A Phase 2 Trial Evaluating the Efficacy of Flotetuzumab for Relapsed Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS) Following Allogeneic Hematopoietic Cell Transplantation (allo-HCT)

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Protocol #: 202011122
Version Date: 28 February 2024

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Study Drug(s):	Flotetuzumab (MGD006)
IND#:	153399
IND Sponsor:	Matthew Christopher, M.D., Ph.D.
NCT#:	NCT04582864
Funded by:	MacroGenics, Inc.

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Protocol Revision History

Initial Approval Version	**15 December 2020**
Amendment #1 Version	**23 April 2021**
Amendment #2 Version	**17 September 2021**
Amendment #3 Version	**19 October 2021**
Amendment #4 Version	**19 January 2022**
Amendment #5 Version	**27 July 2022**
Amendment #6 Version (QASMC only)	**31 January 2023**
Amendment #6 Version (PRMC only)	**24 February 2023**
Amendment #6 Version	**31 March 2023**
Amendment #7 Version	**04 May 2023**
Amendment #8 Version (QASMC only)	**21 February 2024**
Amendment #8 Version	**28 February 2024**

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BMT	Bone marrow transplant
CBC	Complete blood count
CFR	Code of Federal Regulations
CNS	Central nervous system
CR	Complete response
CRc	Cytogenetic complete remission
CRi	Complete remission incomplete
CRm	Morphologic complete remission
CRF	Case report form
CST	Central standard time
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DLT	Dose limiting toxicity
DNA	deoxyribonucleic acid
DSM	Data and Safety Monitoring
DSMC	Data Safety Monitoring Committee
ECG (or EKG)	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
FISH	fluorescent in situ hybridization
FWA	Federal wide assurance
GCP	Good Clinical Practice
GVHD	Graft vs. Host Disease
HHS	Department of Health and Human Services
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
IND	Investigational New Drug
IRB	Institutional Review Board
MDS	Myelodysplastic syndrome
MM	Multiple myeloma
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose

NCCN	National Cancer Center Network
NCI	National Cancer Institute
NIH	National Institutes of Health
NSCLC	Non-small cell lung cancer
OHRP	Office of Human Research Protections
ORR	Overall response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PI	Principal investigator
PR	Partial response
PSA	Prostate-specific antigen
QASMC	Quality Assurance and Safety Monitoring Committee
RECIST	Response Evaluation Criteria in Solid Tumors (Committee)
RFS	Relapse free survival
RR	Response rate
SAE	Serious adverse event
SCC	Siteman Cancer Center
SCT	Stem cell transplant
SD	Stable disease
TSH	Thyroid stimulating hormone
TTP	Time to progression
UPN	Unique patient number
US	Ultrasound
VEGF	Vascular endothelial growth factor
WBC	White blood cell (count)

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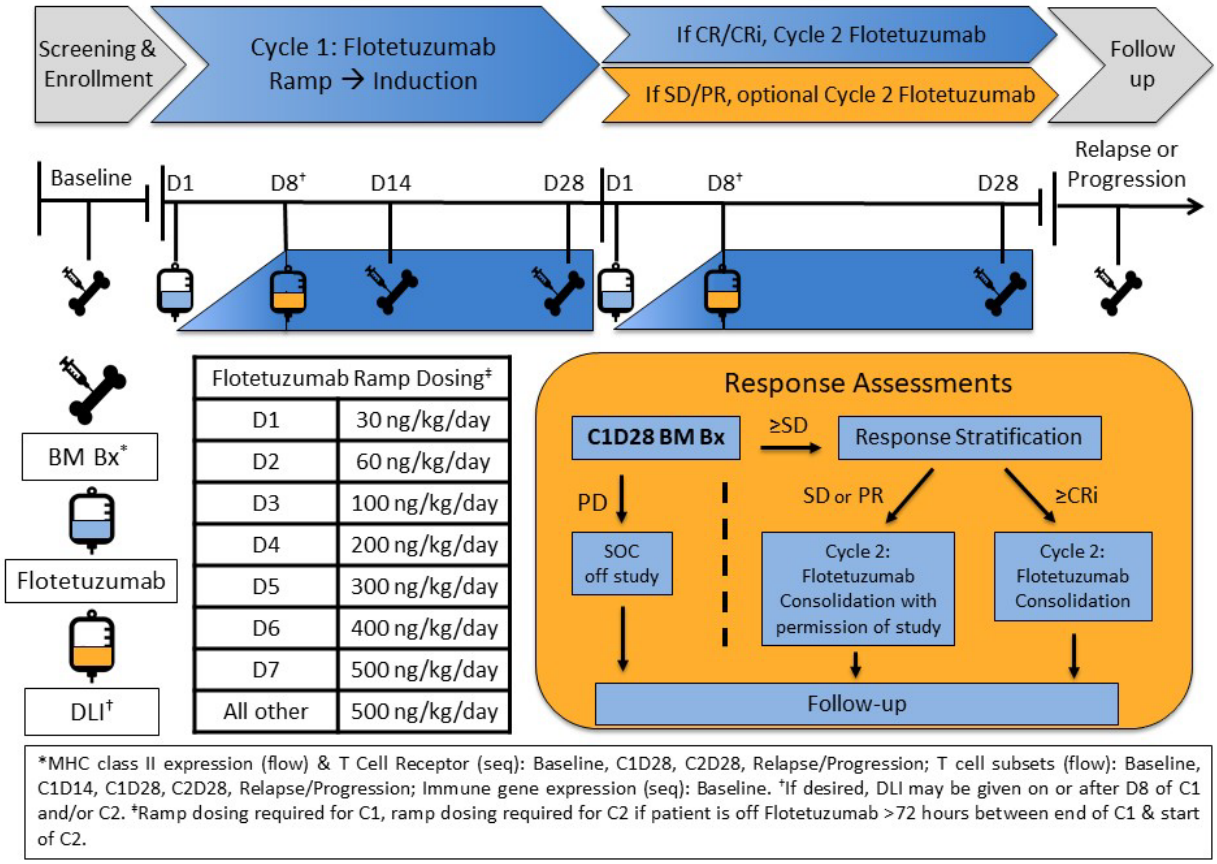
PROTOCOL SUMMARY

Title:	A Phase 2 Trial Evaluating the Efficacy of flotetuzumab for Relapsed Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS) Following Allogeneic Hematopoietic Cell Transplantation (allo-HCT)
Study Description:	We hypothesize that flotetuzumab for relapsed AML and MDS following allo-HCT will be safe, tolerable and may facilitate preferential immune effector cell retargeting of leukemic cells resulting in improved patient outcomes. Furthermore, administration of a donor lymphocyte infusion (DLI) (if available) in combination with flotetuzumab will be safe, tolerable and may provide additional therapeutic efficacy.
Objectives:	Primary Objectives: • To evaluate the efficacy of flotetuzumab in post-allo-HCT relapsed AML and MDS following Cycle 1 of flotetuzumab (Cycle 1 Day 28 assessment). Secondary Objectives: • To evaluate the efficacy of flotetuzumab monotherapy in post-allo-HCT relapsed AML and MDS following Cycle 2 • To evaluate the safety and tolerability of flotetuzumab +/- DLI in the post-allo-HCT setting.
Endpoints:	Primary Endpoint(s): Efficacy assessed via composite endpoint of CR and CRi (Cycle 1 Day 28 assessment) Secondary Endpoints: o Efficacy assessed at Cycle 2 Day 28 (or at Cycle 1 Day 28 if patient receives only one cycle): • Composite endpoint of CR and CRi at Cycle 2 Day 28 • Overall Response (OR) Rate (defined as PR or greater) • Morphologic leukemia-free state (MLFS) Rate • Partial Response (PR) Rate • Stable Disease (SD) Rate • Progression Free Survival (PFS) • Overall Survival (OS) o Safety and Tolerability as assessed by: • AE Grading per CTCAE v5.0 • CRS & Neurotoxicity Grading per ASTCT Consensus Guidelines • Acute GvHD Grading per MAGIC criteria • Chronic GvHD Grading per NIH severity score

Study Population:	The study will enroll adult patients who have been diagnosed with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) and have relapsed after allogeneic HCT.
Phase:	Phase 2
Description of Sites/ Facilities Enrolling:	This is a single institution open label study being conducted at Washington University in St. Louis.
Description of Study Intervention:	Phase 2, single-center, single-arm, open-label trial design evaluating the efficacy of flotetuzumab for the indication of relapsed AML and MDS after allo-HCT
Study Duration:	• Time from study opening to accrual completion – 18 months • Time from accrual completion to primary data analysis – 3 months • Total time from study opening to primary data analysis – 21 months • Time from accrual completion to final data analysis (survival analysis) – 24 months • Total time from study opening to final data analysis – 42 months
Participant Duration:	Patients will receive the investigational treatment for the first 28 days of the study (Cycle 1). Based on Day 28 response assessment, patients with CR/CRi will continue investigational treatment for another 28 days (Cycle 2); patients with SD or PR may also continue investigational treatment for another 28 days (Cycle 2) with permission by the investigator. All patients may receive a donor lymphocyte infusion (DLI) concurrent with flotetuzumab in either cycle, at the discretion of the treating physician and investigator; patients with PD at the end of Cycle 1 will come off study. All patients will be followed for 2 years after receiving investigational treatment.

SCHEMA



*MHC class II expression (flow) & T Cell Receptor (seq): Baseline, C1D28, C2D28, Relapse/Progression; T cell subsets (flow): Baseline, C1D14, C1D28, C2D28, Relapse/Progression; Immune gene expression (seq): Baseline. †If desired, DLI may be given on or after D8 of C1 and/or C2. ‡Ramp dosing required for C1, ramp dosing required for C2 if patient is off Flotetuzumab >72 hours between end of C1 & start of C2.

SCHEDULE OF ACTIVITIES

	Screening [12]	Pre-Meds	Day0	C1D1	C1D7	C1D14	C1D21	C1D28	C2D1	C2D7	C2D14	C2D21	C2D28	Follow-up/End of Treatment [13]
Medical History	X													
Physical Exam	X		X	X----------daily while inpatient----------X										X
Vital Signs (temp, RR, Spo2, HR and BP)	X	X	X	X ---------- see VS frequency/timing as below[1] ---------- X										X
Weight[16]	X		X	X ---------- daily while inpatient (standing preferred) ---------- X										X
ECOG	X			X					X					
AE Evaluation[10]	X[11]			AEs are assessed from start of treatment through 28 days after last day of treatment[10]										
Formal GVHD Assessment[14]	X			X				X		X	X	X	X	X
Disease Response Assessment (per IWG)[2]	X							X					X	X
CBC with differential	X		X	X ---------- daily while inpatient[3] ---------- X										X
CMP	X		X	X ---------- daily while inpatient[3] ---------- X										X
CRP	X		X	X ---------- daily while inpatient[3] ---------- X										X
PT/INR and PTT	X			X	X	X	X	X	X	X	X	X	X	
B-HCG	X			X										
TSH	X													
HIV, Hep B, Hep C	X													
Immune Subset Analysis (peripheral blood)[4]				X	X	X	X	X[5]	X	X	X	X	X[5]	X[6]
EKG	X			X					X					
TTE	X													
PFTs	X													
LP (only required if suspicion of CNS leukemia)	X													
Bone Marrow Biopsy[7]	X					X[17]		X					X	X[6]
Flotetuzumab[15]				X -------- continuous infusion -------- X					X[8] ------ continuous infusion ------ X					
DLI					X[9]					X[9]				

Notes:

[1]For Cycle 1 Day 1, check vital signs (VS) pre-infusion, at 1 hour, and 4 hours after infusion start. On Days 2-7, check VS prior to dose change and at 1 hour and 4 hours after dose change. Check VS on Day 11, approximately 96 hours after bag change, and Days 15, 19, 22, and 26 prior to bag changes as applicable. For all other cycles, check VS at end of infusion/prior to bag change as applicable. Unless otherwise specified, VS will be measured every 4-8 hours, per BMT unit protocol or more frequently as medically indicated

[2]Imaging only required as indicated by the presence of extramedullary disease

[3]Labs daily, or as frequently as medically indicated

[4]Immune subsets to be drawn pre-infusion and immediately prior to dose changes as indicated

[5]In addition to immune subsets by flow, TCR and IgH clonality will be assessed

[6]Follow-up bone marrow biopsy and immune subsets done at time of suspected relapse or progression

[7]Biopsies are done within a +/-3 day window

[8]Patients may continue with flotetuzumab monotherapy in C2 if they achieve SD or better (on C1D28 BmBx)

[9]Patients may receive DLI in combination with continued flotetuzumab during either Cycle 1 and/or 2 at the discretion of the treating physician. DLI may take place on or after Day 8 of either/both cycles

[10]CRS, ICE and ICANS toxicity assessments will be recorded in the EMR flowsheets and daily progress notes, as per BMT protocol for patients receiving cellular therapies and cell-engaging therapies

[11]AE assessment at baseline is intended to establish patient's current medical condition. No regulatory reporting of AEs at baseline is required

[12]Once consent is obtained, screening assessments should take place no more than 28 days prior to C1D1

[13]Patients will be followed every 6 months for 2 years. Follow-up visits should take place within a +/- 14 day window. If the subject is coming off trial the End of Treatment Visit is identical to the follow-up visit.

[14]See Appendices F and G.

[15]DART bag change has window of ± 2hours.

[16]Standing weight should be collected daily as clinically allowed

[17]Collection of C1D14 biopsy is at the discretion of the principal investigator

1.0 INTRODUCTION

1.1 Background

1.1.1 AML and the Current Treatment Paradigm

Acute myeloid leukemia (AML) is a hematopoietic neoplasm characterized by the presence of malignant, clonal cells which typically harbor recurrent chromosomal and genetic abnormalities. AML is projected to account for >21,000 new cases and ~11,000 deaths in the United States in 2019 (1). Clinically, AML often presents with signs/symptoms related to bone marrow dysfunction that can include leukopenia/leukocytosis, anemia and thrombocytopenia; as well as complications from these hematologic abnormalities. The treatment paradigm for AML includes induction chemotherapy, aimed at achieving a remission, followed by consolidation treatment with either high dose chemotherapy or allogeneic hematopoietic cell transplantation (allo-HCT). For those undergoing allo-HCT, 30-50% of patients achieve long-term, disease-free survival at 5 years (2). Meanwhile, post-HCT relapse occurs in ~40% of patients (2) and remains the leading cause of post-HCT mortality, with a median survival of 2-4 months without therapy (3). Standard therapy for post-HCT relapse is reinfusion of donor cells (donor lymphocyte infusion, DLI), sometimes preceded by debulking chemotherapy or hypomethylating agents, and second allo-HCT in select individuals. Unfortunately, many relapsing patients are not candidates for intensive therapy, and those who do undergo further therapy have transient responses at best (3, 4). Therefore, additional novel therapies are needed to improve post-HCT relapse outcomes while reducing the associated toxicity.

1.1.2 Donor Lymphocyte Infusion (DLI)

DLI represents a non-specific form of adoptive cell therapy which involves infusion of a pool of allogeneic immune cells, including CD4+ T cells, CD8+ T cells, regulatory T cells (T Regs), natural killer (NK) cells and professional antigen presenting cells. The DLI is believed to work in part through the graft-versus-leukemia (GvL) effect of allo-HCT, in which donor immune cells control leukemic blasts through both direct cellular cytotoxicity and secretion of inflammatory cytokines such as interferon gamma (IFN-γ). Cytokine release in the tumor microenvironment likely plays a role in promoting tumor antigen expression by major histocompatibility complex (MHC) class I/II, as well as through direct toxicity to leukemia cells. However, while these mechanisms can mediate potent anti-tumor effects, they are not inherently tumor specific, as evidenced by 60-70% rates of acute and chronic graft-versus-host disease (GVHD) following DLI (5). Meanwhile response rates following DLI alone for relapsed AML post-HCT are 15-40% (6-9), with only 15-20% survival at 2 years (7). Therefore, the therapeutic utility of DLI is limited by both toxicity and poor efficacy.

1.1.3 Chemotherapy & Hypomethylating Agent (HMA) Monotherapy

While combined chemotherapy followed by administration of a DLI remains standard therapy for relapsed AML post-HCT, as many as 30-50% of patients are unable to receive a DLI (10, 11). This results from a number of factors, including progressive disease despite chemotherapy, the presence of active GVHD, and the lack of available donor lymphocytes for infusion (10, 11). In such cases, patients are often treated with chemotherapy alone with CR rates of 10-15% and significant toxicity (11). Meanwhile, HMAs have recently emerged as an alternative to chemotherapy. Besides direct anti-leukemic effects, both azacitidine and decitabine are thought to enhance GvL effect, potentially via modulation of T Regs and cytotoxic CD8+ T cells. HMAs have been shown to have a favorable toxicity profile relative to traditional cytotoxic chemotherapy. However, studies evaluating the efficacy of HMA monotherapy in the post-HCT setting have demonstrated CR rates of ~10% or less (10, 11). Therefore, chemotherapy alone and HMA monotherapy for relapsed AML post-HCT are limited by efficacy, toxicity or both. Additional evaluation of alternative agents that may enhance the GvL effect and improve outcomes while mitigating toxicity is warranted.

1.1.4 Flotetuzumab (MGD006)

Flotetuzumab is a CD123 x CD3 (DART®) protein. DART proteins are bispecific, antibody-based molecules that can bind 2 distinct antigens simultaneously. Flotetuzumab is designed to target CD123-positive cells for recognition and elimination by CD3-expressing T lymphocytes as effector cells. CD123 is expressed by leukemic blasts and leukemia stem cells but not by normal hematopoietic stem cells (HSCs). Therefore, flotetuzumab represents a promising therapy for the treatment of AML via redirected T cell targeting of the leukemic cells. Data from early phase studies have shown flotetuzumab was relatively well-tolerated, with the most common AEs being infusion reactions and the majority of AEs being graded as mild to moderate in severity (12). Meanwhile, 69.5% (16/23) of AML patients who received target doses (500 ng/kg/day) of flotetuzumab experienced a clinical benefit (13); and recent data from patients with primary refractory AML treated with target doses of flotetuzumab showed 32.1% (9/28) achieving a CR/CRi (14).

1.1.5 Immune Retargeting in Relapsed AML post-HCT with Flotetuzumab and DLI

A significant driver of long-term remissions in allo-HCT is the GvL effect. Additionally, relapse post-HCT may, in part, be related to loss of GvL. This likely occurs both through exhaustion of donor T cells as well as epigenetic changes in AML cells that promote immune escape (15, 16). The potential of T cell engaging therapies, such as flotetuzumab, to reestablish and retarget GvL in the post-HCT relapsed setting may be an effective approach to treat AML relapse after allo-HCT. In support of this, patients with relapsed/refractory ALL following allo-HCT who were treated with blinatumomab, a CD19 x CD3 bi-specific T cell engager (BiTE),

achieved a 45% CR/CRi rate with 65% of these responses being MRD negative CRs (17). Additionally, GVHD rates were infrequent, mild and did not lead to discontinuation of therapy on trial (17).

Therefore, the administration of a T cell retargeting agent such as the flotetuzumab DART represents a promising approach to restore the GvL effect, putting AML patients relapsing after allo-HCT back in remission. Additionally, administration of fresh donor-derived immune cells through DLI may enhance the ability of flotetuzumab to target AML cells, while reducing post-DLI GVHD by redirecting donor lymphocytes away from normal host tissues.

Finally, one recently identified mechanism for the loss of GvL is the down-regulation of specific MHC class II genes on AML cells, observed in 30-50% of patients (15, 16). Loss of MHC class II expression is predicted to weaken GvL by preventing antigen expression to donor immune cells. In preclinical experiments, treatment of MHC class II-low AML cells with flotetuzumab in the presence of donor T cells restores MHC class II expression on AML cells through IFN-γ release in the tumor microenvironment, leading to AML cell death even in the absence of CD123 expression (unpublished). Therefore, flotetuzumab may be an especially effective treatment for patients relapsing with MHC class II-low AML cells, both through the direct flotetuzumab-mediated T cell engagement with CD123+ AML cells (MHC class-II independent), as well as indirectly through upregulation of MHC class II by IFN-γ release in the tumor microenvironment.

1.1.6 Hypothesis

We hypothesize that flotetuzumab for relapsed AML following allo-HCT will be safe, tolerable and may facilitate preferential immune effector cell retargeting of leukemic cells resulting in improved patient outcomes. Furthermore, administration of a DLI (if available) in combination with flotetuzumab will be safe, tolerable and may provide additional therapeutic efficacy.

1.1.7 Justification for Dose

MGD administered as a continuous IV infusion on 28-day cycles has been studied in a phase 1/2 clinical trial (CP-MGD006-01) in MDS/AML patients.

Dose Selection:
During the phase 1 dose escalation portions of this study, doses of flotetuzumab including 3, 10, 30, 100, 300, 500 and 700 ng/kg/day were evaluated. Based on the safety and tolerability data from this portion of the trial, the 500 ng/kg/day dose was determined to be the maximum tolerated dose (MTD) and recommended phase II dose (RP2D).

Schedule of Administration:
Two schedules of administration were evaluated in the Phase 1 dose escalation and first cohort (n-30) of the Phase 2 cohort expansion, including: 1) a 4-day on/3-day off each week administration schedule for 28 days and 2) a continuous infusion for the entire 28-day cycle. Ultimately, both approaches were shown to be feasible, however the continuous infusion schedule was selected to carry forward for the remainder of the dose expansion cohorts (n=132), because continuous infusion without 3-day breaks offered improved safety with respect to adverse events (CRS) and improved anti-leukemic activity (some patients were observed to have transient increases in peripheral blast counts during the off days).

Week 1, Lead-in Dosing:
Both schedules of administration utilized a lead-in dose in Week 1, after which all cohorts stepped up to their target doses on their assigned schedules of administration for the remainder of the 28-day cycle. Initially the lead-in dose was 100 ng/kg/day for 4 days. This lead-in dose was modified to 30 ng/kg/day for 3 days followed by 100 ng/kg/day for 4 days for the first week of Cycle 1. A subsequent protocol amendment modified the lead-in dose in Week 1, to include an initial dose of 30 ng/kg/day on Day 1, with daily, stepwise up-titration reaching a final step up on Day 7 to the goal of 500 ng/kg/day (see Table 3).

Safety, Tolerability and Efficacy:
As of November, 2019, data have been reported on 50 patients with AML receiving flotetuzumab administered via a stepwise ramp during week 1 (see Table 3) to the RP2D goal of 500 ng/kg/day administered as a 7-day/week continuous infusion on 28-day cycles. Flotetuzumab was well tolerated, with comparable cytokine release syndrome events in primary refractory patients (30% G1, 67% G2, 3% G3) and relapsed patients (26% G1, 58% G2, 16% G3). Meanwhile, 69.5% (16/23) of AML patients who received target doses (500 ng/kg/day) of flotetuzumab experienced a clinical benefit (13); and recent data from patients with primary refractory and early relapsed AML treated with target doses of flotetuzumab showed 32.1% (9/28) achieving a CR/CRi (14).

Based on the above data, we propose dosing flotetuzumab with a stepwise, daily up-titration during week 1 (see Table 3) to a goal RP2D dose of 500 ng/kg/day administered as a continuous IV infusion 7 days/week on 28-day cycles.

1.2 Risk/Benefit Assessment

1.2.1 Known Potential Risks

As of the data cut-off date of 22 January 2021 from the most current MacroGenics IB v11.0, flotetuzumab has been evaluated in the ongoing first-in-human monotherapy clinical study (CP-MGD006-01). Overall, the preliminary safety profile of flotetuzumab does not show any unexpected, significant, or rare safety

findings and confirms the safety observations are consistent with the mechanism of action of flotetuzumab or with the patients' underlying disease.

Infusion-related reaction (IRR, including cytokine release syndrome (CRS)), is the only important identified risk based on the observations from the 81 patients in this study and from the mechanism of action of flotetuzumab. Cumulatively, 71/81 patients (87.6%) experienced at least one IRR or CRS event. The majority of patients experiencing infusion-related reactions (including CRS) had reactions that were mild to moderate in severity (62/71), whereas 9 patients experienced at least 1 severe (Grade 3) event. None of these events have been life-threatening or fatal. Infusion-related reactions (including CRS) typically occurred during the initial 48 hours of the first infusion of flotetuzumab. Infusion-related reaction symptoms typically included pyrexia, chills, hypotension, tachycardia, and/or respiratory symptoms (e.g., tachypnea, hypoxia). Most of these events were considered possibly, probably, or definitely related to flotetuzumab. Infusion-related reaction (including CRS) events have been manageable with treatment interruption, dose reduction, early use of tocilizumab, and medical intervention, with antipyretics, antihistamines, and corticosteroids. Notably, the stepwise lead-in dosing is associated with a reduced frequency and/or severity of IRR/CRS.

Neurological events, defined as AEs of any grade of the Nervous System or Psychiatric Disorders System Organ Class, irrespective of attribution, have been seen in patients exposed to flotetuzumab. The most common of these to date were headache and insomnia. Other neurological events, Grade 3 or higher attributable to flotetuzumab, included delirium, encephalopathy, headache, mental status changes, and syncope. These events generally occurred in the clinical setting of IRR/CRS. Events reported in the Nervous System or Psychiatric Disorders System Organ Class to date have generally been mild to moderate in severity in most patients, and were reversible with no long-term clinical sequelae.

Risk of capillary leak syndrome (CLS) has been associated with T-cell redirecting therapy, including chimeric antigen receptor T-cell (CAR-T) and CD3-engaging bispecific antibody-based molecules, and reported for the CD123-directed cytotoxin, tagraxofusp-erzs (Elzonris®). While CLS has not been frequently observed in patients treated with flotetuzumab, a detailed review of recent clinical data across all flotetuzumab studies identified three patients who received flotetuzumab monotherapy that, concomitant with cytokine release syndrome during multi-step lead-in dosing, exhibited rapid weight gain, decreased albumin, and systemic edema suggestive of CLS. Pleural and pericardial effusions were noted in some cases as well. Of note, CLS may not be associated with hemoconcentration in this patient population.

Acute and chronic GVHD are well-described complications following allo-HCT. Furthermore, new onset or worsening acute and chronic GVHD has been reported is as many as 60-70% of patients receiving DLI alone or in combination with chemotherapy for relapsed AML following allo-HCT. The reported severity of both

acute and chronic GVHD in this setting have ranged from mild to severe, with reports of grade 4 and grade 5 toxicity. Flotetuzumab has been studied in AML patients with primary refractory and relapsed disease in the CP-MGD006-01 study. In addition, other T cell redirecting therapies have been used post-allo-HCT without increased GVHD (17). Nevertheless, flotetuzumab has not been evaluated in AML patients with relapsed disease following allo-HCT and the risk of GVHD in this patient population is unknown.

1.2.2 Known Potential Benefits

Flotetuzumab has demonstrated anti-neoplastic activity. During cohort expansion in the CP-MGD006-01 study, 23 patients treated at the threshold 500 ng/kg/day dose have completed at least one cycle of treatment and had a post-treatment bone marrow biopsy. Anti-leukemic activity was documented in 69.5% (16/23) patients, with 4/23 patients achieving CR (2), CRi (1) or PR (1). Additionally, 12 patients had stable disease or other clinical benefits (defined as a decline in bone marrow leukemic blasts not meeting complete or partial response as classified by modified IWG criteria). Additional, recently reported data as of 11/2019 from the same cohort of patients with primary refractory and early relapsed AML treated with target doses of flotetuzumab showed 32.1% (9/28) achieving a CR/CRi. This compares favorably to historic CR/CRi rates of ~12.5% utilizing best SOC options.

1.3 Overall Study Design and Plan

This is an open-label, single-arm, single-center Phase 2 study to evaluate for efficacy, safety and tolerability of flotetuzumab administered as a single agent and/or in combination with DLI in patients with relapsed AML post-allo-HCT.

The study is designed to include 2 cycles of treatment. During Cycle 1, patients will be administered flotetuzumab as a continuous IV infusion with a week 1 ramp up to goal/target dose of 500ng/kg/day, which will be continued for the remaining 21 days of a 28-day (4-week) cycle (Cycle 1). Patients found to meet the primary endpoint of complete remission with incomplete hematologic recovery (CRi) or better on their C1D28 response assessment (bone marrow biopsy) will continue on to Cycle 2 of flotetuzumab administered as a continuous IV infusion at a target dose of 500ng/kg/day for an additional 28-day cycle (Cycle 2). Patients found to be deriving clinical benefit as evidenced by stable disease (SD) or a partial remission (PR) on their C1D28 response assessment (bone marrow biopsy) may also continue to Cycle 2 of flotetuzumab at the same dose at the discretion of the treating physician and investigator. Patients may receive a DLI concurrently with flotetuzumab treatment in Cycle 1 and/or Cycle 2 at the discretion of the investigator and treating physician. DLI, if administered, will be given on or after Day 8 of the respective cycle. Of note, if patients are off flotetuzumab for >72 hours between end of C1 and beginning of C2, they will require repeat week 1 ramp up to goal/target dose of 500ng/kg/day. Patients found to have progressive disease (PD) on their C1D28 response assessment (bone marrow biopsy) will proceed off study with SOC. All patients who receive investigational agent will then be followed for treatment response and safety monitoring.

1.4 Study Duration and Dates

It is generally expected that ~15-20 patients will be accrued per year. The target accrual for the study is 25 patients. Therefore, it is anticipated that the time from the study opening to completion of accrual with be ~18 months.

Patients will receive the investigational treatment for the first 28 days of the study (Cycle 1). Based on Day 28 response assessment, patients with CR/CRi/PR/SD may continue investigational treatment for another 28 days (Cycle 2); and patients with PD will come off study. All patients will be followed for 2 years after receiving investigational treatment.

1.5 Definition of End of Trial

End of Trial is defined as the date when the last patient on the study completes his or her End of Treatment visit and the last follow-up data is collected.

2.0 OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To evaluate the efficacy of flotetuzumab in post-allo-HCT relapsed AML and MDS following Cycle 1 of flotetuzumab (Cycle 1 Day 28 assessment).	Efficacy assessed via composite endpoint of CR and CRi (Cycle 1 Day 28 assessment)
Secondary	
• To evaluate the efficacy of flotetuzumab monotherapy in post-allo-HCT relapsed AML and MDS following Cycle 2. • To evaluate the safety and tolerability of flotetuzumab +/- DLI in the post-allo-HCT setting.	• Efficacy assessed at Cycle 2 Day 28 (or at C1 D28 in patients who receive only one cycle): o Composite endpoint of CR and CRi at C2 D28 o Overall Response (OR) Rate (defined as PR or greater) o Morphologic leukemia-free state (MLFS) Rate o Partial Response (PR) Rate o Stable Disease (SD) Rate o Progression Free Survival (PFS) o Overall Survival (OS) • Safety and tolerability as assessed by: o AE Grading per CTCAE v5.0 o CRS & Neurotoxicity Grading per ASTCT Consensus Guidelines o Acute GVHD Grading per MAGIC criteria Chronic GVHD Grading per NIH severity score

3.0 STUDY POPULATION

3.1 Inclusion Criteria

1. Histologically or cytologically confirmed relapsed acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS), including any AML subtype, except acute promyelocytic leukemia (APL), and including AML that has evolved from a previous MDS or MPN.

2. Patients must have peripheral blast count ≤ 20,000/mm^3. Use of hydroxyurea to control blast count is permitted.

3. Patients must be status post allo-HCT (including: matched related, matched unrelated, haploidentical, mismatched unrelated; and cord blood HCT).

4. Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 2 (see Appendix A).

5. Adequate organ function, defined as:
   a. Hepatic transaminase (both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels ≤2.5 times the institutional upper limit of normal (ULN),
   b. Total bilirubin level ≤1.5 times the ULN (unless the patient has a history of Gilbert's Syndrome, in which case, total bilirubin must be ≤2.5 times the ULN),
   c. Creatinine clearance of ≥50 ml/min.
   d. Adequate organ reserve including cardiovascular (ejection fraction within institutional normal limits), pulmonary (baseline pulmonary function test [PFT]: carbon monoxide diffusion capacity in the lung [DLCO] > 50%, forced expiratory volume in 1 second [FEV1] > 70%), renal, and hepatic functioning sufficient, in the judgment of the Investigator, to undergo therapy.
   e. Normal thyroid function or stable thyroid tests on supplementation, except euthyroid sick syndrome.

6. Recovery from toxicities of clinical consequence attributed to previous chemotherapy to CTCAE v4.0 Grade ≤ 1 (i.e., certain toxicities such as alopecia will not be considered in this category).

7. Female patients of childbearing potential must test negative for pregnancy at enrollment and during the study. Sexually active women of child-bearing potential, unless surgically sterile, must be willing to use a highly effective method of birth control defined as those which result in a low failure rate (i.e., less than 1% per year) such as implants, injectables, combined oral contraceptives, intra-uterine devices (IUDs) or vasectomized partner. Male patients with partners of childbearing potential must be either vasectomized or agree to use a condom in addition to having their partners use another method of contraception resulting in a highly effective method of birth control defined as those which result in a low failure rate (i.e., less than 1% per

year) such as implants, injectables, combined oral contraceptives, or IUDs. Patients should not have sexual intercourse with females who are either pregnant or lactating without a condom. Contraception should be employed from the time of consent through 12 weeks after flotetuzumab administration. Patients should also abstain from sperm/egg donation during the course of the study.

8. Able to have corticosteroids weaned to ≤0.5mg/kg prednisone/day (or equivalent)

9. Able to have non-steroidal immunosuppression discontinued, including:
   a. mycophenolate (MMF)
   b. calcineurin inhibitors (tacrolimus, cyclosporine)
      i. calcineurin inhibitors must be able to be discontinued at least 14 days prior to enrolling on study.
   c. JAK inhibitors (ruxolitinib)
   d. MTOR inhibitors (sirolimus)

10. At least 18 years of age.

11. Ability to understand and willingness to sign an IRB approved written informed consent document.

3.2 Exclusion Criteria

1. Active GVHD requiring systemic immunosuppression with more than 0.5mg/day prednisone.

2. Currently receiving any other investigational agents.

3. Any active untreated autoimmune disorders (with the exception of vitiligo, resolved childhood atopic dermatitis, prior Grave's disease now euthyroid clinically and with stable supplementation).

4. Second primary malignancy that requires active therapy (adjuvant hormonal therapy is allowed).

5. Antitumor therapy (chemotherapy, radiotherapy, antibody therapy, molecular- targeted therapy, retinoid therapy, or investigational agent) within 5 half-lives of Cycle 1 Day 1.

6. At the time of study entry, steroids >0.5mg/kg of prednisone or equivalent (except steroid inhaler, nasal spray or ophthalmic solution which are allowed).

7. Use of immunosuppressant medications (other than steroids as noted) in the 2 weeks prior to study drug administration (Cycle 1 Day 1).

8. Isolated extramedullary relapse (i.e., no evidence of bone marrow involvement).

9. Known central nervous system (CNS) leukemia. Patients with suspected CNS leukemia must be evaluated by lumbar puncture and be free of CNS disease prior to study entry. Previously treated CNS leukemia is allowed provided adequate treatment has been provided and the patient is free of CNS disease.

10. Any medical or psychiatric condition limiting full compliance or increasing the safety risk, at the discretion of the PI, such as:
    a. active uncontrolled infection (including, but not limited to viral, bacterial, fungal, or mycobacterial infection),
    b. known human immunodeficiency virus infection,
    c. known, active, or chronic hepatitis B or C infection (appropriately treated HBV/HCV infections with documented clearance of viral titer are allowed),
    d. Grade 3 or 4 bleeding,
    e. significant pulmonary compromise including chronic supplemental oxygen use, history of non-infectious pneumonitis (including radiation pneumonitis), pulmonary fibrosis, or severe chronic obstructive pulmonary disease (COPD),
    f. uncontrolled (persistent) hypertension (systolic pressure > 180 mm Hg or diastolic pressure > 100 mm Hg
    g. clinically significant arrhythmia, clinically significant baseline QTcF >480 msec,
    h. unstable angina,
    i. recent myocardial infarction within 6 months prior to study drug administration (Cycle 1 Day 1),
    j. clinically significant heart disease, such as, congestive heart failure, history of pericarditis, myocarditis,
    k. history of stroke or transient ischemic event within 3 months prior to study drug administration (Cycle 1 Day 1),
    l. untreated pulmonary embolism, or non-catheter-related deep-vein thrombosis in the 3 months prior to study drug administration (Cycle 1 Day 1),
    m. pregnancy, or breast feeding,
    n. major surgery or trauma within 4 weeks before enrollment.

11. Known hypersensitivity to murine, yeast, or recombinant proteins; polysorbate 80; recombinant human serum albumin; benzyl alcohol; or any excipient contained in the flotetuzumab drug formulation.

12. Dementia or altered mental status that would preclude sufficient understanding to provide informed consent.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. The registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

4.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomized to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (if applicable).

5.0 STUDY TREATMENT PLAN

5.1 Premedication Administration

Due to the risk of infusion-related reactions (IRRs), specific pre-treatment regimens are described below. Table 1 below presents the premedication and prophylaxis regimens.

Prior to the first dose (Week 1):
All patients are to be treated as described in Table 1 below. No additional steroids should be administered for infusion-related reaction prophylaxis except as detailed below. Patients should receive adequate hydration during study therapy, and the addition of IV fluids to maintain intravascular volume (e.g., 50-100 mL/hr of normal saline) is recommended for at least 48 hours after the start of therapy.

Prior to Week 2 dosing:
The premedication schedule should be followed as described in Table 1 below. In addition, these premedications should be considered after consultation with the Principal Investigator (PI) if patients are resuming flotetuzumab treatment after a dose interruption lasting > 1 day in Cycle 1 or 2:

- Steroids should not be administered for infusion-related reaction prophylaxis except as indicated below, but may be used for the treatment of emerging symptoms as clinically appropriate.
- Tocilizumab (8 mg/kg IV) may be used prophylactically in patients who have had severe IRR or CRS in the first week of dosing upon consultation with the PI.
- Patients should receive adequate hydration during study therapy, and the addition of IV fluids to maintain intravascular volume (e.g., 50-100 mL/hr of normal saline) is recommended for at least 48 hours after the start of therapy in Week 2.

Prior to subsequent doses (after Week 2):

1. The premedication schedule should be followed as described in Table 1 below. Tocilizumab or steroids may be considered after consultation with the PI.
2. For infusion-related reactions during the course of treatment, supportive care measures should be implemented as outlined in Section 6.1.
3. IV fluids should be administered for all patients unless contraindicated.

Table 1: Flotetuzumab Ramp & Continuous Infusion Pre-medications

28 day continuous Flotetuzumab infusion schedule				
Medication	Week 1	Week 2	Week 3	Week 4
Acetaminophen (1000mg PO)	Day 1: 30 minutes prior to dosing, then q8 hrs for 48 hours Day 7 or day of target dose: 30 minutes prior to dose change, then q8 hrs for 48 hours	None	None	None

28 day continuous Flotetuzumab infusion schedule				
Medication	Week 1	Week 2	Week 3	Week 4
Diphenhydramine (25-50mg IV or PO) or equivalent	Day 1: 30 minutes prior to dosing, then q8 hrs for 48 hours Day 7 or day of target dose: 30 minutes prior to dose change, then q8 hrs for 48 hours	None	None	None
Ranitidine (50mg IV) or equivalent	Day 1 or day of target dose: 30 minutes prior to dosing, then q8 hrs for 48 hours Day 7 or day of target dose: 30 minutes prior to dose change, then q8 hrs for 48 hours	None	None	None
Dexamethasone (or equivalent)	Day 1: 10-20mg IV up to 30 minutes before dosing. Then 4mg IV at 12 hours after dosing. Day 7 or day of target dose: 10mg IV up to 30 minutes prior to dose change, then 4mg 12 hrs later.	None	None	None
Tocilizumab (8mg/kg IV)	None	After consultation with PI	None	None

5.2 Study Intervention Description

Patients enrolled on study will be admitted to the hospital for admission assessments, administered premedications and started on Cycle 1 Day 1 on the dose escalation ramp schedule (Table 3) of flotetuzumab as a continuous IV infusion. During the ramp week, patients will be initiated on flotetuzumab at 30 ng/kg/day and have their flotetuzumab dose increased daily to a target goal of 500 ng/kg/day by Day 7.

Patients will then continue on flotetuzumab continuous IV infusion at 500 ng/kg/day for the remaining 21 days of the 28-day cycle.

On Cycle 1 Day 28, patients will undergo repeat bone marrow biopsy for assessment of disease status. Patients not deriving clinical benefit defined as PD (Table 2) will proceed with SOC therapy off study. Patients who have achieved a CR/CRi will proceed to a second cycle per protocol, while patients deriving clinical benefit defined as PR or SD or better may proceed to Cycle 2 with permission of the investigator. Patients with available donor lymphocytes may receive DLI concurrently with flotetuzumab during Cycle 1 and/or Cycle 2. At the discretion of Principal Investigator, on Cycle 1 Day 14 patients will undergo a bone marrow biopsy for tissue banking and correlative studies.

5.2.1 Cycle 1

Cycle 1 will include pre-medications, a dose escalation ramp of flotetuzumab and then a continuous infusion of flotetuzumab (Tables 1 & 3).

- Patients will be admitted to the hospital on day 0 for admission assessments.
- Pre-medications will be administered, beginning on Day 1, as in the previous phase 2 trial NCT02152956.
- The dose escalation ramp will begin on Day 1 and will consist of continuous ramp infusion of flotetuzumab starting at 30 ng/kg/day with increasing dose over 7 days to a target dose of 500 ng/kg/day.
- Patients will then continue on flotetuzumab for additional 21 days at target dose of 500 ng/kg/day (28 days total).
- Patients may receive a DLI at the discretion of the treating physician on or after Day 8 of Cycle 1.
- At mid-Cycle 1 (Day 14), at the discretion of the Principal Investigator, patients will undergo a bone marrow biopsy for correlative studies only (not used for disease response assessments).
- At the end of Cycle 1 (Day 28), patients will undergo bone marrow biopsy for correlative studies **and** disease response assessment.
- Patients not deriving clinical benefit (defined as PD, Table 2) will proceed with SOC therapy off study. Patients with a CR/CRi (Table 2) will proceed to Cycle 2 per protocol. Patients deriving clinical benefit (defined as SD or better, Table 2) may proceed to Cycle 2 with permission of the investigator.

Table 2: Response Criteria

Complete remission (CR)	Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0×10^9/L (1000/μL); platelet count ≥100×10^9/L (100,000/μL), transfusion independence
CR with incomplete hematologic recovery (CRi)	All CR criteria except for residual neutropenia (<1.0×10^9/L [1000/μL]) or thrombocytopenia (<100×10^9/L [100,000/μL])
Morphologic leukemia-free state (MLFS)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5 to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%
Stable disease (SD)	Absence of CR, CRi, PR, MLFS; and criteria for PD not met
Progressive disease (PD)	Evidence for an increase in bone marrow blast percentage (>50% over baseline), and/or increase of absolute blast counts in the blood (>50% to >25×10^9/L) without differentiation syndrome, or new extramedullary disease

Table 3: Flotetuzumab Ramp, Induction, and Consolidation Dosing Guidelines[1]

Ramp + Induction			
Cycle	Weeks	Days	Dose (ng/kg/day)
1	1	Day 1	30
		Day 2	60
		Day 3	100
		Day 4	200
		Day 5	300
		Day 6	400
		Day 7	500
	2	Days 8-14	500
	3	Days 15-21	500
	4	Days 22-28	500
Induction/Consolidation			
2	1-4	Days 1-28	500

[1]Table provided for dosing guidelines. Actual dosing may be altered as clinically indicated.'

5.2.2 Cycle 2 Flotetuzumab Consolidation

Patients meeting the primary endpoint of CR/CRi and patients with PR/SD (with permission of the investigator) will receive Cycle 2 flotetuzumab pre-medications and a continuous infusion of flotetuzumab (Tables 1 & 3), beginning within 2 weeks of completing Cycle 1.

- Patients will be admitted to the hospital and pre-medications administered on Day 1.
  - If patients have been off flotetuzumab for >72 hours, then the dose escalation ramp will begin on Day 1 and will consist of continuous ramp infusion of flotetuzumab starting at 30 ng/kg/day with increasing dose over 7 days to a target dose of 500 ng/kg/day. Patients will then continue on flotetuzumab for additional 21 days at target dose of 500 ng/kg/day (28 days total).
  - If patients have been off flotetuzumab ≤72 hours, then patients will receive flotetuzumab dosed at 500 ng/kg/day continuous infusion for a total of 4 weeks (28 days).
- At the end of Cycle 2 (Day 28), patients will undergo bone marrow biopsy for disease response assessment and correlative studies, and proceed to the follow-up phase of the study.

5.2.3 Donor Lymphocyte Infusion (DLI)

Patients with available donor cells may receive a donor lymphocyte infusion during Cycle 1 and/or Cycle 2 (if given) at the discretion of the investigator and the treating physician.

- DLI should be administered on or after Day 8 of Cycle 1 and/or Cycle 2

- Cell dose of DLI will vary depending on donor source at the discretion of the treating physician and according to institutional practices:
  - For matched unrelated donors and matched related donors DLI containing a range of 0.5 x 10^7 to 1.0 x 10^8 CD3+ cells/kg will be administered.
  - For haploidentical and mismatched donors DLI containing a range of 0.5 x 10^6 to 1.0 x 10^7 CD3+ cells/kg will be administered.

5.3 Response Assessments

Bone Marrow Biopsy (BM Bx) will be performed at the following timepoints for disease assessments and correlative assessments:

- Screening/Baseline BM Bx: performed prior to starting on treatment.
- Cycle 1 D14 (+/- 3 days) BM Bx mid-Cycle 1 (correlative assessments only, no disease response assessment, collected at PI's discretion only).
- Cycle 1 D28 (+/- 3 days) BM Bx following completion of Cycle 1.
- Cycle 2 D28 (+/- 3 days) BM Bx following completion of Cycle 2.
- Final BM Bx performed at the time of suspected disease relapse or progression.

Responses will be assessed via IWG Criteria (Table 2).

See Section 7.0 for additional details regarding bone marrow biopsy schedule and correlative assessments.

5.4 Safety Assessments

Safety assessments will be performed on an ongoing, continual basis to monitor for any AEs. Additionally, formal GVHD assessments will be performed at baseline, on C1 D1 prior to flotetuzumab dose, C1D28; for patients receiving DLI, formal GVHD assessments will be performed weekly following DLI and during follow up.

5.5 Definitions of Evaluability

Endpoint	**To be evaluable for this endpoint, a patient must…**
Efficacy (CR and CRi, C1D28 assessment)	Have completed all Cycle 1 infusions of flotetuzumab (or discontinued treatment due to drug toxicity or disease progression). Patients who are not evaluable for the primary objective will be replaced.
Efficacy (CR and CRi, C2D28 assessment)	Have completed all Cycle 1 and 2 infusions of flotetuzumab (or discontinued treatment due to drug toxicity or disease progression) and undergone C2D28 disease assessment
Efficacy (ORR defined as PR or greater)	Have completed all Cycle 1 infusions of flotetuzumab (or discontinued treatment due to

Efficacy (MLFS rate)	drug toxicity or progression) and undergone C2D28 disease assessment (or C1D28 assessment for patients who only received Cycle 1 treatment).
Efficacy (PR rate)	
Efficacy (SD rate)	
Efficacy (PFS)	
Efficacy (OS)	Have undergone any period of infusion of flotetuzumab
Safety and tolerability (AEs by CTCAE)	
Safety and tolerability (CRS and neurotoxicity by ASTCT)	
Safety and tolerability (acute GVHD by MAGIC criteria)	
Safety and tolerability (chronic GVHD by NIH severity score)	

5.6 Concomitant Therapy and Supportive Care Guidelines

All concomitant medications defined below that are administered during the patient's participation in the study must be recorded in the source document and on the electronic Case Report Form (eCRF).

Concomitant Medications that will be recorded in the eCRF:

- Medications related to the management of IRR, CRS and/or Neurotoxicity administered from admission to the hospital for Cycle 1 of flotetuzumab, during Cycle 1 and Cycle 2, and for the 4 weeks following completion of treatment on protocol will be documented in the eCRF.
- Medications and treatments related to the management of CLS administered from admission to the hospital for cycle 1 of flotetuzumab, during Cycle 1 and Cycle 2, and for the 4 weeks following completion of treatment on protocol will be documented in the eCRF
- Immunosuppressive medications and medications related to management of GVHD from the time of study enrollment through 28 days after the last administration of study drug or End-of-Treatment visit, whichever is later, will be documented in the eCRF.

Patients may not receive the following concurrent therapy:

- Any other therapies for leukemia. Use of hydroxyurea or other cytoreductive agents for the purposes of achieving an initial peripheral blast count $\leq 20{,}000/mm^3$ should have ceased by the time of registration. However, hydroxyurea may be reinstituted at the discretion of the Investigator for the prevention or treatment of signs or symptoms of leukostasis during the time of study participation. If this occurs, hydroxyurea and any other leukemia therapies used must be recorded in the eCRF.
- Patients may not get treatment with any other investigational agent within 14 days or 5 half- lives, whichever is longer, prior to study drug administration (Cycle 1 Day 1).
- Patients may not receive other investigational drugs during the period of study participation.
- Because flotetuzumab employs a mechanism of action dependent upon the engagement

of T lymphocytes, the use of corticosteroids other than those employed for premedication should be limited as much as possible. Corticosteroid pretreatment should follow the recommended dose and schedule. Steroids may be employed in the treatment of suspected flotetuzumab-associated immunoinflammatory AEs, GVHD/autoimmune AEs or leukemia-associated AEs in consultation with the PI.

- Ruxolitinib should not be administered in patients on active treatment with flotetuzumab without approval from the PI.
- Use of granulocyte colony stimulating factor or granulocyte-macrophage colony stimulating factor is prohibited during Cycle 1. Following Cycle 1, the use of hematopoietic growth factors is still discouraged. However, granulocyte colony stimulating factor or granulocyte-macrophage colony stimulating factor may be used in accordance with the practice of medicine and institutional guidelines at the investigator's discretion.
- Vaccinations (with the exception of the annual inactivated influenza vaccine) are prohibited while patients are actively receiving study drug (i.e. during Cycle 1 and Cycle 2).

Patients may receive the following concurrent therapy:

- Antiemetics, antidiarrheals, anticholinergics, antispasmodics, antipyretics, antihistamines, analgesics, antibiotics and other antimicrobials, histamine receptor (H2) antagonists or proton pump inhibitors, and any other medication intended to treat symptoms or signs of disease not explicitly prohibited as above are allowed.

5.7 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum or urine pregnancy test within 7 days prior to the first dose of flotetuzumab.

Female and male patients (along with their female partners) are required to use a highly effective form of acceptable contraception, defined as those which result in a low failure rate (i.e., less than 1% per year) such as implants, injectables, combined oral contraceptives, intra-uterine devices (IUDs) or vasectomized partner. Male patients with partners of childbearing potential must be either vasectomized or agree to use a condom in addition to having their partners use another method of contraception resulting in a highly effective method of birth control defined as those which result in a low failure rate (i.e., less than 1% per year) such as implants, injectables, combined oral contraceptives, or IUDs. Patients must agree to use contraception during participation in the study and for 12 weeks following the last dose of flotetuzumab. Patients should also abstain from sperm/egg donation during the course of the study.

If a patient is suspected to be pregnant, flotetuzumab should be immediately discontinued. In addition, a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within 12 weeks after the last dose of flotetuzumab, the investigator must be notified in order to facilitate outcome follow-up.

5.8 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue for 2 months or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unable to receive further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will still be followed as indicated in the study calendar.

5.9 Duration of Follow-up

Patients will be followed at least every 6 months for 2 years or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Patients will return to the clinic for routine post-transplant visits during the first 6 months of follow-up. After the first 6 months, patients can be followed either in person via clinic visit or through a phone call or medical records review.

5.10 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for 6 weeks following a scheduled visit and is unable to be contacted by the study team.

The following actions must be taken if the participant fails to return to clinic for a required study visit:

- The study team will attempt to contact the participant and reschedule the missed visit within 6 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

6.0 DOSE DELAYS/DOSE MODIFICATIONS

6.1 Infusion-related Reactions including CRS

The predicted mechanism of action of flotetuzumab is the creation of an immunological synapse between the leukemic blast target cell bearing CD123 and immune effectors bearing the T cell specific CD3 complex, leading to T cell activation and killing of the leukemic cell. Activation of T cells is associated with the elaboration of various cytokines. *In vitro* preclinical testing using human PBMCs has suggested that the most vigorous cytokine production is that of IFN-γ, but increases in production of TNF-α, IL-2, IL-6, IL-4, and IL-10 were documented. Cytokine production, primarily of IL-6, was also documented in normal cynomolgus monkeys receiving flotetuzumab - a circumstance in which relatively few target cells (mostly CD123-bearing pDCs) were present in the animal's peripheral blood but elimination of those cells was observed. In the cynomolgus monkey, very high doses of flotetuzumab (i.e., 5 μg/kg/day) were associated with severe cytokine release of multiple cytokines including IL-6, IFN-γ, IL-2, TNF-α and to a lesser extent IL-8 and IL-4, resulting in moribundity or death.

Infusion-related reactions or Cytokine Release Syndrome (CRS) may occur. Anaphylactic or anaphylactoid-type reactions are possible. Precautions for the management of these reactions should be observed during flotetuzumab administration.

Infusion-related reactions (including CRS) associated with flotetuzumab administration should be managed according to the standard practice of medicine. General guidelines for the management of such reactions are provided in this section. However, severe reactions may require extraordinary interventions like those that have been required to extinguish the syndrome of cytokine storm or may have anaphylactoid features.

Patients should be monitored closely for the development of IRRs during flotetuzumab infusion; with CRS, ICE and ICANS toxicity assessments recorded in the EMR flowsheets and daily progress notes, as per BMT protocol for patients receiving cellular therapies and cell-engaging therapies. Medications and supportive measures for the treatment of such

reactions should be available for immediate use for an infusion reaction during study drug administration and may include, but are not limited to: subcutaneous (SC) epinephrine (0.3 to 0.5 mL of a 1:1000 solution), antihistamines (e.g., diphenhydramine 25 to 50 mg IV), corticosteroids (e.g., dexamethasone 10 mg IV push or equivalent), IV fluids, vasopressors, oxygen, bronchodilators, and antipyretics. Resuscitation equipment and other supplies for the emergency management of an allergic/toxic reaction must be available. The patient should be treated according to the best available local practices and procedures. All supportive measures consistent with optimal patient care will be provided throughout the study according to institutional standards.

Should symptoms of fever or chills develop it may be difficult or impossible to distinguish among potential causes of the symptoms including underlying leukemia, neutropenic fever, emerging infection, or infusion reaction. Temporarily halting the flotetuzumab infusion to collect cultures, institute antimicrobials, etc., may provide information regarding etiology. If the fever and chills are considered not related (as described in Section 6.3) to the infusion of study drug, the study drug may be reinstituted at the same rate of administration after administration of acetaminophen, H2 blockers, and diphenhydramine if not administered previously for premedication. More complex symptom composites may require additional intervention. Please refer to Section 6.3 for management of infusion reactions if the event is considered related to flotetuzumab. If during assessment of IRR/CRS a patient undergoes imaging and/or sample collection, e.g., pleural fluid, intra-articular joint aspiration, or ascitic fluid, a sample may be further evaluated in order to help elucidate etiology.

6.2 Premedications and Prophylaxis

Based on the clinical experience to date, similar findings from other bispecific molecules such as blinatumomab and reported clinical experience with CAR-T therapies, all patients will receive premedication to prevent or mitigate potential infusion-related reactions. Specific pre-treatment regimens are described below (Table 4).

Prior to the first dose (Week 1):
All patients are to be treated as described in Table 4 below. No steroids should be administered for infusion-related reaction prophylaxis except as indicated below. Patients should receive adequate hydration during study therapy, and the addition of IV fluids to maintain intravascular volume (e.g., 50-100 mL/hr of normal saline) is recommended for at least 48 hours after the start of therapy.

Prior to Week 2 dosing:
The premedication schedule should be followed as described in Table 4 below. In addition, the same premedications should be considered after consultation with the PI if they are resuming flotetuzumab treatment after a dose interruption lasting > 1 day in Cycle 1.

Steroids should not be administered for infusion-related reaction prophylaxis except as indicated below, but may be used for the treatment of emerging symptoms as clinically appropriate. Tocilizumab (8 mg/kg IV) may be used prophylactically in patients who have had severe IRR or CRS in the first week of dosing upon consultation with the PI. Patients should receive adequate hydration during study therapy, and the addition of IV fluids to

maintain intravascular volume (e.g., 50-100 mL/hr of normal saline) is recommended for at least 48 hours after the start of therapy in Week 2.

Prior to subsequent doses (after Week 2):
The premedication schedule should be followed as described in Table 4 below. Tocilizumab or steroids may be considered after consultation with the PI.

For infusion-related reactions during the course of treatment, supportive care measures should be implemented as outlined in Table 7 below.

IV fluids should be administered for inpatients unless contraindicated and adequate oral hydration should be emphasized for patients treated as outpatients in Cycles 2 and beyond.

Dose interruption:
If dosing is interrupted for > 24 hours during continuous infusion, the premedication schedule should be followed (Table 4).

Table 4: Premedication Guidelines

28 day continuous Flotetuzumab infusion schedule				
Medication	Week 1	Week 2	Week 3	Week 4
Acetaminophen (1000mg PO)	Day 1: 30 minutes prior to dosing, then q8 hrs for 48 hours Day 7 or day of target dose: 30 minutes prior to dose change, then q8 hrs for 48 hours	None	None	None
Diphenhydramine (25-50mg IV or PO) or equivalent	Day 1: 30 minutes prior to dosing, then q8 hrs for 48 hours Day 7 or day of target dose: 30 minutes prior to dose change, then q8 hrs for 48 hours	None	None	None
Ranitidine (50mg IV) or equivalent	Day 1: 30 minutes prior to dosing, then q8 hrs for 48 hours Day 7 or day of target dose: 30 minutes prior to dose change, then q8 hrs for 48 hours	None	None	None
Dexamethasone (or equivalent)	Day 1: 10-20mg IV up to 30 minutes before dosing. Then 4mg IV at 12 hours after dosing. Day 7 or day of target dose: 10mg IV up to 30 minutes prior to dose change, then 4mg 12 hrs later.	None	None	None

28 day continuous Flotetuzumab infusion schedule				
Medication	Week 1	Week 2	Week 3	Week 4
Tocilizumab (8mg/kg IV)	None	After consultation with PI	None	None

6.3 Management of Observed Flotetuzumab Infusion Reactions and Infusion-related Adverse Events

The following are treatment guidelines (which may be modified as needed by the responsible Investigator according to the best practices of medicine) for flotetuzumab IRRs including CRS. Early intervention at the first signs of IRR/CRS, including pyrexia, tachycardia, tachypnea and/or hypotension in the absence of alternative etiologies and in consistent temporal relationship to administration of flotetuzumab, should be undertaken.

IRR/CRS is a commonly observed event during treatment with flotetuzumab 89.6% of the 115 patients receiving flotetuzumab monotherapy experiencing at least 1 event of Grade 1-3 IRR/CRS, as of the data cut-off date of 21 April 2020 from the most current MacroGenics IB v9.0. Based on current understanding gained with patients treated with CAR-T cells (which have a T-cell activating mechanism of action in common with flotetuzumab), CRS is likely to be a common toxicity that can be managed through supportive care and anti-cytokine interventions to allow for full activity of T cells during therapy. In the 2019 ASTCT Consensus Guidelines for grading and management of CRS proposed by Lee, et al (18), intensive supportive care and treatment of the underlying cause of the CRS (excessive cytokine production) is recommended before the event must be considered Grade 3 or Grade 4 severity. Adopting this definition of CRS as described in Table 5, and concomitantly modifying the guidelines for management of IRR/CRS (below), will allow for more flexible management of patients who develop CRS and who may nonetheless experience clinical benefit despite the need to manage through mild or moderate infusion reactions while not jeopardizing overall safety.

IRR/CRS will be graded according to the definitions described below in Table 5.

Table 5: Adapted from the ASTCT CRS Consensus Grading Criteria

Toxicity Grade	**Characteristics**
Grade 1	Temperature ≥38°C, no hypotension or hypoxia.
Grade 2	Temperature ≥38°C, not requiring vasopressors and/or requiring ≤6L/minute oxygen support.
Grade 3	Temperature ≥38°C, requiring a vasopressor (with or without vasopressin) and/or requiring ≥6L/minute oxygen support (via high flow nasal cannula, facemask, non-rebreather or Venturi mask).
Grade 4	Temperature ≥38°C, requiring multiple vasopressors (not including vasopressin) and/or requiring positive pressure ventilation (including CPAP, BiPAP and intubation/mechanical ventilation

Grade 5	Death

Source: Lee DW, et al. (**18**)

The above grading scale (Table 5) should be used to grade all infusion reactions in this study, irrespective of the underlying mechanism of the reaction. Certain AEs may occur in temporal proximity to infusion of study drug and therefore, be considered "infusion-related" while not being considered an "infusion reaction;" such events may be graded separately according to CTCAE specific criteria.

IRR/CRS should be managed as described below, when possible. Every attempt should be made to continue the infusion during management of the reaction, however decisions to change or hold the infusion should ultimately be left to the discretion of the treating physician.

Table 6: Guidelines for Managing Infusion Reactions and Cytokine Release Syndrome*

Grade/Event	Procedures for Management
Grade 1 Infusion Reactions, including Cytokine Release Syndrome (CRS)	• Slow the infusion rate by 10%-20%; • Monitor the patient for worsening of condition; • Administer IV fluids, diphenhydramine 50 mg IV, acetaminophen 1000 mg PO or ibuprofen 400 mg PO for fever, and oxygen and bronchodilators for mild bronchospasm, as appropriate; • Obtain infectious work-up as clinically indicated (UA, blood cultures, imaging, etc) and consider empiric antibiotics per institutional guidelines • Corticosteroids should not be used for Grade 1 IRR/CRS. • Continue infusion at reduced rate and slowly increase infusion rate to the original rate in 2 steps after stabilization or resolution of symptoms every 4-6 hours, as tolerated. A more gradual increase in the rate of infusion may be undertaken after consultation with the PI.
Grade 2 Infusion reactions and infusion-related events	• Slow the infusion rate by 25%-50%; • Administer IV fluids, diphenhydramine 50 mg IV, acetaminophen 1000 mg PO or ibuprofen 400 mg PO for fever, and oxygen and bronchodilators for bronchospasm, as appropriate and if not administered previously; • If not already performed, obtain infectious work-up as clinically indicated (UA, blood cultures, imaging, etc) and consider empiric antibiotics per institutional guidelines • Tocilizumab (8 mg/kg IV) should be used for Grade 2 IRR/CRS that does not resolve with other measures within 2 hours, or that requires the use of supplemental oxygen > 4 L by nasal cannula. Tocilizumab dosing may be repeated in 8 hrs if not improved to Grade 1, with a maximum total of up to 4 doses for each CRS event.

Grade/Event	Procedures for Management
	• Corticosteroids may be used for Grade 2 IRR/CRS that does not respond to other measures, including tocilizumab • Continue infusion at reduced rate and slowly increase infusion rate to the original rate gradually by half-rate increments (i.e., if rate is reduced from 5 mL/hr to 2.5 mL/hr increase to ~3.75 mL/hr, then 4.5 mL/hr, then 5 mL/hr) after stabilization or resolution of symptoms every 4-6 hours, as tolerated. A more gradual increase in the rate of infusion may be undertaken after consultation with the PI. • Report as an immediately reportable event (IRE); • Report the event as a serious adverse event (SAE), if appropriate. • Monitor for worsening condition.
Grade 3 Infusion Reaction and infusion-related events	• Stop the infusion, • TO AVOID EXACERBATION OF INFUSION REACTION OR CRS: DO NOT FLUSH THE TUBING – ASPIRATE RESIDUAL DRUG FROM THE PORT LUMEN; • Administer IV fluids, diphenhydramine 50 mg IV, acetaminophen 1000 mg PO or ibuprofen 400 mg PO for fever, and oxygen and bronchodilators for mild bronchospasm, as appropriate. • Provide appropriate circulatory support including vasopressors as medically indicated; • If not already performed, obtain infectious work-up as clinically indicated (UA, blood cultures, imaging, etc) and consider empiric antibiotics per institutional guidelines • Administer tocilizumab if not administered previously. If administered previously, an additional dose may be administered in 8 hours for prolonged or recurrent episodes, with a maximum total of up to 4 doses for each CRS event. • Grade 3 IRR/CRS that is refractory to tocilizumab should be treated with corticosteroids; doses of dexamethasone (or equivalent) of greater than 30 mg may be required. • Resume the infusion at previously tolerated dose once the infusion reaction has resolved or decreased to Grade 1. Increase dose rate to the original rate, per protocol, e.g., 500 ng/kg/day, by increasing the dose in near double increments as tolerated after stabilization or resolution of symptoms every 4-6 hours. A more gradual increase in the rate of infusion may be undertaken after consultation with the PI. • Report the event as a serious adverse event (SAE), if appropriate.

Grade/Event	Procedures for Management
	• Discontinue the infusion if not resolved to Grade 1 within 72 hours.
Grade 4 Infusion reactions and infusion-related events	• Stop the infusion and disconnect the infusion tubing from the patient; • TO AVOID EXACERBATION OF INFUSION REACTION OR CRS: DO NOT FLUSH THE TUBING – ASPIRATE RESIDUAL DRUG FROM THE PORT LUMEN; • Administer IV fluids, diphenhydramine 50 mg IV, acetaminophen 1000 mg PO or ibuprofen 400 mg PO or paracetamol 1000 mg PO for fever, and oxygen and bronchodilators for mild bronchospasm, as appropriate; • Provide appropriate ventilator and circulatory support as medically indicated; • If not already performed, obtain infectious work-up as clinically indicated (UA, blood cultures, imaging, etc) and consider empiric antibiotics per institutional guidelines • Agents including those listed below have been described in the management of patients with severe complications of cytokine storm and/or severe immune-related adverse events, and may be required: o high doses of corticosteroids above dexamethasone 30 mg (or equivalent) o tocilizumab (anti-IL6 receptor) 8 mg/kg IV, administered every 8 hours as indicated with a maximum total of up to 4 doses for each CRS event • Notify the PI immediately; • Report the event as an SAE. Patients who have a Grade 4 IRR/CRS should not receive further flotetuzumab.
Grade 5 Infusion reactions and infusion-related events	• Notify the PI or designee immediately. • Report the event as an SAE.

* Table provides guidelines but treating physician should follow their discretion to manage and grade CRS/IRR

Other types of adverse reactions may be observed during infusion that are not interpreted as related to cytokine release, but may be related to flotetuzumab. Such reactions should be diagnosed, graded and managed according to best practices.

6.4 Graft versus Host Disease

Use of flotetuzumab has not been evaluated in the post-allo-HCT setting, therefore the impact of flotetuzumab on acute or chronic GVHD is unknown. Theoretically, the mechanism of action of flotetuzumab may redirect allo-reactive T cells toward malignant cells and thus reduce GHVD. In pre-clinical pharmacodynamic data, flotetuzumab displays a 10-fold greater affinity for human CD123 to favor initial binding to the CD123+ target

cell and minimize CD3 engagement in the absence of target cells. It is also possible that there will be no impact of flotetuzumab on the incidence or severity of GVHD, or that it will exacerbate GVHD. The administration of a DLI is associated with GVHD rates as high as 60-70%, depending on cell dose and other factors. Therefore, it is expected that patients who receive a DLI concurrent with flotetuzumab may experience new onset GVHD or worsening of pre-existing GVHD.

Accordingly, patients with active GVHD requiring systemic immunosuppression with more than 0.5mg/day prednisone will be excluded from the trial; in addition, careful monitoring for interval development of new or worsening GVHD for patients on trial is warranted.

Acute and Chronic GVHD assessments (See Appendices F and G) will be performed to evaluate the presence of acute and chronic GVHD at screening/baseline, prior to initiation of Cycle 1 and prior to the initiation of Cycle 2, with documentation as below (Section 7.7.1 and 7.7.2). Additionally, patients should be monitored during treatment for development of new or worsening GVHD with appropriate assessment/grading as clinically indicated in the opinion of Investigators.

6.4.1 Acute GVHD Assessment

Acute GVHD assessment will be documented with date of onset, grade, and organs affected (see Appendix F). Of note, evaluation for alternative etiologies for new or worsening rash, LFT changes or diarrhea should be pursued according to institutional practice and standards of care. Rashes, LFT elevations and diarrhea determined to be explained by an alternative etiology (i.e. not due to aGVHD) should not be included in the Acute GVHD Assessment.

6.4.2 Chronic GVHD Grading/Assessment

Chronic GVHD Assessment will be documented with date of onset, grade, and organs affected. See Appendix G. Of note, evaluation for alternative etiologies for new or worsening findings on the below listed assessments should be pursued according to institutional practice and standards of care. For example, rashes, LFT elevations and diarrhea determined to be explained by an alternative etiology (i.e. not due to cGVHD) should not be included in the Chronic GVHD Assessment.

6.4.3 Dose Delays and Modifications for aGVHD and cGVHD

Table 7: Dose Delays and Modifications for aGVHD

MAGIC Grade I GVHD	The presence of new or worsening MAGIC Grade I GVHD should warrant standard of care therapies for commensurate GVHD level and does not require any dose modifications to the flotetuzumab.

MAGIC Grade II GVHD	The presence of new or worsening MAGIC Grade II GVHD should warrant standard of care therapies for commensurate GVHD and the does not require any dose modifications to the flotetuzumab. Persistent Grade II GVHD not improving with SOC interventions and supportive care within 24-48 hours requires a 25-50% dose reduction in flotetuzumab with continued SOC interventions, supportive care and continued monitoring of aGVHD severity. Uptitration of flotetuzumab to target dose may be attempted as per CRS protocol upon improvement of GVHD Grade to Grade I or better. However, persistent Grade II GVHD not improving with SOC interventions and with 25-50% dose reduction after 48-72 hours requires an immediate hold of flotetuzumab. Reinitiation of flotetuzumab with resolution of GVHD severity to Grade I or better can be attempted, resuming at 25-50% of the target dose and uptitration as per CRS protocol.
MAGIC Grade III GVHD	The presence of MAGIC Grade III GVHD should warrant standard of care therapies for commensurate GVHD and requires immediate hold of flotetuzumab. SOC interventions and supportive cares should be continued with daily assessment of GVHD Grade. Reinitiation of flotetuzumab with resolution of GVHD severity to Grade I or better can be attempted, resuming at 25-50% of the target dose and uptitration as per CRS protocol.
MAGIC Grade IV GVHD	The presence of MAGIC Grade IV GVHD should warrant standard of care therapies for commensurate GVHD and requires immediate hold of flotetuzumab. SOC interventions and supportive cares should be continued with daily assessment of aGVHD Grade. Reinitiation of flotetuzumab should not be attempted.

Table 8: Dose Delays and Modifications for cGVHD (see NIH cGVHD Severity Score in Appendix G)

Mild cGVHD	The presence of new or worsening Mild cGVHD should warrant standard of care therapies for commensurate GVHD level and does not require any dose modifications to the flotetuzumab.
Moderate cGVHD	The presence of new or worsening Moderate cGVHD when baseline assessment prior to flotetuzumab was rated as Mild or better should warrant standard of care therapies

	for commensurate GVHD and the does not require any dose modifications to the flotetuzumab. Persistent Moderate cGVHD not improving with SOC interventions and supportive cares within 24-48 hours requires a 25-50% dose reduction in flotetuzumab with continued SOC interventions, supportive cares and continued monitoring of cGVHD severity. Uptitration of flotetuzumab to target dose may be attempted as per CRS protocol upon improvement of cGVHD to Mild or better. However, persistent Moderate cGVHD not improving with SOC interventions and with 25-50% dose reduction after 48-72 hours requires an immediate hold of flotetuzumab. Reinitiation of flotetuzumab with resolution of cGVHD severity to Mild or better can be attempted, resuming at 25-50% of the target dose and uptitration as per CRS protocol.
Severe cGVHD	The presence of Severe cGVHD should warrant standard of care therapies for commensurate GVHD and requires immediate hold of flotetuzumab. SOC interventions and supportive cares should be continued with daily assessment of cGVHD Grade. Reinitiation of flotetuzumab should not be attempted.

6.5 Tumor Lysis Syndrome

Tumor lysis syndrome (TLS) is the most common disease-related emergency encountered by physicians caring for children or adults with hematologic cancers. Characteristic findings are hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia. These electrolyte and metabolic disturbances can progress to clinical toxic effects, including renal insufficiency, cardiac arrhythmias, seizures, and death due to multi-organ failure.

Prophylaxis for TLS should be administered for patients with AML. Aggressive hydration, allopurinol, and oral phosphate binders should be implemented starting preferably 24 hours before flotetuzumab administration. Serum chemistries should be closely monitored during flotetuzumab administration in accordance with institutional standards for the treatment of acute leukemia. If TLS is observed, rasburicase should be considered for management as per institutional standards. Patients who develop TLS should be monitored closely for abnormal serum chemistries or signs of end organ damage and treated appropriately.

6.6 Neutropenic Sepsis/Fungemia

While there is no evidence that flotetuzumab targets and depletes neutrophils in vitro in human samples or decreases circulating neutrophils in monkeys receiving the drug, caution should be exercised in assessing the possibility of infections. Early initiation of empiric broad-spectrum antimicrobials is recommended in the event of fever.

Several monotherapy or multicomponent regimens have been employed. Investigators should employ these agents in compliance with best medical practices and institutional guidelines and standards. If fever persists for 4 to 7 days, an echinocandin or liposomal amphotericin B should be considered. The use of recombinant hematopoietic growth factors in AML is controversial. Their use is specifically prohibited in Cycle 1. However, beyond Cycle 1, while discouraged, their use is not specifically prohibited by this protocol and they may be employed at the discretion of the Investigator.

6.7 Immune-related Adverse Experiences

Immune checkpoint blockade has been associated with several syndromes resulting from the breaking of immunological tolerance. Although not observed in non-clinical studies to date, similar immunomodulation might be expected with flotetuzumab. These syndromes include: pneumonitis, colitis, autoimmune hepatitis, arthritis, glomerulonephritis, myocarditis and cardiomyopathy, hypophysitis, or thyroiditis. Their occurrence dictates interruption, and potentially discontinuation, of study drug administration pending further evaluation.

6.8 Neurotoxicity Monitoring

Neurotoxicity, including changes in mental status, has been reported with other T-cell directed therapies including chimeric-antigen receptor (CAR)-T cells and CD3xCD19 based bispecific antibodies such as blinatumomab. The exact mechanism of the toxicity is unknown. As a result, additional monitoring of patients receiving flotetuzumab is indicated. An alteration in mental status refers to general changes in brain function, such as confusion, amnesia, loss of alertness, disorientation, defects in judgment or thought, and disruption in perception and psychomotor skills. Patients should be monitored for changes in mental status or other potential neurotoxic events and should be evaluated for orientation to time, place and person at baseline prior to treatment with flotetuzumab and at regular intervals during therapy.

Any suspected neurotoxic event should prompt full evaluation including imaging studies, lumbar puncture and neurological consultation as indicated to rule out other causes. The incidence of CNS involvement by AML is low and routine lumbar puncture is not generally considered indicated. Patients with known, active CNS leukemia are excluded from the study and those with suspected disease must be evaluated by lumbar puncture prior to enrollment. See Appendix E for grading, evaluation and management of neurotoxicity.

7.0 CORRELATIVE STUDIES

Patient material will be obtained at Barnes-Jewish Hospital or in the Siteman Cancer Center. Samples will be identified with the patient's name, date of birth and clinic or hospital number. The Stem Cell Transplantation Core analyst or a designated person will be responsible for removal of this information from the sample container and its replacement by a label containing a Unique Patient Number (UPN) and sample number. Any paper copies of the master code listing UPN and

sample numbers with the patient names will be kept in a locked storage. Any electronic copies of the master code will be kept in a secured network. Access to either paper or electronic copies will be restricted to the Principal and Sub-Investigators of this study and associated study personnel.

Stem Cell Transplantation Core personnel will be responsible for transport of the specimens to the pre-defined laboratories for processing. Specimens will be identified by UPN and sample number and will be processed and stored at the pre-defined lab. Each lab will be responsible for sample processing, storage, and delivery of specimens according to established standard operating procedure documents generated for the purpose of this trial. The pre-defined lab will be responsible for establishing and maintaining standardized operating procedures as it relates to sample handling.

All samples should be sent to:
Siteman Cancer Center Tissue Procurement Core Facility
425 S. Euclid Ave., Rm 5120
St. Louis, MO 63110
Phone: 314-454-7615
Fax: 314-454-5525
Email: tbank@pathology.wustl.edu

OR

Dr. Matt Christopher's Laboratory
Washington University School of Medicine
4940 Parkview
St. Louis, MO 63110
Contact Person: Tyler Elmendorf
Phone: 314-273-0286
Email: elmendorft@wustl.edu

During each BM Bx, samples for correlative studies will be obtained and banked at the following time points: (2-3 ml bone marrow aspirate in 1 EDTA tube per time point):

- Screening/Baseline
- Cycle 1 Day 14 (optional, at discretion of Principal Investigator.)
- Cycle 1 Day 28
- Cycle 2 Day 28
- At relapse or progression of disease

In addition, peripheral blood will be obtained from patients at these time points:

- Cycle 1 Day 1 (pre-flotetuzumab dose)
- Cycle 1 Day 7 (pre-week 2 infusion)
- Cycle 1 Day 14 (pre-week 3 infusion)
- Cycle 1 Day 21 (pre-week 4 infusion)
- Cycle 1 Day 28 (with C1D28 bone marrow biopsy)
- Cycle 2 Day 1 (pre-flotetuzumab)
- Cycle 2 Day 7 (pre-week 2 infusion)

- Cycle 2 Day 14 (pre-week 3 infusion)
- Cycle 2 Day 21 (pre-week 4 infusion)
- Cycle 2 Day 28 (with C2D28 bone marrow biopsy)
- At the time of disease relapse/progression.

These de-identified samples will be analyzed at a later time point at the discretion of the principal investigators and/or MacroGenics.

8.0 PHARMACEUTICAL INFORMATION

8.1 Flotetuzumab

8.1.1 Flotetuzumab Description

Flotetuzumab is a CD123 x CD3 bi-specific antibody-based molecular construct referred to as a DART molecule. Its purpose is to bring into close proximity immune effector cells bearing the CD3 antigen, which is a component of the T cell antigen receptor complex, and target cells bearing the CD123 antigen, which is a component of the interleukin-3 receptor. As a result of opposing the two cells and activating the T cell, it is hypothesized that the neoplastic cells will be killed.

Flotetuzumab drug product (DP) is provided as a clear to slightly opalescent, colorless to pale yellow 5 mL sterile aqueous solution with a protein concentration of 0.1 mg/mL in a buffer composed of 10 mM sodium phosphate at pH 6.0, 150 mM sodium chloride, and 0.1 mg/mL polysorbate 80.

The concentration, function, and grade of each component in the DP are summarized below.

Flotetuzumab Drug Product Composition

Name of Ingredient	Concentration (mg/mL)	Nominal Amount per Vial (mg) [a]	Function
Flotetuzumab DS	0.10	0.50	Active ingredient
Sodium phosphate monobasic monohydrate	1.13	5.65	Buffer component
Sodium phosphate dibasic	0.26	1.3	Buffer component
Sodium chloride	8.78	43.9	Tonicifier
Polysorbate 80	0.10	0.50	Surfactant

Abbreviations: DS, drug substance
a. Amount based on 5.0 mL fill volume; vial contains approximately 0.30 mL overfill to deliver 5 mL.

8.1.2 Clinical Pharmacology

The predicted mechanism of action of flotetuzumab is the creation of an immunological synapse between the leukemic blast target cell bearing CD123 and immune effector cells bearing the T cell-specific CD3 complex, leading to T cell activation and killing of the leukemic cell.

8.1.3 Pharmacokinetics and Drug Metabolism

See Section 5.4 of the Investigator's Brochure for preliminary PK results from Study CP-MGD006-01. Overall, initial review of the data shows that there was high interpatient variability, but no obvious deviation of linearity across different doses. Preliminary analysis of flotetuzumab serum concentration indicates flotetuzumab PK is best fit by a two compartment PK model. In addition, flotetuzumab demonstrates a high clearance rate and a short half-life, as expected. Steady state volumes suggest there is high tissue distribution and/or binding. These data continue to be evaluated.

8.1.4 Supplier(s)

Flotetuzumab will be provided by MacroGenics Inc.

Flotetuzumab Drug Product
Flotetuzumab Drug Product is supplied as 0.5 mg/5 mL (0.1 mg/mL) sterile aqueous solution packaged in a USP and Ph. Eur. conforming Type I borosilicate, 5 mL clear glass vial with a 20 mm FluroTec-coated 4432/50 gray butyl rubber serum stopper. The vial is sealed with a 20 mm TruEdge aluminum closure with a plastic overseal.

MGV004 Vehicle
The MGV004 IV Solution Stabilizer is supplied as a sterile solution of The MGV004 IV Solution Stabilizer is packaged in a USP and Ph. Eur. conforming Type I borosilicate, 50 mL clear glass vial with a 20 mm FluroTec and B2-40-coated 4023/50 gray butyl rubber serum stopper and sealed with a 20 mm TruEdge aluminum closure with a plastic overseal.

MGV002 Vehicle
MGV002 IV Solution Stabilizer is a sterile, clear-colorless solution presented in a single use glass vial. The MGV002 IV Solution Stabilizer is supplied as a sterile solution containing 0.9% sodium chloride solution with 0.1 mg/mL polysorbate 80. The MGV002 IV Solution Stabilizer is packaged in a USP and Ph. Eur. conforming Type I borosilicate, 50 cc clear glass vial with a 20 mm -4432/50 gray butyl rubber serum stopper and sealed with a 20 mm TruEdge aluminum closure with a plastic overseal.

8.1.5 Dosage Form and Preparation

Flotetuzumab DP is provided as a clear to slightly opalescent, colorless to pale yellow 5 mL sterile aqueous solution with a protein concentration of 0.1 mg/mL in a buffer composed of 10 mM sodium phosphate at pH 6.0, 150 mM sodium chloride and 0.1 mg/mL polysorbate 80.

The flotetuzumab dose solution can be prepared for administration from a syringe, an ambulatory cassette or a standard infusion bag. The administration options include the use of a syringe pump, dual ambulatory pumps, a single ambulatory pump, or standard IV infusion pump. Instructions for the preparation and administration of flotetuzumab are detailed in the Pharmacy Manual.

Important: Flotetuzumab MUST NOT BE ADMINISTERED AS AN IV PUSH OR BOLUS.

Flotetuzumab should not be mixed with other drugs or diluted with any solution other than MGV004 Vehicle or MGV002 Vehicle when administering the drug by 24-hour infusion.

Errors in dilution could result in fatal cytokine release syndrome; please refer to the Pharmacy Manual for further details on dosing schedule and dose preparation.

8.1.6 Storage and Stability

Vials containing flotetuzumab and MGV004 Vehicle or MGV002 Vehicle should be stored upright under refrigeration at 2°–8°C (36°–46°F) in an appropriate, locked room accessible only to pharmacy personnel, the Investigator, or a duly designated person. flotetuzumab and MGV004 Vehicle or MGV002 Vehicle must not be frozen. Monitor temperature and document and report any excursions as instructed in the Pharmacy Manual. Protect from light during storage as described in the Pharmacy Manual. DO NOT SHAKE. Use standard laboratory practices for avoidance of contact. Recommended safety measures for handling and preparation include masks, protective clothing, gloves, and vertical laminar airflow safety cabinet.

Follow standard hygiene practices, such as hand washing after handling. If material is released or spilled, soak up material with absorbent material and wash spill area thoroughly with soap and water. Dispose of collected material in accordance with applicable waste disposal regulations.

8.1.7 Administration

When administered by syringe pump or dual ambulatory pump configuration over 24 hours, flotetuzumab must be diluted prior to administration with appropriate custom vehicle, MGV004 Vehicle (2.2 mg/mL sodium phosphate monobasic

monohydrate, 0.52 mg/mL sodium phosphate dibasic anhydrous, 13.2 mg/mL benzyl alcohol, 4.25 mg/mL methyl 4-hydroxybenzoate sodium salt (methyl paraben), and 0.25 mg/mL polysorbate 80, pH 8.2) or MGV002 Vehicle (0.9% sodium chloride solution with 0.1 mg/mL polysorbate 80).

8.1.8 Special Handling Instructions

Precautions: Patients with known hypersensitivity to recombinant proteins, polysorbate 80, recombinant human serum albumin, benzyl alcohol, or any excipient contained in the flotetuzumab drug formulation should not receive flotetuzumab.

Infusion-related reactions including hypersensitivity/anaphylactic/anaphylactoid reactions or cytokine release syndrome may occur. Precautions for the management of these reactions should be observed during flotetuzumab administration.

9.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
On-Study Form Concomitant Meds Form Medical History Form Treatment History Form	Prior to starting treatment (meds given to treat CRS and ICANS collected throughout treatment)
Treatment Form	Every cycle
Acute GVHD form Chronic GVHD form	C1D1, C1D28, C2D7, C2D14, C2D21, C2D28, Follow-up
Toxicity Form Stopping Rules form	Continuous
Treatment Summary Form	Completion of treatment
Follow Up Form	Every 6 months for 2 years
Research Bone Marrow Collection Form	Baseline, C1D14, C1D28, C2D28, suspected disease relapse or progression.
Response Assessment Form	Baseline, C1D28, C2D28, end of treatment
Progression Form	Time of disease progression
Death Form	Time of death
MedWatch Form	See Section 13.0 for reporting requirements

10.0 MEASUREMENT OF EFFECT

Disease activity will be assessed using CBC and peripheral blood cell morphological examination, examination of bone marrow biopsy, and physical examination. For AML, modifications to the Revised Recommendations of the International Working Group for Diagnosis (IWG), Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia will be used. See table below.

Complete remission (CR)	Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/μL); platelet count ≥100 × 10^9/L (100,000/μL), transfusion independence
CR with incomplete hematologic recovery (CRi)	All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1000/μL]) or thrombocytopenia (<100 × 10^9/L [100,000/μL])
Morphologic leukemia-free state (MLFS)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5 to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%
Stable disease (SD)	Absence of CR, CRi, PR, MLFS; and criteria for PD not met
Progressive disease (PD)	Evidence for an increase in bone marrow blast percentage (>50% over baseline), and/or increase of absolute blast counts in the blood (>50% to >25 × 10^9/L) without differentiation syndrome, or new extramedullary disease

Time to response (first or best response) will be calculated from the time of first dose of study drug to the time of initial response. Response duration and time to response will be calculated for patients who achieve an objective response. Progression free survival and overall survival will be calculated as the time from the start of the first dose of study drug until the occurrence of disease progression or death from any cause, respectively.

11.0 STATISTICAL CONSIDERATIONS

11.1 Hypothesis

Flotetuzumab for relapsed AML following allo-HCT will result in improved response rates compared to historical response rates with chemotherapy or HMA monotherapy

11.2 Primary Endpoint

The primary endpoint will be efficacy assessed via composite endpoint of CR and CRi (Cycle 1 Day 28 assessment).

Efficacy Endpoint will be defined as a combined endpoint of complete response (CR) and complete response with incomplete hematologic recovery (CRi), per International Working Group criteria (Table 2, and above.)

11.3 Sample Size Estimate

Twenty-five evaluable patients will be enrolled for this study. Assuming a single treatment arm compared to historical controls treated with chemotherapy alone or HMA monotherapy using a dichotomous endpoint of CR/CRi vs not CR/CRi (as defined by the Primary Endpoint, as above), based on Simon's Minimax two-stage design, we estimate a sample size of 25 patients would provide 80% power (alpha 0.05) to detect a difference between a 10% CR/CRi rate in historical controls treated with chemotherapy or hypomethylating agents alone (10, 11) compared to 30% CR/CRi rate in the treatment arm (effect size 20%).

11.4 Stopping rules for futility

Fifteen patients will be enrolled in the first stage. This cohort will include all patients who have been treated, including those who withdraw from the study. If at least 2 CR/CRi are observed out of the 15 patients, then an additional 10 patients will be enrolled. If 6 or more CR/CRi are observed from this cohort of 15 patients, we would conclude that there is evidence of efficacy to warrant further investigation. There is less than 5% chance to stop the trial at stage 1 if the "true" response is 30% or higher. Conversely, there will be 55% chance to terminate the study early if the true rate is only 10% or less.

11.5 Safety monitoring and determination of excess toxicity

To minimize risk to patients on study, we will perform interval analyses to determine if excess treatment-associated toxicity is observed during the trial. Toxicity may occur due to treatment with flotetuzumab, DLI administration, or unknown factors. Since GVHD may be an expected result of DLI, there will be separate stopping rules for excess non-GVHD toxicity and for GVHD toxicity.

11.5.1 Excess non-GVHD toxicity

Excess non-GVHD toxicity will be defined as a <u>combined</u> incidence of > 20% of <u>any</u> of the following:

- ≥ Grade 3 non-hematologic AE at least possibly attributable to protocol treatment, with the following exceptions:
  - Grade 3 fatigue, asthenia, fever, constipation or anorexia (if anorexia does not result in hospitalization, tube-feeding or use of total parenteral nutrition [TPN])
  - Grade 3 nausea, vomiting or diarrhea not requiring tube feeding, TPN, or requiring or prolonging hospitalization
  - Infection, bleeding, or other expected direct complications of cytopenias due to active underlying leukemia
  - Grade 3 infusion reaction including CRS, if successfully managed clinically and resolves within 7 days without end-organ damage
  - Grade 3 or 4 isolated electrolyte abnormalities that last < 72 hours

- Grade 4 neutropenia or thrombocytopenia lasting ≥ 42 days from the start of the cycle in the absence of MDS/AML
- Evidence of drug-induced liver injury based on presence of any of the following abnormalities without an alternate explanation:
  - ALT or AST >8x ULN
  - ALT or AST >5x ULN for more than 2 weeks
  - ALT or AST >3x ULN and (TBL >2xULN or INR >1.5)
  - ALT or AST >3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

11.5.2 Excess GVHD toxicity

Excess GVHD toxicity will be defined as a > 25% incidence of Grade III-IV acute GVHD or severe chronic GVHD occurring at any time during treatment.

11.6 Stopping rules for safety

A Bayesian sequential monitoring rule will be implemented for safety. The stopping rule is defined as $Pr(\theta > \theta_T | data) > 0.8$, where θ denotes the proportion of toxicities (as defined in Section 11.5) and θ_T denotes the expected serious toxicity rate for study treatments (set as $\theta_T = 20\%$ for non-GVHD toxicity and 25% for GVHD toxicity). Interval analyses will be performed after every five evaluable patients (see 11.8 for patient evaluability). If the accumulated observed data suggests a high chance (i.e., >80% probability) for the "true" toxicity (as defined in Section 11.5) to be >20% for non-GVHD events or 25% for GVHD events, enrollment will be paused and data for assessment of early stopping will be sent to QASMC for review to consider study termination. The following tables show the number of patients with the number of adverse events that would result in a pause of enrollment to assess premature stopping of the trial. The stopping boundaries were obtained using Multc99 version 2.1, a free-download software from M.D. Anderson Cancer Center (https://trialdesign.org/one-page-shell.html#BTOX). Specifically, we assume that the parameter θ follows a prior distribution of beta (1,1), i.e., θ with mean = 0.5 but with relatively large variability (non-informative prior). Early stopping will be recommended if we observe 2 non-GVHD events out of the first 6 or 2 GVHD events out of first 5 patients, etc. (see Tables below), in patients treated in this trial. A simulation study is also performed to assess the operating characteristics for the above stopping rule (all scenarios are based on 10,000 simulated trials). For non-GVHD events, there will be only 15% chance to stop early if the "true" toxicity rate is 10%. In contrast, we will have 88% chance to stop early (with a median of 10 patients treated) if the "true" toxicity rate on flotetuzumab monotherapy is 30%. For GVHD events, there will be only 21% chance to stop early if the "true" rate is 15% and there will be 86% chance to stop early (with a median of 5 patients treated) if the "true" rate is 35%.

Stopping rules for non-GVHD toxicity:

# Patients treated	5	10	15	20	25
Stop if the # Grade ≥ 3 non-hematologic toxicity	2	3	4	5	6

Stopping rules for GVHD toxicity:

# Patients treated	5	10	15	20	25
Stop if the # Grade ≥ III aGVHD or severe cGVHD	2	4	5	6	8

11.7 Statistical analyses

The data will be analyzed in both intent-to-treat (ITT) and per-protocol (PP) approaches. The ITT cohort will include all patients who have received at least one dose of flotetuzumab. The PP cohort will only include evaluable patients. Patients who withdraw before completing Cycle 1 for a reason unrelated to drug toxicity will not be evaluable for the primary objective and will be replaced. The data analyses will be descriptive in nature. Demographic and clinical characteristics of the sample, as well as response to treatment, and loss to follow up will be summarized using descriptive statistics. The CR rate, overall response rate, PR rate, SD rate, morphologic leukemia-free state (MLFS) rate, and AEs that occur during flotetuzumab monotherapy and their 90% confidence intervals will be presented. Rate of CR, overall response rate, PR rate, SSD rate, MLFS rate, and AEs with subsequent treatment will also be documented with exact 90% confidence intervals. Overall survival (OS) and progression-free survival (PFS) will be described using Kaplan-Meier product limit method.

11.8 Analysis Populations

Safety analysis will include all patients who receive at least one dose of treatment drug.

Efficacy analysis will include patients who complete at least 1 cycle of treatment or patients who withdraw before completing Cycle 1 for a reason at least possibly related to drug toxicity.

12.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least one patient has been enrolled) or one year after accrual has opened (if no patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

13.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to Appendix C for definitions and Appendix D for a grid of reporting timelines.

Adverse events will be tracked from start of treatment through 28 days following discontinuation of study drug flotetuzumab. All adverse events—including adverse events associated with administration of DLI—must be recorded on the toxicity tracking case report form (CRF) with the exception of baseline adverse events, including any events related to allogenic hematopoietic cell transplantation (allo-HCT), which shall be recorded on the medical history CRF.

Refer to the data submission schedule in Section 9 for instructions on the collection of AEs in the EDC.

13.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

13.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Sponsor Investigator (or designee) is required to notify the QASMC of any unanticipated problems involving risks to participants or others occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

13.3 Reporting to MacroGenics, Inc.

Immediately reportable events (IREs) are events that must be reported immediately to MacroGenics Product Safety or designee within 24 hours of being identified. IREs include:

- SAEs
- Pregnancy in a study patient or partner (Note: If the female partner of a male patient becomes pregnant, the partner must be requested to complete a Pregnant Partner Consent Form so that pregnant partner, fetal and/or newborn information can be collected.) Upon confirmation of serum pregnancy testing, the patient will be followed for the outcome of pregnancy. All live newborns will be followed six months after the birth, and all necessary information will be collected to assess the effects of study drug on the newborn. If necessary, the follow-up period will be extended for the newborn.

See Appendix C for definitions of AE, SAE, SUSAR.

Contact information for MacroGenics Product Safety is below:

- **Email:** SAEReports@macrogenics.com **(preferred transmission) or**
- Fax: (301) 354-3800

13.4 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the

responsibility of the Washington University Sponsor-Investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix C for definitions) no later than **7 calendar days** after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix C) no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix C) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
  - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
  - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
  - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within **15 calendar days** after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Siteman Cancer Center Protocol Development team of each potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Siteman Cancer Center Protocol Development team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

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Acknowledgements
We would like to thank the Alvin J. Siteman Cancer Center at Washington University School of Medicine and Barnes-Jewish Hospital in St. Louis, Missouri, for the use of the Clinical Trials Core, which provided protocol development services. The Siteman Cancer Center is supported in part by an NCI Cancer Center Support Grant #P30 CA91842.

APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.

4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: Revised International Prognostic Scoring System (IPSS-R)

IPSS-R Prognostic Score Values							
Prognostic Variable	Score						
	0	0.5	1.0	1.5	2.0	3.0	4.0
Cytogenetics*	Very good		Good		Intermediate	Poor	Very poor
Bone marrow blast (percent)	≤2		>2 to <5		5 to 10	>10	
Hemoglobin (g/dL)	≥10		8 to <10	<8			
Platelets (cells/microL)	≥100	50 to <100	<50				
ANC (cells/microL)	≥0.8	<0.8					

*See cytogenetics in table below (MDS Cytogenetic Scoring System) for classifications.

MDS Cytogenetic Scoring System	
Prognostic subgroups	Cytogenetic Abnormalities
Very good	−Y, del(11q)
Good	Normal, del(5q), del(12p), del(20q), double including del(5q)
Intermediate	del(7q), +8, +19, i(17q), any other single or double independent clones
Poor	−7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex: 3 abnormalities
Very poor	Complex: > 3 abnormalities

IPSS-R Prognostic Risk Categories/Scores	
Risk Category	Risk Score
Very low	≤ 1.5
Low	>1.5 – 3
Intermediate	> 3 – 4.5
High	> 4.5 – 6
Very high	> 6

Taken from: Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sole F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454-65.

APPENDIX C: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event

- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study

APPENDIX D: Reporting Timelines

Expedited Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**MacroGenics, Inc.**
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	Report within 24 hours of being identified to SAEReports@macrogenics.com
Unexpected fatal or life-threatening suspected adverse reaction			Report no later than 7 calendar days after initial receipt of the information	Report within 24 hours of being identified to SAEReports@macrogenics.com
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Report via email after IRB acknowledgment		
Pregnancy				Report within 24 hours of being identified to SAEReports@macrogenics.com
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.			
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.			
Protocol exception	Approval must be obtained prior to implementing the change			

Expedited Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**MacroGenics, Inc.**
Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.			
Breach of confidentiality	Within 10 working days.			
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			

Routine Reporting Timelines			
Event	**HRPO**	**QASMC**	**FDA**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.
Minor deviation	Report summary information at the time of continuing review.		
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.		
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.		

APPENDIX E: Neurotoxicity Grading and Management

Grade 1	Grade 2	Grade 3	Grade 4
Decreased LOC, awakens spontaneously ICE score 7-9 (see table below for ICE scoring parameters)	Decreased LOC, awakens to voice ICE score 3-6	Decreased LOC, awakens only to tactile stimulus ICE score 0-2 Seizure: transient Cerebral Edema on imaging	Decreased LOC, unarousable ICE score 0 Seizure: prolonged (>5 minutes) or recurrent Cerebral Edema on imaging Focal neurologic weakness/deficits
Slow infusion by 10-20% Frequent neuro checks Supportive care <u>Consider</u> seizure prophylaxis (e.g. Levetiracetam 1000mg twice daily)	Slow infusion by 25-50% Obtain brain MRI, LP (with opening pressure) and EEG Neuro checks, including fundoscopy and Glasgow coma scale assessments Start telemetry and continuous SpO2 monitoring If concurrent CRS, administer Tocilizumab and <u>Consider</u> steroids per CRS algorithm <u>Administer</u> seizure prophylaxis, or increase dose if already started	Stop infusion Manage as Grade 2, plus: If concurrent CRS, administer Tocilizumab and <u>administer</u> steroids per CRS algorithm Consider ICU care	Manage as Grade 3, plus: Consider high dose steroids plus CRS algorithm management (e.g. Methylprednisolone 1000mg IV daily x3 days, then rapid taper over 6 days) Treat cerebral edema Transfer to ICU care with aggressive supportive care

LOC: level of consciousness; MRI: magnetic resonance imaging; LP: lumbar puncture; EEG: electroencephalogram

Immune Effector Cell-Associated Encephalopathy (ICE) Score

Orientation: oriented to year, month, city, hospital: 1 point each, 4 points total				
Naming: ability to name 3 objects (e.g. clock, pen, button): 1 point each, 3 points total				
Follow Commands: can follow a simple command (e.g. show me 2 fingers): 1 point total				
Writing: can write standard sentence (e.g. Our national bird is the bald eagle): 1 point total				
Attention: can count backwards from 100 by 10: 1 point total				
Scoring				
10 = no impairment	7-9 = ICANS grade 1	3-6 = ICANS grade 2	0-2 = ICANS grade 3	0 = ICANS grade 4

ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells (*Biol Blood Marrow Transplant 2019;25: 625*).

APPENDIX F: Acute GVHD Grading/Assessment

MAGIC CRITERIA FOR STAGING AND GRADING FOR ACUTE GVHD

Stage	Skin (Active Erythema Only)	Liver (Bilirbuin)	Upper GI	Lower GI (Stool Output/Day)
0	No active (erythematous) GVHD rash	< 2 mg/dL	No or intermittent nausea, vomiting, or anorexia	Adult: < 500 mL/day or < 3 episodes day Child: < 10 mL/kg per day or < 4 episodes/day
1	Maculpapular rash < 25% BSA	2-3 mg/dL	Persistent nausea, vomiting, or anorexia	Adult: 500-900 mL/day or 3-4 episodes day Child: 10-19.9 mL/kg per day or 4-6 episodes/day
2	Maculpapular rash 25-50% BSA	3.1-6 mg/dL	-	Adult: 1000-1500 mL/day or 5-7 episodes day Child: 20-30 mL/kg per day or 7-10 episodes/day
3	Maculpapular rash > 50% BSA	6.1-15 mg/dL	-	Adult: > 1500 mL/day or > 7 episodes day Child: > 30 mL/kg per day or > 10 episodes/day
4	Generalized erythroderma (> 50% BSA) plus bullous formation and desquamation > 5% BSA	> 15 mg/dL	-	Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume)

Overall Clinical Grade

Grade 0:
No stage 1-4 of any organ

Grade I:
Skin Stage 1-2 (without liver, upper GI, or lower GI)

Grade II:
Stage 3 rash and/or Stage 1 liver and/or Stage 1 upper GI and/or Stage 1 lower GI

Grade III:
Stage 2-3 liver and/or Stage 2-3 lower GI with Stage 0-3 Skin and/or Stage 0-1 upper GI

Grade IV:
Stage 4 skin, liver, or lower GI with Stage 0-1 upper GI

Acute GVHD Specifications	Chronic GVHD Specifications
• _____%BSA • Stool Output: _____mL/day OR episodes/day. • Grade: _____ • ECOG: _____	Does the subject have signs/symptoms of **Chronic** GVHD? _____ • If yes, please circle overall grade and describe: Mild / Moderate / Severe • Describe:_____ _____.

Signature Date

Date of Acute GVHD staging and grading	
Skin rash extent (% of BSA)	
Skin rash stage	□ Stage 0 □ Stage 1 □ Stage 2 □ Stage 3 □ Stage 4
Stool output (episodes per day)	
Stool output (estimated total volume in mL per day)	
Lower GI stage	□ Stage 0 □ Stage 1 □ Stage 2 □ Stage 3 □ Stage 4
Upper GI stage	□ Stage 0 □ Stage 1 □ Stage 2 □ Stage 3 □ Stage 4
Upper GI features due to GVHD	□ Persistent nausea □ Persistent vomiting □ Persistent anorexia □ None
Liver stage	□ Stage 0 □ Stage 1 □ Stage 2 □ Stage 3 □ Stage 4
MAGIC Criteria Overall Clinical Grade	□ Grade 0 □ Grade I □ Grade II □ Grade III □ Grade IV
Did the subject experience a GVHD flare since the last visit?	□ No □ Yes

APPENDIX G: Chronic GVHD Assessment (NIH Severity Score)

	0	1	2	3
Skin Score	❑ No Symptoms	❑ <18% BSA with disease signs but **NO** sclerotic features	❑ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	❑ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
Mouth Score	❑ No symptoms	❑ Mild symptoms with disease signs but not limiting oral intake significantly	❑ Moderate symptoms with signs with **partial** limitation of oral intake	❑ Severe symptoms with disease signs on examination with **major** limitation of oral intake
GI Tract Score (symptoms averaged over the last 3 days)	❑ No symptoms	❑ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	❑ Symptoms associated with mild to moderate weight loss (5-15%)	❑ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
Eye Score	❑ No symptoms	❑ Mild dry eye symptoms not affecting ADL (requiring eye drops ≤3x per day) **OR** asymptomatic signs of kerato-conjunctivitis sicca	❑ Moderate dry eye symptoms partially affecting ADL (requiring eye drops >3x per day or punctual plugs) **WITHOUT** vision impairment	❑ Severe dry eye symptoms significantly affecting ADL (special eyewear to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by kerato-conjunctivitis sicca
Joint and Fascia Score	❑ No symptoms	❑ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	❑ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	❑ Contracture WITH significant decrease of ROM **AND** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)

Genital Tract Score (score even if no GYN exam, required for men too) ❑ **No GYN Exam**	❑ No symptoms	❑ Symptomatic with mild distinct signs on exam AND no effect on coitus and minimal discomfort with GYN exam	❑ Symptomatic with distinct signs on exam AND with mild dyspareunia or discomfort with GYN exam	❑ Symptomatic WITH advanced signs (stricture, labia agglutination or severe ulceration) AND severe pain with coitus or inability to insert vaginal spectrum
Lung Score	❑ No symptoms	❑ Mild symptoms (shortness of breath after climbing one flight of steps)	❑ Moderate symptoms (shortness of breath after walking on flat ground)	❑ Severe symptoms (shortness of breath at rest; requiring O_2)
Liver Score	❑ Normal LFTs	❑ Elevated bilirubin, alkaline phosphatase, AST or ALT <2x ULN	❑ Bilirubin > 3 mg/dl or bilirubin, AST or ALT 2-5x ULN	❑ Bilirubin, AST or ALT > 5x ULN

Liver score to be completed using most recent LFTs from within +/- 2 weeks of the assessment

Date LFT sample obtained _____

PFT values from within one month of the assessment

% FEV1 _____% Date of FEV1 _____ ☐ Not done

% DLCOc _____% Date of DLCOc _____ ☐ Not done

Is an erythematous or maculopapular rash present? ☐ Yes ☐ No

Does the patient have nausea, vomiting or diarrhea? ☐ Yes ☐ No

Date of diagnosis of cGVHD _____

Previous assessment of NIH severity grade _____

Please rate the severity of this person's chronic GVHD											
on this scale →	❑ None (1)			❑ Mild (2)			❑ Moderate (3)			❑ Severe (4)	
and on this scale → *(circle one)*	cGVHD symptoms are not at all severe									cGVHD symptoms are most severe possible	
	0	1	2	3	4	5	6	7	8	9	10

APPENDIX H: Institutional Criteria for Donor Screening

Donor Guidelines
(adopted from Donor Screening Policy dated 5/2021, refer to this for complete guidelines)

Patients with SD/PR on their C1D28 response assessment may receive a DLI from their original donor at the discretion of the treating physician. Patients may receive cryopreserved cells from the original donation, or the previous donor must be willing to donate again. Donor selection will be in compliance with 21 CFR Part 1271 and with our institutional guidelines governing donor eligibility.

If requesting additional collections from an unrelated donor, donor assessment and consent process will occur in the apheresis center or similar donor facility. For matched related or haploidentical donors, donor assessment take place at Washington University School of Medicine. In cases where the donor medical evaluation occurred more than 6 months prior to re-donation, evaluation will be repeated per our institutional guidelines and will include the following testing:

- Complete health history and physical examination including vaccination history if greater than 6 months since original donor evaluation
- Required Tests:
  - CMP, CBC with manual differential, PT/PTT
  - HSV serology, qualitative HCG (Females of childbearing potential only)
  - BMT Donor Evaluation Test (panel includes the following tests: hepatitis B surface antigen, hepatitis C antibody, hepatitis B core total, Hep B NAT, HCV NAT, HIV NAT, WNV NAT, HIV 1-2, HTLV-I/II, *trypanosome cruzi* antibody, RPR, CMV titer. These tests must be performed by the American Red Cross).
  - Hemoglobinopathy Assessment: G-CSF mobilized, leukapheresis donors, must have a negative sickle cell test or be cleared for leukapheresis by the transplant physician.
  - ABO/Rh typing, red cell antibody screen

- All infectious disease testing must be done within seven days of an unstimulated DCI collection and within 30 days within a stimulated DCI collection
- A negative pregnancy assessment result is required within seven days prior to initiation of donor starting collection

Collection of unmobilized peripheral blood lymphocytes from donors is performed by leukopheresis. Unmanipulated donor cells are washed, counted, analyzed for viability, and cryopreserved.

APPENDIX I: Study-Specific DSM Tables

Protocol Objectives and Subject Evaluability	
Objective	**# of patients evaluable for this endpoint to date**
Primary	
To evaluate the efficacy of flotetuzumab in post-allo-HCT relapsed AML and MDS following Cycle 1 of flotetuzumab (Cycle 1 Day 28 assessment).	
Secondary	
To evaluate the efficacy of flotetuzumab monotherapy in post-allo-HCT relapsed AML and MDS following Cycle 2 (or following Cycle 1 for patients that receive only one cycle).	CR and CRi at C2D28 = ORR = MLFS = PR = SD = PFS = OS =
To evaluate the safety and tolerability of flotetuzumab +/- DLI in the post-allo-HCT setting	AEs = CRS and neurotoxicity = Acute GVHD = Chronic GVHD =

Interim Analysis and Early Stopping Rules
Does the study design include an interim toxicity analysis? No
Does the study design include an interim futility analysis? Yes
If yes, please insert text describing interim futility analysis from the protocol Fifteen patients will be enrolled in the first stage. This cohort will include patients who withdraw from the study for reasons unrelated to drug toxicity. If at least 2 CR/CRi are observed out of the 15 patients, then an additional 10 patients will be enrolled. If 6 or more CR/CRi are observed at the end of study, we would conclude that there is evidence of efficacy to warrant further investigation. There is less than 5% chance to stop the trial at stage 1 if the "true" response is 30% or higher. Conversely, there will be 55% chance to terminate the study early if the true rate is only 10% or less.
If yes, please provide data from the interim futility analysis when completed:
Are there early stopping rules that outline circumstances under which the study must be suspended or closed? Yes
If yes, please insert text describing early stopping rules from the protocol A Bayesian sequential monitoring rule will be implemented for safety. The stopping rule is defined as $Pr(\theta > \theta_T \mid data) > 0.8$, where θ denotes the proportion of toxicities (as defined in Section 11.5) and θ_T denotes the expected serious toxicity rate for study treatments (set as θ_T = 20% for non-GVHD toxicity and 25% for GVHD toxicity). Interval analyses will be performed

after every five patients has completed the first cycle of treatment (see tables below). If the accumulated observed data suggests a high chance (i.e., >80% probability) for the "true" toxicity (as defined in Section 11.5) to be >20% for non-GVHD events or 25% for GVHD events, enrollment will be paused and data for assessment of early stopping will be sent to QASMC for review to consider study termination. The following tables show the number of patients with the number of adverse events that would result in a pause of enrollment to assess premature stopping of the trial. The stopping boundaries were obtained using Multc99 version 2.1, a free-download software from M.D. Anderson Cancer Center (https://trialdesign.org/one-page-shell.html#BTOX). Specifically, we assume that the parameter θ follows a prior distribution of beta (1,1), i.e., θ with mean = 0.5 but with relatively large variability (non-informative prior). Early stopping will be recommended if we observe 2 non-GVHD events out of the first 6 or 2 GVHD events out of first 5 patients, etc. (see Tables below), in patients treated in this trial. A simulation study is also performed to assess the operating characteristics for the above stopping rule (all scenarios are based on 10,000 simulated trials). For non-GVHD events, there will be only 15% chance to stop early if the "true" toxicity rate is 10%. In contrast, we will have 88% chance to stop early (with a median of 10 patients treated) if the "true" toxicity rate on flotetuzumab monotherapy is 30%. For GVHD events, there will be only 21% chance to stop early if the "true" rate is 15% and there will be 86% chance to stop early (with a median of 5 patients treated) if the "true" rate is 35%.

Stopping rules for non-GVHD toxicity:

# Patients treated	5	10	15	20	25
Stop if the # Grade ≥ 3 non-hematologic toxicity	2	3	4	5	6

Stopping rules for GVHD toxicity:

# Patients treated	5	10	15	20	25
Stop if the # Grade ≥ III aGVHD or severe cGVHD	2	4	5	6	8

Definitions of Excess Toxicity

Excess non-GVHD toxicity will be defined as a combined incidence of > 20% of any of the following:

- ≥ Grade 3 non-hematologic AE at least possibly attributable to protocol treatment, with the following exceptions:
  - Grade 3 fatigue, asthenia, fever, constipation or anorexia (if anorexia does not result in hospitalization, tube-feeding or use of total parenteral nutrition [TPN])
  - Grade 3 nausea, vomiting or diarrhea not requiring tube feeding, TPN, or requiring or prolonging hospitalization
  - Infection, bleeding, or other expected direct complications of cytopenias due to active underlying leukemia

- Grade 3 infusion reaction including CRS, if successfully managed clinically and resolves within 7 days without end-organ damage
- Grade 3 or 4 isolated electrolyte abnormalities that last < 72 hours

- Grade 4 neutropenia or thrombocytopenia lasting ≥ 42 days from the start of the cycle in the absence of MDS/AML
- Evidence of drug-induced liver injury based on presence of <u>any</u> of the following abnormalities without an alternate explanation:
  - ALT or AST >8x ULN
  - ALT or AST >5x ULN for more than 2 weeks
  - ALT or AST >3x ULN and (TBL >2xULN or INR >1.5)
  - ALT or AST >3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

Excess GVHD toxicity will be defined as a > 25% incidence of Grade III-IV acute GVHD or severe chronic GVHD occurring at any time during treatment.

If yes, please provide data describing whether any patients have met the stopping rules:

UPN	Did pt experience GVHD toxicity? (y/n)	Did pt experience non-GHVD toxicity? (y/n)	If yes, enter toxicity and grade

Response						
UPN	**On treatment date**	**# cycles completed**	**C1D28 response**	**C2D28 response (or N/A)**	**Date of progression**	**Patient replaced? (y/n)**

Treatment Discontinuation and Survival				
UPN	**Off treatment date**	**Reason for discontinuation of treatment**	**Vital status**	**If dead, cause**

Aggregated GVHD Data			
# of pts who experienced aGVHD grades I/II	**# of pts who experienced aGVHD grades III/IV**	**# pts who experienced none/mild/moderate cGVHD**	**# pts who experienced severe cGVHD**

Summary of Specimen Collections			
Type of specimen	**Time point**	**# of patients eligible for collection at this time point**	**% of patients who have reached this time point and had the specimen collected**
Bone marrow aspirate	Screening/baseline		
	C1D28		
	C1D14		
	C2D14		
	C2D28		
	Relapse/progression		
Peripheral blood	C1D1		
	C1D7		
	C1D14		
	C1D21		
	C1D28		
	C2D1		
	C2D7		
	C2D14		
	C2D21		
	C2D28		
	Relapse/progression		