability, confusion, somnolence, and coma). Less common signs or symptoms include electrocardiographic signs of myocardial ischemia or right ventricular overload, worsening renal insufficiency, priapism, acute limb ischemia, or bowel infarction.

In addition to the above rules, the study may be temporarily or permanently discontinued at any time. Reasons for study discontinuation may include, but are not limited to, the following:

a. Safety concerns
b. Poor enrollment
c. Request to discontinue the trial by a regulatory or health authority or an IRB
d. Manufacturing difficulties/concerns

4.4 Alternatives

If patients do not participate in this study, they may undergo HSC mobilization with the two common standard of care strategies to achieve HSC collection as described in Section **2.1.2.** These include chemotherapy-based mobilization (typically cyclophosphamide) along with G-CSF or G-CSF mobilization with plerixafor, in all patients or in a risk-adaptive algorithm.[20] Cyclophosphamide mobilization can take two to three weeks and result in complications like neutropenic fever and significant cytopenias.[2-4] G-CSF and plerixafor mobilization requires up to 4 days of G-CSF injections before mobilization can begin **(Figure 1)**. Injections are continued through collection, which can be as long as 4 sessions, therefore requiring 5-8 days for collection.[5]

5 INVESTIGATIONAL AGENT INFORMATION

5.1 Investigational Agent MGTA-145

Complete information on investigational study agent is provided in the accompanying **Investigator's Brochure**. This includes the mechanism of action, summaries of animal and clinical studies, non-clinical and clinical pharmacokinetics, major route of elimination, safety profile, and the rationale for the starting dose and regimen chosen. This also includes information on the metabolism of MGTA-145 in humans.

5.2 Availability

MGTA-145 investigational agent will be provided by Magenta Therapeutics. Plerixafor will be acquired commercially based on standard institutional procurement for patients undergoing stem cell mobilization.

5.3 Agent Ordering

MGTA-145 will be delivered to Stanford Health Care Investigational Drug Pharmacy by Magenta Therapeutics. Two doses of MGTA-145 must be available before attempted mobilization for a given patient on this protocol. Plerixafor will be obtained commercially per institutional standard procedures.

Shipping address of the investigational pharmacy:

Stanford Health Care

Attention: Investigational Pharmacy

[redacted]

Stanford, CA 94305

[redacted]

5.4 Agent Accountability/Preparation/Administration

The investigational agent will be secured by the Stanford Health Care investigational pharmacy per standard procedures.

a. **Formulation:** MGTA-145 is formulated at 20 mg/mL in 20 mM sodium acetate, 150 mM sodium chloride, 0.02% (w/v) Polysorbate 80 at pH 4.0. MGTA-145 is supplied in a single-dose 2 mL Type I amber glass vial. Each vial contains not less than 1 mL. MGTA-145 (injection) is a sterile solution for IV use supplied as a single-dose vial packaged in a carton.

b. **Storage:** Vials of MGTA-145 should be stored at -20°C ± 5°C in the carton to protect from light. Vials of MGTA-145 should be thawed overnight at 4°C prior to use and are stable at 4°C for up to 30 days. Once thawed, MGTA-145 should not be refrozen.

c. **Thawing:** The thawing will be conducted per investigational pharmacy standard procedures and drug hand off to Stanford Health Care apheresis unit at 875 Blake Wilbur Drive will occur in the morning before administration per institutional procedures.

d. **Preparation:** MGTA-145 will be diluted in 0.9% saline in accordance with the pharmacy manual. Solutions of MGTA-145 (0.036 to 1.44 mg/mL) prepared in 0.9% saline may be stored for no more than 8 hours at room temperature prior to administration.

e. **Administration:** MGTA-145 will be administered as an IV infusion over 3 to10 minutes, as clinically indicated.

6 DOSE MODIFICATIONS

There are no proposed dose modifications of MGTA-145 or plerixafor as described in section 4 under treatment plan. If any patient experiences grade 3 or higher non-hematological toxicity, not reversible to grade 1 or baseline using standard of care interventions within 24 hours, and is considered possibly, probably or definitely related to MGTA-145, the study drug will be discontinued and patient will go to event monitoring. The patient can continue to receive plerixafor if that is felt appropriate by the treating physician. The patients will be followed for 30 days from last dose of MGTA-145or till start of transplant conditioning, whichever comes first.

7 ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

7.1.1 MGTA-145

Study 145-HV-101, Phase 1 study with MGTA-145 and plerixafor in healthy volunteers: Overall, 77 out of 107 subjects (65 who received MGTA-145 and 12 who received placebo) experienced at least 1 AE during the study. The most frequent treatment-emergent adverse events (TEAEs) (occurring in > 5 subjects) among subjects randomized to receive MGTA-145 were back pain (48 subjects), nausea (13 subjects), dizziness (13 subjects), diarrhea (8 subjects), and headache (8 subjects). A total of 63 subjects experienced a TEAE considered by the Investigator to be related to MGTA-145.

The most frequent TEAEs (occurring in > 1 subject) were back pain, diarrhea, nausea, vomiting, fatigue, abdominal discomfort, abdominal pain, injection site pruritus, headache, paresthesia, musculoskeletal chest pain, and dizziness. The majority of all AEs reported were described as mild (grade 1) in NCI CTCAE severity, without an apparent trend across dose levels or study part. The consistent observation associated with MGTA-145 infusion was grade 1 bone pain in the back that begins during the 10-minute infusion and generally approximately 10 minutes later **(Table 7**: Summary of Acute Back Pain **).** All of the reported AEs of back pain were considered by the Investigator to be related to study treatment.

Table 7: Summary of Acute Back Pain (Table source: *Investigator's Brochure*)

Parameter	MGTA-145								Pooled Placebo (N=28)
	0.0075 mg/kg (N=4)	0.015 mg/kg (N=14)	0.030 mg/kg (N=25)	0.070 mg/kg (N=10)	0.075 mg/kg (N=12)	0.15 mg/kg (N=10)	0.30 mg/kg (N=4)	All MGTA-145 (N=79)	
Duration (min)									
n	4	8	13	5	10	5	3	48	2
Mean	9.5	10.3	17.2	14	28.6	12.8	125	23.7	15.5
Standard Deviation	4.51	3.69	14.63	3.74	37.07	3.90	201.79	52.90	13.44
Median	11.0	9.5	11.0	14.0	21.0	11.0	10.0	11.0	15.5
Minimum	3	5	4	10	6	9	7	3	6
Maximum	13	17	55	20	132	17	358	358	25

Note: Acute back pain is defined as back pain that occurs on the dosing date.

The known toxicities of plerixafor were observed and generally assessed as mild. For this study, a dose-limiting toxicity (DLT) was defined as a grade 2 or higher TEAE based on the NCI CTCAE v5.0 that is not a commonly expected TEAE associated with plerixafor (≥5% of plerixafor administrations) (see below in **Section 7.1.2**).

There were no grade 3 or grade 4 TEAEs in the study. A DLT of 1 event of grade 2 back pain was observed at 0.075 mg/kg MGTA-145 mg/kg, administered 2 hours after plerixafor (Part B). After dose reduction to 0.070 mg/kg, no DLTs were observed at that dose or at subsequent doses of 0.030 mg/kg or 0.015 mg/kg when MGTA-145 was administered 2 hours after plerixafor. Notably, grade 2 back pain was not reported for subjects administered MGTA-145 at 0.075 and 0.150 mg/kg immediately following plerixafor in Part B, or for subjects administered MGTA-145 at 0.070 mg/kg on 2 days in Part C.

Seventy-seven subjects (65 in a MGTA-145 group and 12 in the placebo group) reported at least 1 grade 1 TEAE; 4 subjects experienced a moderate (grade 2) event, and no ≥ grade 3 events were observed. One subject (Subject 101-393) experienced an AE that led

to study treatment withdrawal. This grade 2 event of back pain occurred following administration of 0.075 mg/kg MGTA-145 which was given 2 hours after plerixafor and met the criteria for a DLT per the protocol. As such, a maximum dose of 0.07 mg/kg MGTA-145 Tablein combination with plerixafor was used as the highest dose for the remainder of the study dosing. No subject in the study was discontinued from an MGTA-145 study due to an AE, and no serious adverse events (SAEs) or deaths were reported.

There were no changes in any laboratory investigation that were assessed as clinically significant. Isolated elevations in bilirubin from 1.4 to 3.2 mg/dL (normal 0 to 1.2 mg/dL) were seen on Days 1 to 3 in 8 subjects (6 received MGTA-145 + plerixafor, 1 received placebo + plerixafor, 1 received MGTA-145 alone). Elevations in bilirubin are known to be associated with plerixafor dosing. No elevation was assessed as clinically significant, and all values returned to normal before study completion. The 1 subject who received MGTA-145 alone in Part A who had an elevated bilirubin to 1.4 mg/dL on Day 2 also had an elevated level of 1.3 mg/dL at baseline. Thus, the elevation on Day 2 was not attributed to study drug. Elevations in WBCs and WBC subsets are expected as part of the PD action of MGTA-145 + plerixafor. Prothrombin time/activated partial thromboplastin time (aPTT), fibrinogen, and d-dimer tests were added to the laboratory investigations for the final subjects in the study (subjects receiving MGTA-145 0.015 mg/kg 2 hours after plerixafor in Part B and Part D) after a subject at the 0.03 mg/kg dose level in Part D failed to establish adequate venous flow for apheresis after mobilization. For these subjects receiving the MGTA-145 0.015 mg/kg dose 2 hours after plerixafor, transient elevations were seen in d-dimer to 0.59 to 1.44 mg/L in 5 of 9 subjects (normal range 0 to 0.49 mg/L) 6 hours after MGTA-145 infusion. D-dimer returned to normal in all subjects at the next observation at 48 hours. Transient changes in d-dimer are consistent with what is observed during other mobilization regimens. There was no clinically significant change in vital signs with MGTA-145 dosing. Transient elevations in systolic blood pressure ≥ 140 mmHg were noted in 10 subjects who experienced back pain.

Changes in electrocardiogram (ECG) measurements were considered not clinically significant, and no clinically important increase in the frequency of QTc abnormalities after initiation of treatment was observed.

Overall, the incidence of AEs was similar between the treatment cohorts. No increase in severity or frequency of adverse effects associated with plerixafor was observed when it was given in combination with MGTA-145.

Please refer to accompanying *Investigator's Brochure* for a detailed description of adverse events in each of the 4 arms of the study as well as AEs seen in the ongoing study of MGTA-145 in patients with renal impairment.

7.1.2 Plerixafor:

The potential adverse event associated with plerixafor as obtained from the FDA approved **package insert** are as follows:

> **Clinical Trials Experience:** The most common adverse reactions (≥10%) reported in patients who received plerixafor in conjunction with G-CSF regardless of causality and more frequent with plerixafor than placebo during HSC mobilization and apheresis were diarrhea, nausea, fatigue, injection site reactions, headache, arthralgia, dizziness, and vomiting.
>
> Safety data for plerixafor in combination with G-CSF were obtained from two randomized placebo-controlled studies (301 patients) and 10 uncontrolled studies (242 patients). Patients were primarily treated with Plerixafor at daily doses of 0.24 mg/kg SC. Median exposure to Plerixafor in these studies was 2 days (range 1 to 7 days).
>
> In the two randomized studies in patients with NHL and MM, a total of 301 patients were treated in the Plerixafor and G-CSF group and 292 patients were treated in the placebo and G-CSF group. Patients received daily morning doses of G-CSF 10 micrograms/kg for 4 days prior to the first dose of Plerixafor 0.24 mg/kg SC or placebo and on each morning prior to apheresis. The adverse reactions that occurred in ≥5% of the patients who received Plerixafor regardless of causality and were more frequent with Plerixafor than placebo during HSC mobilization and apheresis are shown in **Table 8.**
>
> In the randomized studies, 34% of patients with NHL or MM 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 allergic reactions** were observed in less than 1% of patients within approximately 30 min after Plerixafor administration, including 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 ≤0.24 mg/kg. The majority of these events occurred within 1 hour of Plerixafor administration. Because of the potential for these reactions, appropriate precautions should be taken.
>
> Other adverse reactions in the randomized studies that occurred in <5% of patients but were reported as related to plerixafor during HSC mobilization and apheresis included abdominal pain, hyperhidrosis, abdominal distention, dry mouth, erythema, stomach

discomfort, malaise, hypoesthesia oral, constipation, dyspepsia, and musculoskeletal pain.

Hyperleukocytosis: In clinical trials, white blood cell counts of 100,000/mcL or greater were observed, on the day prior to or any day of apheresis, in 7% of patients receiving plerixafor and in 1% of patients receiving placebo. No complications or clinical symptoms of leukostasis were observed.

Post-marketing Experience: In addition to adverse reactions reported from clinical trials, the following adverse reactions have been reported from postmarketing experience with Plerixafor. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

- Blood and Lymphatic System: Splenomegaly and splenic rupture
- Immune System Disorders: Anaphylactic reactions, including anaphylactic shock
- Psychiatric Disorders: Abnormal dreams and nightmares

Table 8: Adverse Reactions in ≥5% of Non-Hodgkin's Lymphoma and Multiple Myeloma Patients Receiving Plerixafor and More Frequent than Placebo during HSC Mobilization and Apheresis

	Percent of Patients (%)					
	Mozobil and G-CSF (n=301)			Placebo and G-CSF (n=292)		
	All Grades*	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Gastrointestinal disorders						
Diarrhea	37	<1	0	17	0	0
Nausea	34	1	0	22	0	0
Vomiting	10	<1	0	6	0	0
Flatulence	7	0	0	3	0	0
General disorders and administration site conditions						
Injection site reactions	34	0	0	10	0	0
Fatigue	27	0	0	25	0	0
Musculoskeletal and connective tissue disorders						
Arthralgia	13	0	0	12	0	0
Nervous system disorders						
Headache	22	<1	0	21	1	0
Dizziness	11	0	0	6	0	0
Psychiatric disorders						
Insomnia	7	0	0	5	0	0

* Grades based on criteria from the World Health Organization (WHO)

7.2 Adverse Event and Serious Adverse Event Reporting

7.2.1 Definitions

Adverse Event:

An adverse event (AE) is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the subject, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All adverse events will be graded according to NCI CTCAE v5.0.

Toxicities present at the initiation of study drug will be considered baseline conditions.

Serious Adverse Event

An AE or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- In-patient hospitalization or prolongation of existing hospitalization [Note: Hospitalizations related to scheduling issues (e.g. evening or weekend evaluations) and convenience considerations do not constitute a serious adverse event.]
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the subject or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.2.2 Serious Adverse Event Reporting

Adverse events and Serious Adverse Events in this study will be graded for severity according to National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (**CTCAE v5.0).**

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 Serious Adverse Event (SAE) to determine whether it is unexpected according to the Reference Safety Information section of the Investigator's Brochure for MGTA-145 or the package insert for plerixafor.

All Serious Adverse Events (SAEs) will be tracked from pre-mobilization evaluation (baseline AE assessment, can occur up to 7 days before mobilization as described in schedule of events) until resolution, or until 30 days after the last dose of the study intervention i.e. mobilization agents. Study intervention related AEs/ SAEs should continue to be followed until resolution or stabilization. Any SAE occurring after the reporting period must be promptly reported if a causal relationship to the investigational drug is suspected.

When assessing whether an adverse event is related to the investigational intervention, the following attribution categories are utilized:

Definite - The adverse event is clearly related to the agent(s).

Probable - The adverse event is likely related to the agent(s).

Possible - The adverse event may be related to the agent(s).

Unlikely - The adverse event is doubtfully related to the agent(s).

Unrelated - The adverse event is clearly NOT related to the agent(s).

For the purpose of regulatory reporting requirements, causal relationships of definite, probable, and possible will be considered treatment related, while unlikely and unrelated will be considered not treatment related.

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 investigational product. 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.

All SAEs occurring during stem cell mobilization and up to post-mobilization visit, regardless of relationship to study treatment, must be reported immediately to Magenta Therapeutics with the timeframe not to exceed 24 hours of the Investigator becoming aware of the event. Initial SAE notification should be made by emailing or faxing the SAE report form to Magenta Therapeutics at the email or fax number provided by Magenta Therapeutics. Any pregnancy in a female patient or partner of a male patient must also be reported to Magenta Therapeutics, within 24 hours of the investigator becoming aware of the event using a Pregnancy Report Form.

SAEs occurring after the patient discontinues MGTA-145, i.e. beyond the post-mobilization visit, will be collected and reported according to above protocol if they are at least possibly related to study agent, and have not been previously reported (unexpected).

As study sponsor, the Investigator is responsible for all communications with the FDA. The Investigator will report to the FDA, any serious adverse event that meets the FDA's criteria for expedited reporting following reporting requirements and timelines set by the FDA.

Magenta will notify Investigators of all suspected unexpected serious adverse reactions (SUSARs 7/15 Day Safety Reports) that occur during any clinical studies that are using the investigative compound. Each site is responsible for notifying their IRB/ EC of these additional SUSARs in accordance with local regulations.

7.2.3 Baseline and Follow-up Adverse Event Evaluations:

a. Adverse events to be graded at baseline, after each dose of MGTA-145, in a timely manner of study intervention for stem cell mobilization and 30 days after the last dose of study intervention, or until initiation of transplant procedures, whichever is first. If the AE occurs after initiation of transplant procedures but before the conclusion of the 30 days, AEs will be collected if they are serious and possibly, probably or definitely related to the study agent.

Transplant related AEs will NOT be collected on this study.

b. Death within 30 days of last investigational agent and death at any time at least possibly related to study agent will also be reported on the Event Monitoring Form and reported to DSMC, Magenta Therapeutics using an SAE Form and the FDA.

c. Adverse events will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grading unless otherwise stated.

d. The following pre-treatment symptoms/conditions (**Table 9**) are to be graded at baseline and adverse events are to be graded at each evaluation using CTCAE v5.0 grading (document 0 if absent).

Table 9: Adverse Event Assessment

CTCAE SYSTEM/ ORGAN/CLASS	Adverse event/Symptoms	Baseline	Mobilization Days	Post-mobilization
Investigations	Creatinine increased	X	X	X
	Neutrophil count	X	X	X
	Neutrophil count	X	X	X
	Platelet count decreased	X	X	X
	ALT increased	X	X	X
	AST increased	X	X	X
	Bilirubin increased	X	X	X
General disorders and	Fatigue	X	X	X
	Flu like symptoms	X	X	X
	Pain	X	X	X
	Injection site reaction	X	X	X
Procedural	Infusion related reaction	X	X	X
Gastrointestinal Disorders	Nausea	X	X	X
	Vomiting	X	X	X
	Abdominal Pain	X	X	X
	Baseline number of stools	X		

	Diarrhea	X	X	X
Blood and lymphatic	Anemia	X	X	X
Musculoskeletal	Bone Pain	X	X	X
	Back Pain	X	X	X
	Arthralgia	X	X	X
	Muscle Cramp	X	X	X
Nervous System Disorders	Paresthesias	X	X	X
	Headache	X	X	X
	Dizziness	X	X	X

e. The following AEs experienced by a patient and not specified in Section 7.2.2 will be recorded in the study database using Case Report Forms.

- Grade 2 AEs deemed possibly, probably, or definitely related to the study treatment or procedure at above defined time points.
- Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure at above defined time points.
- Grade 5 AEs (Deaths). Any death within 30 days of the patient's last study intervention regardless of attribution to the study agent. Any death more than 30 days after the patient's last study agent that is felt to be at least possibly related to the study agent must also be reported as a Grade 5 AE, with the reason for death, and attribution assigned.

8 LABORATORY CORRELATIVE/SPECIAL STUDIES

8.1 (Apheresis Product Composition – Laboratory Correlative Study #1a , #1b, #1c)

8.1.1 Collection of Specimen(s): Two 2-4 mL samples of cryopreserved peripherally mobilized apheresis product will taken from the mobilized fresh product from the first day of apheresis delivered to the Stanford BMT Cellular Therapy Facility (CTF). A third fresh sample (2-3 mL) will be collected for minimal residual disease analysis.

8.1.2 Handling of Specimens(s): Samples will be processed, aliquoted and cryopreserved in the Cellular Therapy Facility at Stanford and stored in liquid nitrogen until ready for batched analysis. For MRD testing (study #1c), fresh sample will be aliquoted in a EDTA (purple top) tube to be shipped to Mayo Clinic for processing and analysis.

8.1.3 Shipping of Specimen(s): Cryopreserved samples will be hand delivered Dr. Shizuru's lab for analysis for flow cytometry and Human Immune Monitoring Center at Stanford for CyTOF analysis. Samples will be thawed per laboratory protocol prior to batched testing. Fresh sample (de-identified) for MRD testing will be shipped overnight in a styrofoam container with chilled or ambient packs.

8.1.4 Site(s) Performing Correlative Study: Stanford University (Shizuru lab and Human Immnue Monitoring Center - HIMC), Mayo Clinic (Shaji Kumar lab) for MRD testing.

8.1.5 Coding of specimens for privacy protection: For specimens being tested internally at Stanford University: specimens may be labeled with patient's name, medical record number and study ID. For samples to be to be sent outside of Stanford University, PHI information will be removed and samples will be labeled with patients initials and Study ID only. The latter convention (study ID and initials) may be adopted for all internal samples as well.

8.2 (CFU assay– Laboratory Correlative Study #2)

8.2.1 Collection of Specimen(s): Samples of peripherally mobilized apheresis product will taken from the mobilized product delivered to the CTF from apheresis unit. CFU can be done on both fresh and thawed samples. Fresh samples will be used. CFU testing per Stanford BMT CTF Facility.

8.2.2 Handling of Specimens(s): Processing and testing will be done in the Stanford BMT CTF lab per institutional SOP.

8.2.3 Shipping of Specimen(s): None

8.2.4 Site(s) Performing Correlative Study: Stanford BMT Cellular Therapy Facility

8.2.5 Coding of specimens for privacy protection: Specimens will be labeled with patient's name, medical record number and study ID. Samples will be analyzed within Stanford University. In case samples need to be sent outside of Stanford University, PHI information will be removed and samples will be labeled with patients initials and Study ID only. The latter convention (study ID and initials) may be adopted for all internal samples as well.

8.3 (Immune Reconstitution at day 28 and 100 by flow cytometry and at day 100 by CyTOF – Laboratory Correlative Study #3a and 3b)

8.3.1 Collection of Specimen(s): Peripheral blood samples, two aliquots of 3-4 mL each will be collected in conjunction with the clinical lab and/or clinical trials research unit (CTRU). Samples will be delivered to the CTF.

8.3.2 Handling of Specimens(s): Samples will be processed at the Cellular Therapy Facility, where they will be stored until ready for batched analysis by the Shizuru lab and HIMC.

8.3.3 Shipping of Specimen(s): None, samples will be hand delivered to Dr. Shizuru's lab for analysis for flow cytometry and Human Immune Monitoring Center at Stanford for CyTOF analysis.

8.3.4 Site(s) Performing Correlative Study: Stanford university Shizuru Lab, Stanford HIMC8.1.

8.3.5 Coding of specimens for privacy protection: Specimens will be labeled with patient's name, medical record number and study ID. Samples will be analyzed within Stanford University. In case samples need to be sent outside of Stanford University, PHI information will be

removed and samples will be labeled with patients initials and Study ID only. The latter convention (study ID and initials) may be adopted for all internal samples as well.

9 STUDY CALENDAR

Table 10A: Schedule of Study Assessments

	All patients				No transplant	Undergoing Upfront Transplant		
Study period procedures	**Pre-mobilization**		**Mobilization** [a]	**Post-Mobilization**	**30 days post-mobilization (±5)**	**Day -2 to 30**[b]	**Day 28 (± 5)**	**Day +100 (± 10)**
	≤ 30 days	**≤ 7 days**	**Daily**	**Within 7 days of mobilization**				
Complete medical history	X							
Inclusion/exclusion criteria review	X							
Prior antineoplastic therapy review	X							
Bone marrow biopsy and aspirate results review for response assessment and cellularity [c]	X							(X)[d]
SPEP, FLC and immunofixation review for response assessment from chart review [c]	X							(X)[d]
UPEP and immunofixation review for response assessment (as clinically indicated)[c] from chart review	X							(X)[d]
Height	X							
Weight, BSA		X		X				
Physical examination [e]	X			X				X
Vital signs		X	X	X				

	All patients				No transplant	Undergoing Upfront Transplant		
Study period procedures	**Pre-mobilization**		**Mobilization [a]**	**Post-Mobilization**	**30 days post-mobilization (±5)**	**Day -2 to 30[b]**	**Day 28 (± 5)**	**Day +100 (± 10)**
	≤ 30 days	**≤ 7 days**	**Daily**	**Within 7 days of mobilization**				
ECOG score	X							
Serum/urine βHCG (women of childbearing potential)		X						
12-Lead ECG		X						
Urinalysis		X						
Chemistry[f]		X	X[k]	X			X	X
Hematology[f]		X	X[k]	X			X	X
Peripheral blood CD34+ counts			X (30 mins ± 15 mins after MGTA-145 infusion start and at end of apheresis)					
Apheresis parameters [g,h]			X					
MGTA-145 administration[R]			X					
Plerixafor administration			X					
QoL/Pain measures[R]		X	X	X				
Adverse event assessment[R]		X	X	X[l]				
Concomitant medications[R]		X	X	X				

	All patients				No transplant	Undergoing Upfront Transplant		
Study period procedures	**Pre-mobilization**		**Mobilization [a]**	**Post-Mobilization**	**30 days post-mobilization (±5)**	**Day -2 to 30[b]**	**Day 28 (± 5)**	**Day +100 (± 10)**
	≤ 30 days	**≤ 7 days**	**Daily**	**Within 7 days of mobilization**				
Correlative study samples[R]		X	X				X	X[j]
Follow-up Phone Call[i R]					X			
Data collection and reporting for secondary outcomes (engraftment, progression, death) [R]						X	X	X

Abbreviations: ECOG: Eastern Cooperative Oncology Group, EOS: end of study; FLC: serum free light chains, HIV: Human immunodeficiency virus, QoL: Quality of life, SPEP: serum protein electrophoresis, UPEP: urine protein electrophoresis

[a] Mobilization with apheresis collection will occur over 1 or 2 days, depending on Day 1 $CD34^+$ cell yield. Timing of mobilization and relative to HSCT is at the Investigator discretion.

[b] Timing of transplant will vary and can be up to 60 days following start of collection per treating physician discretion. Management during transplant, including labs and other clinical evaluation will be per Stanford BMT institutional standardized clinical practice. Data on outcomes will be collected by study staff from electronic medical record or labs done locally on case report forms at periodic intervals up to day +30. In case patients have not engrafted by day 30, data will continue to be collected until engraftment. After Day 30, data on these outcomes from day 30 to 100 will be collected on day 100 by study staff.

[c] Bone marrow biopsy, SPEP, UPEP and immunofixation are standard of care procedures and may be done at non-Stanford local labs. Results of these tests should be reviewed per medical records and documented at the clinic visit

[d] Restaging studies may be done as standard of care between day 60 and 100 visit and may be done locally. Results of the restaging should also be collected at the day 100 Visit.

[e] A complete physical examination will be conducted at Pre and Post-Mobilization Visit. An abbreviated physical examination will be conducted at all other time points.

[f] Clinical chemistry: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.

[f] Hematology is complete blood count with platelets and differential.

[g] $CD34^+$ cell count in absolute numbers and per kg.

[h] Procedure start/end times, blood volume processed, volume of product collected, procedure-related AEs

[i] Follow-up phone call by study team at day 30 to discuss any ongoing concerns, including resolution of any investigational treatment related toxicities

[j] Corelative research bone marrow biopsy aspirate sample to be done only in patients undergoing a BM biopsy by day 100 for routine clinical care after transplant at Stanford.

[k] Chemistry and Hematology may be done within 24 hours of starting apheresis, including on the day prior

[l] Post-Mobilization adverse event assessment will be conducted in a timely manner (reference Section 7.2.3 for complete information)

All tests and procedures are standard of care unless marked R, R= research

Table 10B: Correlative Lab Study Schedule

	Correlative Studies	Timing	Source	Amount	Collecting Tube
Apheresis product	Flow Cytometry CyTOF CFU assay MRD assay Additional sample for banking	Apheresis day 1	Apheresis product	15-20 mL	Sample obtained from apheresis collection bag. At least 5 aliquots (2-4mL). These will be used for flow cytometry, CyTOF, MRD testing and CFU analysis. At least 1 aliquot to be banked (If large amount of sample remaining for banking, can divide in more than 1 cryovial) CFU assay and MRD testing on fresh sample. Rest will be on cryopreserved samples.
Peripheral Blood	Flow Cytometry CyTOF Additional sample for banking	**Baseline sample: all** **Upfront Transplant Group:** Post-transplant day 28 visit and day 100 visit	Peripheral Blood	10-20 mL	**Baseline sample:** 10 mL (fresh) in a green top tube **Day 28 sample:** 10 mL (fresh) in a green top tube for flow cytometry Additional 5 mL processed and cryopreserved in 1 aliquot, which will be banked

					Day 100 sample: 10 mL (fresh) in a green top tube. Rest of the sample processed and stored in at least two cryopreserved aliquots of 5 mL each. 1 aliquot will be used for CyTOF and an additional aliquot will be banked.
Bone Marrow Biopsy	CyTOF	**Upfront Transplant Group** (Done only in patients undergoing BM biopsy by day 100 timepoint for clinical care following transplant), Timing: By day 100. Timepoint. Usually done day 60-100 post transplant	Bone marrow aspirate	5 mL	Sample will be processed and cryopreserved in one aliquot of 5 mL.

10 MEASUREMENTS

Primary Outcome Measure Definition: CD34 yield of collected HSCs expressed as CD34+ cells/kg of patient body weight.

- **Time Frame:** Day one and two of stem cell mobilization and apheresis
- **Safety Issue:** Is this outcome measure assessing a safety issue? No

10.1 HSC yield in apheresis product (Primary and Secondary Outcome measures)

The primary outcome is collection of 2.0 x 10^6 CD34 cells/kg in one or two days of apheresis after mobilization with MGTA-145 and plerixafor. Secondary outcomes also include day one yield of 2.0 x 10^6 CD34 cells/kg and 4.0 x 10^6 CD34 cells/kg and total yield of 4.0 x 10^6 CD34 cells/kg and 6.0 x 10^6 CD34 cells/kg in up to two days of apheresis

10.1.1 Relevant Subset: All patients enrolled in the study who undergo stem cell mobilization, N=25

10.1.2 Measurement definition and time points: CD34 yield of apheresis product on day 1 and 2 of apheresis will estimated through flow cytometric evaluation of the apheresis product. The apheresis product volume and the absolute number of CD34+ cells per unit volume are to be measured to calculate the total yield of CD34+ cells. This will be done following Stanford BMT/Apheresis and CTF standard operating procedures.

Total yield reporting includes the combined yield from day 1 and 2 (if patient undergoes a second day of collection).

10.2 Other Secondary Outcome Measures

10.2.1 Infusion related toxicities with MGTA-145; Adverse events with mobilization of HSCs with MGTA-145 and plerixafor.

Relevant Subset: All patients enrolled in the study who undergo stem cell mobilization, N=25.

Measurement definition and time points: This will be assessed by NCI CTCAE version 5.0 as described under section 7 of the protocol. For a given AE, the maximal grade AE on that day will be reported.

AE assessment will occur at the following time points, (except infusion related AEs, which will only assessed on each day of mobilization):

- Baseline (within 3 days of mobilization)
- On each day of mobilization

- Within 7 days after mobilization

10.2.2 Neutrophil and platelet engraftment

Relevant Subset: Only patients proceeding with upfront transplant will be assessed.

Measurement definition and time points:

Time to primary engraftment is defined as time in days from the day of stem cell infusion to the first day of engraftment.

Ongoing successful engraftment at 30- and 100-days post-transplant after successful primary engraftment will also be assessed by CBC done as part of routine clinical care of patients undergoing transplant.

Engraftment definitions are as follows:

- Neutrophil engraftment is defined as the first day of ANC ≥0.5 x 10^9/L for 3 days following stem cell infusion. This is assessed by absolute neutrophil counts obtained as part of CBC with differential. It is standard practice to follow patients regularly in the inpatient unit/outpatient transplant unit until they achieve engraftment.
- Platelet engraftment is defined as the first day of platelet count more than or equal to 20 x 10^9/L without transfusion in the last 7 days and with platelet count ≥20 x 10^9/L on 2 separate, subsequent days. This is assessed by platelets counts obtained as part of CBC with differential. It is standard practice to follow patients regularly in the transplant inpatient unit/outpatient transplant until they achieve engraftment.
- We will also determine time to first day of platelet count more than or equal to 50 x 10^9/L, without transfusion within 48 hours.

10.2.3 Transplant related mortality

Relevant Subset: Only patients proceeding with upfront transplant will be assessed.

Measurement definition and time points: Transplant related mortality is described as death from any cause within the first 100 days of HSC infusion.

10.2.4 Non-relapse related mortality

Relevant Subset: Only patients proceeding with upfront transplant will be assessed.

Measurement definition and time points: Transplant related non-relapse mortality is described as death from any cause except disease relapse/progression within the first 100 days of HSC infusion.

10.2.5 Progression Free Survival (PFS):

Relevant Subset: Only patients proceeding with upfront transplant will be assessed.

Measurement definition and time points: Duration from start of the ASCT to disease progression or death (regardless of cause of death), whichever comes first. Progression will be assessed using day 100 post-transplant response and PFS rates will be reported at day 100 in patients undergoing upfront transplant. After day 100, these patients will be followed for PFS off study under the BMT research database under a separate IRB approved protocol.

10.2.6 Overall Survival (OS):

Relevant Subset: Only patients proceeding with upfront transplant will be assessed.

Measurement definition and time points: Duration from start of the ASCT to death (regardless of cause of death). This will be assessed from start of transplant and OS rates will be reported at day 100 following transplant in patients undergoing upfront transplant. After day 100, these patients will be followed for PFS off study under the BMT research database under a separate IRB approved protocol.

10.2.7 Response Assessment to Determine Progression:

Relevant Subset: Response before mobilization will be assessed in all patients. Response/progression following transplant will be assessed in patients undergoing upfront transplant.

Measurement definition and time points Myeloma response before stem cell mobilization and at day 100 will be assessed per the International Myeloma Working Group Uniform Response Criteria.[27]

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). ny 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

11.2.1 Principal Investigator and Study Research Team

The clinical research team and investigator sponsor will meet on a regular basis during patient screening and while patients are actively receiving the investigational agent on this trial to discuss eligibility questions, trial accrual and toxicities.

All data will be collected in a timely manner and reviewed by the investigator-sponsor or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the DSMC, sponsor-investigator and FDA as needed.

The principal investigator will review adverse event and outcome data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

11.2.2 Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC)

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.

11.2.3 Data Management Plan

The Protocol Director, or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study, in accordance with the established practices of the Stanford Division of Blood and Marrow Transplantation. Study specific electronic Case Report Forms (CRFs) will document treatment outcomes for data analysis. Data will

be transcribed into eCRFs using Medrio software. Medrio software is compliant with 21 Code of Federal Regulations Part 11 FDA requirements.

Data will be entered for this study utilizing one or a combination of the following methods:

- Data may be captured electronically, without use of paper.
- Data may be transcribed from the Electronic Medical Record source documents into the EDC system without the use of paper
- Data may be captured on paper (source documents) and transcribed into the EDC system. In this case paper documentation will be retained and will be available for review.

DSMC of the Stanford Cancer Center will oversee this study and will receive regular reporting of SAEs and monitoring. This Committee involves knowledgeable physicians, scientists and statisticians who oversee all phase I/II trials conducted under the auspices of the Stanford Cancer Center. Individuals who serve on this Committee who are associated with this trial will recuse themselves from discussions of study results, and decisions about revision or closure. Audits will be conducted as requested. Data will also be submitted to the FDA as required by our IND and follow the appropriate reporting rules and regulations including an annual report. Data will be shared with Magenta Therapeutics according to a data sharing and use agreement.

12 COLLABORATIVE AGREEMENTS

A collaborative research agreement exists for receipt of MGTA-145 and financial support from Magenta Therapeutics. This collaborative agreement is disclosed in the informed consent document. In this agreement, Magenta Therapeutics has access to study data and study information and may receive study samples for analysis.

13 STATISTICAL CONSIDERATIONS

13.1 Statistical Design

This is a single center, single arm phase II proof of concept study designed to assess the success rate of stem cell mobilization with a novel regimen of an investigational drug (MGTA-145) with plerixafor in patients with multiple myeloma. The sample size of this phase II proof of concept study is 25 patients as described below. Additional patients may be screened to account for patients who screen fail or withdraw prior to start of study intervention i.e administration of MGTA-145.

13.2 Safety Run-In/Futility Rule

Early Safety Run-in: The first six patients accrued to this study will be patients who are planned for upfront transplant following collection. Additional patients will not begin mobilization until these three patients have completed stem cell mobilization and are at least 21 days following infusion of stem cells after myeloablative melphalan conditioning. Safety review will be done to evaluate study drug-related adverse events, in particular for any unexpected grade 3 or higher adverse events drug during this period. Engraftment data will also be reviewed. If any unexpected grade 3 or higher

adverse events probably or definitely related to the study drug are noted in the first three patients, the PI will review the study with the FDA to determine whether study can continue.

13.3 Descriptive Statistics and Exploratory Data Analysis

Descriptive statistics will be used to describe study patient population and exploratory data analysis. Categorical variables will be summarized in frequencies. Continuous variables will be summarized in mean and median, or median and interquartile range when they are appropriate. Ninety-five confidence intervals will be presented.

13.4 Primary Analysis

The **primary endpoint** of this study is to assess the rate of successful collection of ≥2.0 x 10^6 $CD34^+$ cells/kg in up to two days of apheresis after MGTA-145 and plerixafor dosing in patients with MM. (as described in section **1.1** and **10.1**)

a. **Analysis Population:** Patients proceeding with stem cell mobilization after receiving the novel regimen of MGTA-145 and plerixafor. This includes 15 patients in segment one. If the primary endpoint is met in the first segment of the study, this endpoint will also be evaluated in the total sample size of 25 patients (segment one and two).

b. **Analysis Plan:** Apheresis product cell counts are reported as a standard of care in all patients undergoing stem cell collection using flow cytometry per institutional guidelines. The successful achievement of the primary objective of efficacy of this regimen will be assessed by calculating the proportion of patients meeting this endpoint after up to two days of investigational drug regimen followed by apheresis.

   HSCs counts will be added to get the final HSC count per kg of patient body weight from HSCs collected in different bags and on different days as is standard operating procedure. As described under sample size, we hypothesize that at least 95% of patients will meet the primary endpoint. Thus, we expect that at least 13 out of 15 subjects in the first segment of the study will achieve this HSC collection target.

13.5 Secondary Analysis

13.5.1 Secondary Endpoint #1:

To assess the rate of successful collection of **(a)** ≥4.0 x 10^6 $CD34^+$ cells/kg in up to two apheresis sessions after MGTA-145 and plerixafor dosing in patients with MM. **(Key Secondary Endpoint) (b)** ≥6.0 x 10^6 $CD34^+$ cells/kg with up to two apheresis sessions after MGTA-145 and plerixafor dosing in patients with MM. **(c)** ≥2.0 x 10^6 $CD34^+$ and ≥4.0 x 10^6 $CD34^+$ cells/kg with one apheresis session after MGTA-145 and plerixafor dosing.

Analysis Population: All patients proceeding with stem cell mobilization after receiving the novel regimen of MGTA-145 and plerixafor. This includes 25 patients including segment one and two.

Analysis Plan: Apheresis product cell counts are reported as a standard of care in all patients undergoing stem cell collection using flow cytometry per institutional guidelines. The successful achievement of the secondary endpoint 1a and 1b will be assessed by calculating the proportion of patients meeting the targets listed above after receiving investigational drug regimen followed by apheresis. HSCs counts will be added to get the final HSC count per kg of patient body weight from HSCs collected in different bags and on different days as is standard operating procedure. Proportion of patients meeting the endpoints will be reported.

13.5.2 Secondary Endpoint #2:

To assess infusion related toxicities with MGTA-145 and adverse events with mobilization of HSCs with MGTA-145 and plerixafor.

Analysis Population: All patients proceeding with stem cell mobilization after receiving the novel regimen of MGTA-145 and plerixafor. This includes 25 patients including segment one and two.

Analysis Plan: Adverse events will be reported using NCI CTCAE version 5.0 as described in section 7. Proportion of patients experiencing each side effect and grade of side effect will be analyzed and reported.

13.5.3 Secondary Endpoint #3:

To assess time to neutrophil (defined as the first day of ANC $\geq 0.5 \times 10^9$/L for 3 days following stem cell infusion) and platelet engraftment (defined as first day of platelet count more than or equal to 20×10^9/L without transfusion in the last 7 days and with platelet count $\geq 20 \times 10^9$/L on 2 separate, subsequent days and to also determine time to first day of platelet count more than or equal to 50×10^9/L, without transfusion within 48 hours in patients undergoing upfront ASCT.

Analysis Population: All patients proceeding with upfront transplant (defined as within 60 days of HSC mobilization and collection) will be evaluated for this endpoint.

Analysis Plan: Number and percentage of patients achieving engraftment by above parameters. Time to engraftment will be evaluated for each patient and summarized as median, inter-quartile range, range and proportion of patients achieving engraftment by a given time point.

13.5.4 Secondary Endpoint #4:

To assess rates of ongoing successful engraftment at 30- and 100-days post-transplant after successful primary engraftment in patients undergoing upfront ASCT.

Analysis Population: All patients proceeding with upfront transplant (defined as within 60 days of HSC mobilization and collection) will be evaluated for this endpoint.

Analysis Plan: Proportion of patients achieving primary engraftment by above parameters will also be assessed for ongoing secondary engraftment by CBC with differential on day 30 and 100 post-transplant

13.5.5 Secondary Endpoint #5:

To assess rate of transplant related mortality, non-relapse mortality, progression free survival and overall survival at 100 days in patients with undergoing upfront ASCT.

Analysis Population: All patients proceeding with upfront transplant (defined as within 60 days of HSC mobilization and collection) will be evaluated for this endpoint.

Analysis Plan: Number and percentage of patients experiencing death related to any cause (Transplant related mortality), death related to any cause, except disease progression (Non-relapse mortality) and patients remaining progression free and alive (Day 100 progression free survival), and patients alive at day 100 (day 100 OS) will be reported. Kaplan-Meier survival curves will be generated. Whenever possible, median times to the above events will be estimated with 95% confidence intervals.

13.6 Sample Size

13.6.1 Accrual estimates

The sample size for this single center, single arm study is 25 patients who undergo mobilization as described below. Additional patients may be screened to determine eligibility for the trial.

We expect to complete study accrual in 9-12 months as 100-120 patients with multiple myeloma undergo ASCT annually in our division.

If accrual falls short of expectations, then we will continue to enroll patients for additional 6-9 months to complete accrual.

13.6.2 Sample size justification

For this single-arm trial with a binomial primary endpoint, the maximum sample size is 25 evaluable patients. Based on historical data with HSC mobilization with G-CSF[5,8-10], we hypothesize that at least 95% of subjects who undergo mobilization with this novel regimen of MGTA-145 and plerixafor will meet the primary end-point of collecting $\geq 2.0 \times 10^6$ $CD34^+$ cells/kg in two days of apheresis. We model the primary endpoint as a binomial random variable. The statistical design is based on the following one-sided hypothesis test. The null hypothesis is that the primary endpoint is less than or equal to 70%. The alternative hypothesis is that the primary endpoint is equal to or greater than 95%.

The trial will be conducted in two sequential segments: the first for evaluation of safety and feasibility, and then if successful, the number will be expanded to further evaluate safety and feasibility. In the first segment, 15 patients will be evaluated for the primary endpoint. In the second segment, which will be contingent upon achieving success in the first segment, 10 patients will be enrolled to gain additional safety and feasibility experience with this regimen and to evaluate the key secondary endpoint. Accounting for both segments, the total sample size is 25 patients.

We model the primary endpoint as a binomial random variable. A responder is defined as a patient meeting the primary endpoint. The statistical design for the primary endpoint is based on a binomial exact test for the null hypothesis of a responder rate (i.e meeting the primary endpoint) being 70% or less and the alternative hypothesis of a responder of 95% or more. The criterion for success of the

trial is at least 13 out of 15 patients are responders. The type I error rate is 5% and statistical power is 85%.

This design features an **implicit futility stopping rule**. Patients will be enrolled continuously to the first segment after initial safety run-in. The criterion for success is that at least 13 out of 15 patients are responders. This implies that if 3 or more patients are non-responders at any point, then the trial will be stopped for futility.

The collection of ≥4.0 x 10^6 CD34+ within 2 days a **key secondary endpoint** of this trial. We hypothesize that at least 75% of the 25 patients will meet this secondary endpoint The statistical design for the secondary endpoint is based on enrolling enough patients in the second segment such that the total sample size will produce a precision estimate with a lower binomial confidence limit that excludes the response rate of the historical controls (i.e., 50%). For this secondary endpoint, we compute a precision estimate of our hypothesized response rate for the scenario that both segments are completed. We model the secondary endpoint as a binomial random variable. We define precision to be the length of the binomial confidence interval for the hypothesized response rate of 75%. We compute precision using the method of Wilson at the significance level of 90% which is appropriate for an early-phase trial. Based on these specifications, the precision is 26.8%.

Enrolled patients may be replaced if they withdraw consent or if there is a change in treatment plan for any reason before start of study treatment to allow for 25 evaluable patients.

13.6.3 Effect size justification

Historical data from clinical studies of mobilization with different regimens particularly G-CSF alone in patients with multiple myeloma (**Table 11**) was evaluated to determine effect size. Success rate of collecting ≥2.0 x 10^6 $CD34^+$ cells/kg in up to two days of apheresis after mobilization with MGTA-145 and plerixafor was estimated to be at least 95% based on data below and from results of the phase 1 study with MGTA-145 and plerixafor in healthy volunteers [5,7,9,10,22,53] Differences in regimens and variable primary and secondary endpoint targets were taken into account.

Table 11: Stem cell mobilization data from prior studies of G-CSF and GCSF+/-plerixafor in myeloma

Study	Mobilization Regimen	Results
Flomerberg et al., 2005	G-CSF + Plerixafor and G-CSF alone	Comparison of HSCs mobilized with G-CSF + Plerixafor vs. G-CSF alone, 9 of 25 patients (36%) did not meet 2 million CD34+ cells/kg collection goal in the G-CSF alone attempt. Note: Mobilization was allowed beyond 2 apheresis sessions
Di Persio et al., 2009	G-CSF + Plerixafor vs. G-CSF alone	Primary endpoint was collection of 6 million CD34 cells/kg in 2 apheresis days. Primary endpoint met by 71% of patients in G-CSF+ Plerixafor arm vs. 34%of patients in G-CSF only arm.

Micallef et al 2012	Comparison of different mobilization regimens	In patients receiving mobilization without plerixafor, (i.e G-CSF alone or G-CSF with chemotherapy), failure to collect at least 2 million CD34+ cells was seen in 19% of patients. Note: Mobilization was allowed beyond 2 apheresis sessions
Sinha et al 2012	Different mobilization regimens in patients receiving lenalidomide based induction	11% of patients in the GCSF arm did not meet collection goal of 2 million CD-34+ cells/kg. Note: Mobilization was allowed beyond 2 apheresis sessions
Shaughnessy et al., 2013	G-CSF + SC Plerixafor	2 million CD34+ cells/kg in up to 3 days of apheresis was achieved by 98% of patients with MM. Note: Mobilization was allowed beyond 2 apheresis sessions

Institutional Data (unpublished): Amongst patients undergoing ASCT for myeloma at Stanford University Medical Center from Jan 2017-March 2020, we analyzed 69 consecutive patients who underwent collection with GCSF 10 mcg/kg and as needed plerixafor (which was given when CD34 count on day 4 was < 10). Plerixafor was needed in 55% (n=38) patients. Median number of apheresis was 2, with range of 1 to 5. 98.5% patients (68 of 69) collected ≥2.0 x 10^6 CD34+ cells/kg. 78% of patients (55 of 69) collected ≥4.0 x 10^6 CD34+ cells/kg. 64% (44 out of 69) patients collected ≥4.0 x 10^6 CD34+ within 2 days.

13.7 Criteria for future studies

If the study meets its primary endpoint, future studies will be planned with this combination regimen for stem cell mobilization prior to ASCT in patients with myeloma.

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15 APPENDICES

15.1 APPENDIX A: Participant Eligibility Checklist

A Participant Eligibility Checklist must be completed in its entirety for each subject prior to registration. The completed, signed, and dated checklist must be retained in the patient's study file and the study's Regulatory Binder.

The study coordinator, treating physician and an independent reviewer must verify that the participant's eligibility is accurate, complete, and legible in source records. A description of the eligibility verification process should be included in the EPIC or other Electronic Medical Record progress note.

The following is the Participant Eligibility checklist template that will be used for this protocol

BMT 362: Phase II Study of MGTA-145 in combination with plerixafor in the mobilization of hematopoietic stem cells for autologous transplantation in patients with multiple myeloma
Surbhi Sidana, MD
Subject Name/MRN:
Gender: ☐ Male ☐ Female

Inclusion Criteria

____ Diagnosis of multiple myeloma per the International Myeloma Working Group (IMWG) criteria.

____ Age: 18 to 70 years

____ Eligible for ASCT per institutional guidelines

____ Within one year of start of myeloma therapy

____ Cardiac and pulmonary status sufficient to undergo apheresis and transplantation per institutional transplant guidelines.

____ Calculated creatinine clearance > 30 mL/min according to the Modification of Diet in Renal Disease (MDRD) formula

____ Absolute neutrophil count $\geq$ 1500 x10^6/L and platelets $\geq$ 100,000 x10^6/L

____ Ability to understand and the willingness to sign a written informed consent document.

____ Agreement to use an approved form of contraception if of childbearing potential

Exclusion Criteria

____ History of prior stem cell transplant for multiple myeloma or other indications

____ Planned tandem stem cell transplant

____ Prior history of failure to collect HSCs.

____ Liver function tests: Total bilirubin >1.5x upper limit of normal (ULN) in the absence of a documented history of Gilbert's syndrome and/or AST/ALT > 3x ULN

____ Known allergy to MGTA-145 or plerixafor

____ Lifetime exposure to lenalidomide greater than 6 cumulative months of treatment i.e more than six 28-day cycles or more than eight 21-day cycles

____ Pregnant or lactating women

Treating Physician Signature:	Date:
Printed Name:	

Secondary Reviewer Signature:	Date:
Printed Name:	

Study Coordinator Signature:	Date:
Printed Name:	

15.2 APPENDIX B: **PRO Questionnaire**

1. Have you noticed any pain, swelling or redness at a site of drug injection or IV today? (Adapted from PRO-CTCAE)

   - Yes
   - No

2. If yes, what was the severity of the symptoms at worse? (Adapted from PRO-CTCAE)

   - None
   - Mild
   - Moderate
   - Severe
   - Very Severe

If you answered yes to experiencing pain today, please answer the following questions

3. How long did the pain last?

   - < 20 mins
   - 20 mins- 1 hour
   - More than 1 hour

Brief pain inventory tool (Short Form, Sample):

STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #: _____

Brief Pain Inventory (Short Form)

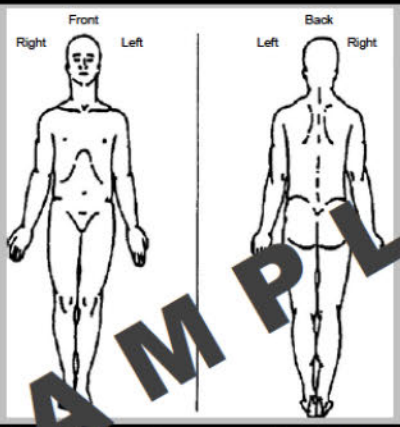
Date: ____/____/____ Time: _____

Name: _____ _____ _____
Last First Middle Initial

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these every-day kinds of pain today?

1. Yes 2. No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by circling the one number that best describes your pain at its worst in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

4. Please rate your pain by circling the one number that best describes your pain at its least in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

5. Please rate your pain by circling the one number that best describes your pain on the average.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

6. Please rate your pain by circling the one number that tells how much pain you have right now.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

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STUDY ID #:_____ DO NOT WRITE ABOVE THIS LINE HOSPITAL #:_____

Date:____/____/____ Time:_____

Name:_____ _____ _____

Last First Middle Initial

7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0% No Relief 10% 20% 30% 40% 50% 60% 70% 80% 90% 100% Complete Relief

9. Circle the one number that describes how, during the past 24 hours, pain has interfered with your:

A. General Activity

0 Does not Interfere 1 2 3 4 5 6 7 8 9 10 Completely Interferes

B. Mood

0 Does not Interfere 1 2 3 4 5 6 7 8 9 10 Completely Interferes

C. Walking Ability

0 Does not Interfere 1 2 3 4 5 6 7 8 9 10 Completely Interferes

D. Normal Work (includes both work outside the home and housework)

0 Does not Interfere 1 2 3 4 5 6 7 8 9 10 Completely Interferes

E. Relations with other people

0 Does not Interfere 1 2 3 4 5 6 7 8 9 10 Completely Interferes

F. Sleep

0 Does not Interfere 1 2 3 4 5 6 7 8 9 10 Completely Interferes

G. Enjoyment of life

0 Does not Interfere 1 2 3 4 5 6 7 8 9 10 Completely Interferes

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