CLINICAL TRIAL PROTOCOL: CP-MGD006-01
PROTOCOL AMENDMENT 13

Study Title:	A Phase 1/2, First-in-Human, Dose Escalation Study of MGD006, a CD123 x CD3 Dual Affinity Re-Targeting (DART) Bi-Specific Antibody-Based Molecule, in Patients with Relapsed or Refractory Acute Myeloid Leukemia or Intermediate-2/High Risk Myelodysplastic Syndrome
Study Number:	CP-MGD006-01
Development Phase:	1/2
Product Name:	Flotetuzumab
Product Number:	MGD006 (S80880)
IND Number:	
EudraCT Number:	[redacted]
Indication:	Primary induction failure (PIF)/early relapse (ER) Acute Myeloid Leukemia (AML)
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Confidentiality Statement

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Study Number: CP-MGD006-01

This clinical study protocol has been approved by the Sponsor:

Signed: ***[See appended electronic signature page]*** Date: _____

SVP Clinical Development
Chief Medical Officer
MacroGenics, Inc.

Signed: ***[See appended electronic signature page]*** Date: _____

Executive Director, Biostatistics
MacroGenics, Inc.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

21CFR	United States Code of Federal Regulations, Title 21
ADA	anti-drug antibody
ADR	adverse drug reaction
AE	adverse event
AESI	adverse event of special interest
ALB	albumin
ALK-P	alkaline phosphatase
ALL	acute lymphoblastic leukemia
ALT	alanine aminotransferase
AML	acute myeloid leukemia
ANC	absolute neutrophil count
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BID	twice daily
BP	blood pressure
bpm	beats per minute
BUN	blood urea nitrogen
Ca	calcium
CAR	chimeric antigen receptor
CBC	complete blood count
CDR	complementary-determining region
CFR	Code of Federal Regulations
CI	confidence interval
CK	creatine kinase
Cl	chloride
C_{max}	maximum concentration
CNS	central nervous system
COVID-19	coronavirus disease-2019
CR	complete remission (mCR, CRc, or CRm)
CR1	initial CR
CRc	cytogenetic complete remission
CRh	complete remission with partial hematologic recovery
CRi	complete remission with incomplete blood cell recovery
CRm	molecular complete remission
CRn	CR with incomplete neutrophil recovery
CRp	CR with incomplete platelet recovery
CRS	cytokine release syndrome
CTCAE	common terminology criteria for adverse events
DLCO	carbon monoxide diffusion capacity in the lung

DLT	dose-limiting toxicity
DP	drug product
DSM	Data Safety Monitor
EBV	Epstein-Barr virus
EC	effective concentration
EC_{10}	concentration at 10% of the maximum effect
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EFS	event-free survival
ELISA	enzyme-linked immunosorbent assay
EMD	extramedullary disease
EOTV	End of Treatment Visit
ER	early relapse
E:T	effector to target ratio
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
FIH	first-in-human
FISH	fluorescence in situ hybridization
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GM-CSF	granulocyte macrophage–colony-stimulating factor
GvHD	graft versus host disease
Hct	hematocrit
HSCT	hematopoietic stem cell transplantation
Hgb	hemoglobin
HIPAA	United States Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IFN-γ	interferon gamma
IHC	immunohistochemistry
IL-3Rα	interleukin-3 receptor alpha chain
IND	Investigational New Drug
IPSS	International Prognostic Scoring System
IRB/IEC	Institutional Review Board/Independent Ethics Committee
IRE	immediately reportable event
IRR	infusion-related reaction
IUD	intrauterine device
IV	intravenous

IWG	International Working Group
K	potassium
LSC	leukemic stem cells
LVEF	left ventricular ejection fraction
mAb	monoclonal antibody
MABEL	minimum anticipated biological effect level
mCR	morphologic CR
MDS	myelodysplastic syndrome
MLFS	morphologic leukemia-free state
MRD	minimal residual disease
MTD	maximum tolerated dose
MTDS	maximum tolerated dose and schedule
MUGA	multiple-gated acquisition scan
Na	sodium
NCI	National Cancer Institute
NK	natural killer
NOAEL	no observed adverse effect level
NOD/SCID	non-obese diabetic/severe combined immunodeficient
OB	other benefit
OCRR	overall complete response rate
OS	overall survival
PBMC	peripheral blood mononuclear cell
PD	progressive disease
pDC	plasmacytoid dendritic cell
PFT	pulmonary function test
PIF	primary induction failure
PK	pharmacokinetics
PO	by mouth
PR	partial remission
PS	performance status
PT	prothrombin time, preferred term
qw	once weekly
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SC	subcutaneous
SD	stable disease
TCR	T-cell receptor
TEAE	treatment-emergent adverse event
TLS	tumor lysis syndrome
ULN	upper limit of normal
US	United States

USP	United States Pharmacopeia
WBC	white blood cell (count)
WHO	World Health Organization

1 SYNOPSIS

Sponsor: MacroGenics, Inc.	**IND Number:** 116832 **EudraCT Number:** 2015-003813-11
Name of Finished Product: Flotetuzumab, MGD006 (CD123 x CD3 DART protein)	
Study Title: A Phase 1/2, First-in-Human, Dose Escalation Study of MGD006, a CD123 x CD3 Dual Affinity Re-Targeting (DART) Bi-Specific Antibody-Based Molecule, in Patients with Relapsed or Refractory Acute Myeloid Leukemia or Intermediate-2/High Risk Myelodysplastic Syndrome	
Study Number: CP-MGD006-01	
Investigator(s)/Centers: The study will be carried out at approximately 50 institutions globally, preferably with experience in the conduct of studies in patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).	
Study Phase: 1/2	
Primary Objective: To assess the anti-neoplastic activity of flotetuzumab in patients with PIF/ER AML, as determined by the proportion of patients who achieve CR/CRh.	
Secondary Objectives: • Assessment of CR rate, CRh rate, overall complete response rate (OCRR; CR, CRh, CRi [CRn, CRp], or MLFS), objective response rate (CR, CRh, CRi [CRn, CRp], MLFS, or PR), time to response and duration of response (DOR). • To measure early mortality rates, overall survival (OS) and event-free survival (EFS). • To determine the rate of eligible patients, per institution criteria, that transition to successful stem cell transplant after achieving overall complete response (CR, CRh, CRi [CRn, CRp], or MLFS). • To assess rate of conversion to and maintenance of transfusion independence. • To evaluate duration of initial hospitalization for flotetuzumab administration. • To evaluate incidence and duration of hospitalizations subsequent to initial discharge. • To monitor the safety and tolerability of flotetuzumab. • To characterize the PK and immunogenicity of flotetuzumab. • To determine safety and efficacy of tocilizumab in the treatment of IRR/CRS.	
Exploratory Objectives: Refer to **Section 3.3** for complete list of exploratory objectives.	

Study Design:

This is an open-label, multi-dose, single-arm, multi-center, Phase 1/2, dose-escalation and expansion study to define an MTDS; describe preliminarily safety; and assess the PK, immunogenicity, immunomodulatory activity, and potential anti-neoplastic activity of flotetuzumab in patients with AML and MDS whose disease is not expected to benefit from cytotoxic chemotherapy.

This study is designed in three segments: the Single Patient Dose Escalation Segment (completed), followed by the Multi-patient Dose Escalation Segment (completed), and the MTDS Expansion Cohort Segment (ongoing). Two expansion cohorts were planned, one in AML and one in MDS. As of Amendment 7, the Sponsor stopped enrolling patients with MDS into this study. The primary objective of the study going forward is to assess anti-neoplastic activity of flotetuzumab in AML, as determined by the proportion of patients who achieve CR/CRh. Trial design aspects relating to continuation of the ongoing Cohort Expansion Segment, as of Amendment 11, are presented in this synopsis. For a summary of prior study development see **Section 2.3.2**.

During Cohort Expansion, flotetuzumab is dosed as a continuous infusion following a multi-step lead-in dose in Cycle 1 Week 1. With Amendment 11 all cycles of flotetuzumab, whether induction or consolidation, will be administered as a continuous infusion in 28 day cycles (see **Figure 5**).

Starting with Cycle 2, patients who are benefiting from flotetuzumab treatment may continue to receive additional cycles of treatment with flotetuzumab, up to a maximum of 8 cycles in total. Benefit will be defined as a response of CR, CRh, CRi (CRn, CRp), morphologic leukemia-free state (MLFS), and partial response (PR). Patients with evidence of PD at the end of Cycle 1 may receive one further cycle to establish evidence of response (treat one cycle beyond PD), if tolerated and at the discretion of the investigator, prior to determining treatment failure.

Flotetuzumab dosing during the Cohort Expansion Segment consists of 2 treatment phases, “Induction” and “Consolidation” (see **Figure 5**).

Induction is comprised of Week 1 multi-step lead-in dosing of 30, 60, 100, 200, 300, 400, and 500 ng/kg/day flotetuzumab for 24 hours each for Days 1 through 7, followed by 500 ng/kg/day continuous IV infusion from Days 8 to 28 (Cycle 1). Patients who do not achieve a CR or CRh after the initial induction cycle (Cycle 1) may receive additional cycles (Cycles 2 – 6) of induction therapy. Further induction therapy consists of flotetuzumab at a dose of 500 ng/kg/day as a 7-day continuous IV infusion each week in each 28-day cycle, which does not include the lead-in dosing as in Cycle 1. Treatment may continue in 4-week cycles until:

- attainment of a CR or CRh,
- no evidence of anti-leukemic activity (PR or better, defined as 50% decrease in BM blasts or a decrease to between 5 and 25% blasts in the BM) after at least 2 successive cycles,

- evidence of progressive disease after one subsequent cycle of flotetuzumab following disease progression at the end of Cycle 1 (one cycle beyond progression),
- evidence of disease progression after achieving a response of PR,
- transition to stem cell transplant,
- the occurrence of DLT,
- death,
- exercise of Investigator discretion, or
- withdrawal of consent by the patient.

Consolidation: Patients who achieve a complete remission with complete or partial hematologic recovery (CR or CRh) after the induction cycle(s) may then receive up to 2 cycles of consolidation treatment consisting of flotetuzumab at 500 ng/kg/day dosed as a 7-day continuous IV infusion each week of a 28-day cycle.

Based on the judgment of the investigator, patients who achieve a CRi may receive one additional cycle of induction (not to exceed a total of 6 induction cycles), and then be considered for transition to consolidation.

At any point during induction or consolidation therapy a patient may transition to receive a stem cell transplant, if eligible per individual institution criteria. Patients who have completed treatment and are scheduled for a stem cell transplant may continue to receive flotetuzumab treatment until the time of the transplant.

Treatment after Cycle 1 Day 8 (and after a minimum of 24 hours at the 500 ng/kg/day maximum dose) may take place in the outpatient setting based on the patient's tolerance of therapy.

Safety evaluations will also be conducted on an ongoing basis during the Cohort Expansion Segment of the study. Review by an Independent Data Monitoring Committee (IDMC, replacing the DSM previously involved in safety reviews), consisting of two physicians and one biostatistician, will be conducted in regular intervals. The interval analysis will utilize data from all cycles of treatment. If during the conduct of the interval cohort analysis, the Bayesian posterior probability is greater than 80% that the DLT event rate is more than 20%, enrollment will be paused and an ad hoc meeting of the IDMC will be held to consider dose reduction or study termination. The IDMC will also review aggregate efficacy data for two planned interim analyses to determine enrollment continuation according to futility and efficacy boundaries.

DLT will be defined employing the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 4.0 (CTCAE v4.0), except CRS which will be defined by the modified criteria proposed by Lee et al. (**26**) as described in **Section 6.4.1.2**.

DLT is defined as:

- Grade 4 neutropenia lasting ≥ 42 days from start of cycle in absence of evidence of active AML (< 5% blasts).
- Grade 3−5 nonhematologic toxicity not clearly resulting from the underlying disease and at least possibly related to protocol indicated treatment EXCEPT:
  - Grade 3 fatigue, asthenia, fever, anorexia, or constipation
  - Grade 3 nausea, vomiting or diarrhea not requiring tube feeding, total parenteral nutrition, or requiring or prolonging hospitalization, and resolve, with or without intervention, to ≤ Grade 2 levels in < 7 days will not be considered DLT
  - Infection, bleeding, or other expected direct complication of cytopenias due to active underlying disease
  - Grade 3 or 4 tumor lysis syndrome (TLS) if it is successfully managed clinically and resolves within 7 days without end-organ damage
  - Grade 3 or 4 isolated biochemical laboratory abnormalities (i.e., those occurring without clinical consequence) that resolve, with or without intervention, to ≤ Grade 2 levels in < 72 hours will not be considered DLT
  - Grade 3 rashes, myalgias or arthralgias that resolve within 96 hours and respond to medical intervention
  - Grade 3 infusion-related reaction or CRS that lasts < 72 hours and responds to medical intervention
- For patients in the ruxolitinib cohort, an increase of ≥ 2 CTCAE v4.0 grades in either hemoglobin or platelet count in patients while on ruxolitinib will also be considered a DLT
- Any treatment-related death

Study Population:

A total of 47 patients were enrolled in the completed Dose Escalation segments of the study.

A total of approximately 283 patients will ultimately be enrolled in the Cohort Expansion segment, including:

- approximately 271 patients treated with flotetuzumab at 500 ng/kg/day
- up to 12 patients in an exploratory mini-cohort treated with flotetuzumab at 500 ng/kg/day plus ruxolitinib

Patient Population/Key Entry Criteria:

The patient population to be enrolled will consist of adult patients with a confirmed diagnosis of primary or secondary AML that meet criteria for primary induction failure (PIF) or early relapse (ER) AML (see **Inclusion Criterion 2**). Patients must have an ECOG performance status ≤ 2, adequate hepatic and renal function, adequate organ reserve

sufficient to undergo therapy, recovery from toxicities of clinical consequence attributed to previous chemotherapy to CTCAE v4.0 Grade ≤ 1, and no serious concurrent illnesses that would increase the risk to the patient or confound the results of the study.

Test Product, Dose, and Mode of Administration:

Patients in Cohort Expansion will receive flotetuzumab as a multi-step-up lead-in dose in Week 1 Cycle 1. This comprises an initial dose of 30 ng/kg/day on Day 1, followed by doses of 60, 100, 200, 300, and 400 ng/kg/day on Days 2, 3, 4, 5, and 6, respectively. On Day 7, the final step up occurs to 500 ng/kg/day (defined as the MTD), given as a continuous infusion through Day 28 of Cycle 1. Throughout subsequent induction and consolidation cycles, dosing continues at 500 ng/kg/day as a 7-day continuous IV infusion each week of a 28-day cycle. Patients that have a dosing interruption > 72 hours will reinitiate dosing with a modified multi-step lead-in dose starting at 30 ng/kg/day (followed by steps up to 60, 100, 200, 300, 400, and 500 ng/kg/day) with dose changes every 4-6 hours as tolerated.

Duration of Treatment and Study Duration:

Patients who benefit as described above in the Study Design Section may continue to receive treatment with flotetuzumab following Cycle 1 for a maximum of up to 8 cycles in total.

The Single Patient Dose Escalation Segment of the study occurred over an 18-month period, and the Multi-patient Dose Escalation Segment of the study occurred over a 20-month period. The Cohort Expansion Segment of the study is expected to occur over an approximately 5-year period. End of study will occur after the last patient has received the last dose of study drug and has been followed until disease progression, stem cell transplant, withdrawal of consent, or until death, whichever occurs first, and the data collection process is complete (time of study database lock).

Total time for conduct of the trial is expected to be approximately 8 years. These estimates of timing for study conduct may vary from that observed in the actual conduct of the trial.

Criteria for Evaluation

Safety Assessments:

- Safety is based on evaluation of AEs and serious adverse events (SAEs) from the time of study drug administration until either 28 days after last administration of study drug or until the start of a subsequent systemic anticancer therapy, if earlier. SAEs considered related to study drug may be reported at any time, even after the patient's final visit. Additionally, protocol-related AEs and SAEs are collected from the time the patient has consented to study participation. Progression of the underlying neoplasm resulting in hospitalization or death (e.g., patient hospitalized for or dies from disease progression only, without any other SAE) will be documented as an efficacy outcome of the study and not as an SAE. If an SAE occurs in a patient and it is unclear if the event is due to progressive disease, the SAE should be reported.

PK and Immunogenicity Assessments:

- Pharmacokinetics: Serum concentrations of flotetuzumab will be monitored with frequent sampling during Cycle 1 and less frequent sampling thereafter for flotetuzumab using an electrochemiluminescence-based sandwich assay, and PK analyses will be performed using industry standard software. Population PK modeling will be performed, and an appropriate model and model parameters will be described.
- Immunogenicity: Anti-drug antibody (ADA) production will be monitored using a bridging enzyme-linked immunosorbent assay (ELISA). The proportion of patients who become positive in this assay will be reported.

Efficacy Assessments:

- Response Assessments: Disease activity will be assessed by the study investigators, by using CBC and peripheral blood cell morphological examination, examination of bone marrow aspiration (or biopsy if required), and physical exam. Modifications to the Revised Recommendations of the International Working Group (IWG) for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia will be used (**Appendix 3**). Response assessment will be based on bone marrow aspirate/biopsy and the best CBC up to 14 days post bone marrow aspirate/biopsy. Responses will be categorized as:
  - CR (mCR [morphologic CR], CRc [cytogenetic CR], or CRm [molecular CR]);
  - CRh (CR with partial hematologic recovery);
  - CRi (CR with incomplete blood count recovery): CRn (CR with incomplete neutrophil recovery) or CRp (CR with incomplete platelet recovery);
  - MLFS;
  - PR;
  - OB;
  - SD;
  - PD.

Other Assessments:

The following exploratory studies may be performed in all or a subset of patients:

- CD123 expression on leukemic blasts in bone marrow and peripheral blood at baseline and over time
- Flow cytometric analysis of bone marrow and peripheral blood cells to describe the status of the leukemia and monocytes, natural killer (NK) cells,

plasmacytoid dendritic cells (pDC), lymphocytes, and lymphocyte subsets and activation markers at baseline and over time

- Minimal residual disease (MRD)
- T-cell immune function (TCR repertoire, in vitro immune assessments)
- Transcriptomic bone marrow tumor microenvironment immune contexture
- Cytokine production at baseline and over time

Analysis Populations:

Three populations will be used for analysis, as defined below:

- **Efficacy population** – All patients that have been enrolled under Protocol Amendment 11 or later and treated with flotetuzumab at the MTD of 500 ng/kg/day as a continuous 7-day per week IV infusion during Cycle 1, have received any portion of one dose of flotetuzumab, and met the definition of PIF/ER AML based on inclusion criteria as revised in Amendment 11 (see **Section 5.1**). This population will be used for the primary analysis of efficacy endpoints.
- **Safety population** – All patients who received any portion of any dose of flotetuzumab. AML and MDS will be analyzed as pooled and separate populations for safety and PK. The analysis of all safety endpoints will be based on the safety population.
- **Response evaluable population** – All patients in the efficacy population that have a baseline bone marrow assessment, have at least one post-infusion assessment of their disease status or were removed from the study for reason of documented evidence of disease progression, death or treatment-related adverse event. This population will be used in the summary of response rate as a secondary analysis.

Statistical Methods:

Summary statistics will consist of absolute and relative frequencies of each category of discrete variables as well as of means, standard deviations, medians, minimum and maximum values of continuous variables.

A three-stage design with two interim analyses will be employed to test the null hypothesis (H0) of CR/CRh rate = 14.7% against the alternative hypothesis (H1) of CR/CRh rate = 23%. Demonstration of CR/CRh rate improvement (H1) by rejecting H0 at a 1-sided type I error of 0.025 with adequate power (80%) in a sequential design requires 170 patients. Specifically, the planned number of patients and the futility and efficacy boundaries at each stage are as follows:

a. **First Interim analysis:** The first interim analysis will be performed after 40 patients have been enrolled and either have had their responses evaluated or were discontinued for any reason from study treatment prior to their first response assessments. This interim analysis is for futility only, that is, the study enrollment will continue if at least 5 (12.5%) out of 40 patients have response of CR/CRh.

b. **Second Interim analysis:** If the enrollment continues after the first interim analysis, a second interim analysis will be performed after additional 60 patients have been enrolled (100 patients in total) and either have had their response evaluated or were discontinued for any reason from study treatment prior to their first response assessment. This interim analysis is for both futility and efficacy. Specifically,

    i. If the number of CR/CRh is less than 15 (15%) out of 100 patients, then the study is considered futile and enrollment will be stopped.

    ii. If the number of CR/CRh is 15 (15%) or greater out of 100 patients, then enrollment will continue.

    iii. If the number of CR/CRh is 25 (25%) or greater out of 100 patients, then H0 is rejected and the study is considered positive. The enrollment will still continue.

c. **Final Analysis:** If enrollment continues after the second interim, then the final analysis will be performed after additional 70 patients have been enrolled (170 patients in total) and either have had their response evaluated or were discontinued for any reason from study treatment prior to their first response assessments. If the number of CR/CRh is at least 35 (20.6%) out of the total of 170 patients, then H0 is rejected and the study is considered positive.

The above design yields a 1-sided alpha of 0.005 for the efficacy boundary at the second interim analysis and an overall 1-sided alpha of 0.024 with approximately 80% power at the final analysis. The probability of early termination under H0 (futility) in the first two stages is 55%.

The primary efficacy endpoint for the AML expansion cohort is the CR/CRh rate, calculated as the proportion of patients in the efficacy population that has achieved a best response of CR (mCR, CRc, or CRm) or CRh at any point during treatment by investigator's assessment, according to IWG AML response criteria (**Appendix 3**). CR/CRh rate will be summarized for the total combined PIF/ER patients as the primary analysis, as well as for PIF and ER patients separately, and for PIF/ER patients in the response-evaluable population as a sensitivity analyses. A two-sided exact 95% confidence interval will be calculated around response rates.

For the second interim analysis of 100 patients, as well as for the final analysis of 170 patients, all treated patients will be followed for at least 6 months or until death from any cause, whichever occurs first, prior to data cutoff for the analysis. If the trial passes the pre-specified efficacy boundary (25% observed CR/CRh rate) at the second interim analysis, a two-sided exact 95% confidence interval as described by Jennison and Turnbull (**21**) will

be produced to account for the bias in the conventional exact confidence interval for fixed sample test.

The key secondary efficacy endpoints include CR rate, CRh rate, duration of response, post-baseline transfusion independence rate, hematopoietic stem cell transplantation (HSCT) rate, and incidence and duration of hospitalization. Additional secondary efficacy endpoints include overall complete response rate, objective response rate, subgroup analyses of CR/CRh, CR, CRh and overall complete response rates, overall survival, event-free survival, time to response, mortality rate at 30, 60, 90, and 180 days, six-month and one-year survival. Counts, percentages, and two-sided exact 95% confidence intervals will be used to describe categorical secondary variables. Kaplan-Meier (KM) methods will be used to estimate time-to-event endpoints, unless otherwise specified. All secondary efficacy endpoints will be summarized in both efficacy and response evaluable population by PIF, ER, and total.

2 BACKGROUND INFORMATION

2.1 Disease Background

2.1.1 Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a heterogeneous group of diseases characterized by infiltration of the blood, bone marrow, and other tissues by neoplastic cells of hematopoietic origin (**47**). The disease occurs at a rate of 3.5 per 100,000 people per year in the United States (US). Incidence increases with age and the median age at onset is 67 years. Heredity, radiation, chemical and occupational exposures, and drugs (particularly chemotherapeutic agents) have been implicated in the etiology of AML. A number of chromosomal and molecular genetic abnormalities have been described in this disease and subclassification based on these abnormalities is now commonplace. Patients often present with nonspecific symptoms, particularly fatigue and/or unexplained fevers resulting from anemia, leukocytosis, leukopenia or leukocyte dysfunction, and/or thrombocytopenia. Initial physical findings may include splenomegaly, hepatomegaly, lymphadenopathy, sternal tenderness, bleeding or hemorrhage, and/or soft tissue infiltration. Diagnosis depends upon examination of peripheral blood elements or, more importantly, characterization of bone marrow cellularity and composition. Therapy for AML has changed little in the last 30 years. Standard induction therapy usually consists of the "7 + 3" regimen of cytarabine and daunorubicin. This is followed by consolidation therapy that may include hematopoietic stem cell transplantation (HSCT) in high-risk patients. However, outcomes from standard therapy are disappointing. Age limits aggressive treatment for many patients and older patients subjected to aggressive therapy have more resistant disease and a greater likelihood of early demise (**1**), and even in patients younger than 60 years, remission rates to standard induction therapy are only 65-75%. Half of those who do not reach complete remission experience a fatal complication related to bone marrow aplasia or impaired recovery of normal hematopoiesis and half have chemotherapy resistant disease. Even when patients enter complete remission, the occurrence of relapse is high. Salvage therapies, including stem cell transplantation, have limited impact, and most patients succumb to this disease in short order (**5**,**25**,**46**). New therapies are clearly needed.

Acute myeloid leukemia is thought to arise in and be perpetuated by a small population of leukemic stem cells (LSCs) (**4**). These cells are generally dormant, resist apoptosis, and are relatively resistant to conventional chemotherapeutic agents (**45**). They are characterized phenotypically as CD34+, CD38-. The CD34+, CD38- population of AML cells has been examined for other cellular characteristics. These cells were found to express high levels of CD123, the alpha chain of the interleukin 3 receptor (IL-3Rα), while the CD34+, CD38- fraction of cells from normal bone marrow demonstrated no detectable expression of CD123 (**24**). Based on these observations, it was suggested that targeting CD123 could be a promising strategy in the preferential ablation of AML cells. A monoclonal antibody (mAb) (7G3) targeting CD123 was shown to impair homing of AML-LSCs to bone marrow, reduce AML-LSC engraftment, and improve survival in a non-obese diabetic/severe combined immunodeficient (NOD/SCID) mouse model of human AML (**22**). However, in a Phase 1 study in high-risk AML patients sponsored by CSL Limited in Australia, the anti-CD123

mAb, CSL360, exhibited little anti-leukemic activity (**38**). The antibody was administered with an acceptable safety profile, i.e., little on-target toxicity, at doses from 0.1 to 10 mg/kg. CD123 saturation and down-regulation were accomplished and ex-vivo proliferative responses to IL-3 were eliminated at doses of 3 mg/kg. The investigators in the Phase 1 study suggested that re-engineering the mAb to improve its ability to mediate antibody dependent cellular cytotoxicity (ADCC) could result in an efficacious anti-leukemic agent. Such an agent is in Phase 1 testing. Other investigators have described the development and use, in preclinical models, of CD123 chimeric antigen receptor (CAR) containing T cells. These CAR T cells exhibited leukemic blast cell killing in vitro and anti-leukemic activity in a xenogeneic model of disseminated AML (**29**). While CAR T cells have shown promise in the treatment of patients with advanced chemotherapy resistant chronic lymphocytic leukemia (**34**,**41**), acute lymphocytic leukemia (**18**,**25**), and mantle cell lymphoma (**41**), these approaches are labor intensive, require transfection of autologous lymphocytes, and have proven to have a difficult-to-manage safety profile. MacroGenics believes that DART® molecules like flotetuzumab, described below may provide the benefits of CAR T cell therapy without many of the problems associated with the CARs.

2.1.2 Myelodysplastic Syndromes

Myelodysplastic syndromes (MDS) are a group of heterogeneous myeloid neoplasms characterized by ineffective hematopoiesis in one or more bone marrow lineages leading to abnormal proliferation and differentiation, with high risk of evolving into acute myeloid leukemia (**39**). The median age of diagnosis is 70 years old and there are an estimated 15,000 new cases a year in the US and 25,000 new cases a year in Europe (**28**).

Myelodysplastic syndromes are classified according to either the French-American-British (FAB) or newer World Health Organization (WHO) schemes with respect to morphological characteristics. Risk stratification is done using histopathological, chromosomal and clinical criteria. Using the International Prognostic Scoring System (IPSS) (**16**,**17**), one of three commonly used methods of risk stratification, patients are assigned either Low, Intermediate-1, Intermediate-2 or High Risk, with markedly different median OS. Patients with Intermediate-2 or High risk have median survivals of 1.2 and 0.4 years, respectively. In addition, the likelihood of evolution to frank AML increases with higher risk categorization.

National Comprehensive Cancer Network guidelines (**32**) for patients with Intermediate-2 or High risk MDS stratify patients by the suitability for stem cell transplant, this being the only available curative therapy. Patients who are not eligible for transplant are usually treated with a demethylating agent, but will relapse and overall have a poor outcome with a median OS of less than 6 months (**35**). The need for additional therapies is acute.

Expression of CD123 on stem cells in MDS has not been as extensively studied as in AML. Preliminary data with small patient numbers suggest that about 50% of samples assayed express CD123 on CD34+CD38- cells (**48**) and the proportion of CD123+ cells may increase with worsening disease (**50**). Together, these data suggest that CD123 is a reasonable target for a T-cell based targeted immunotherapy such as flotetuzumab.

2.2 Flotetuzumab Background

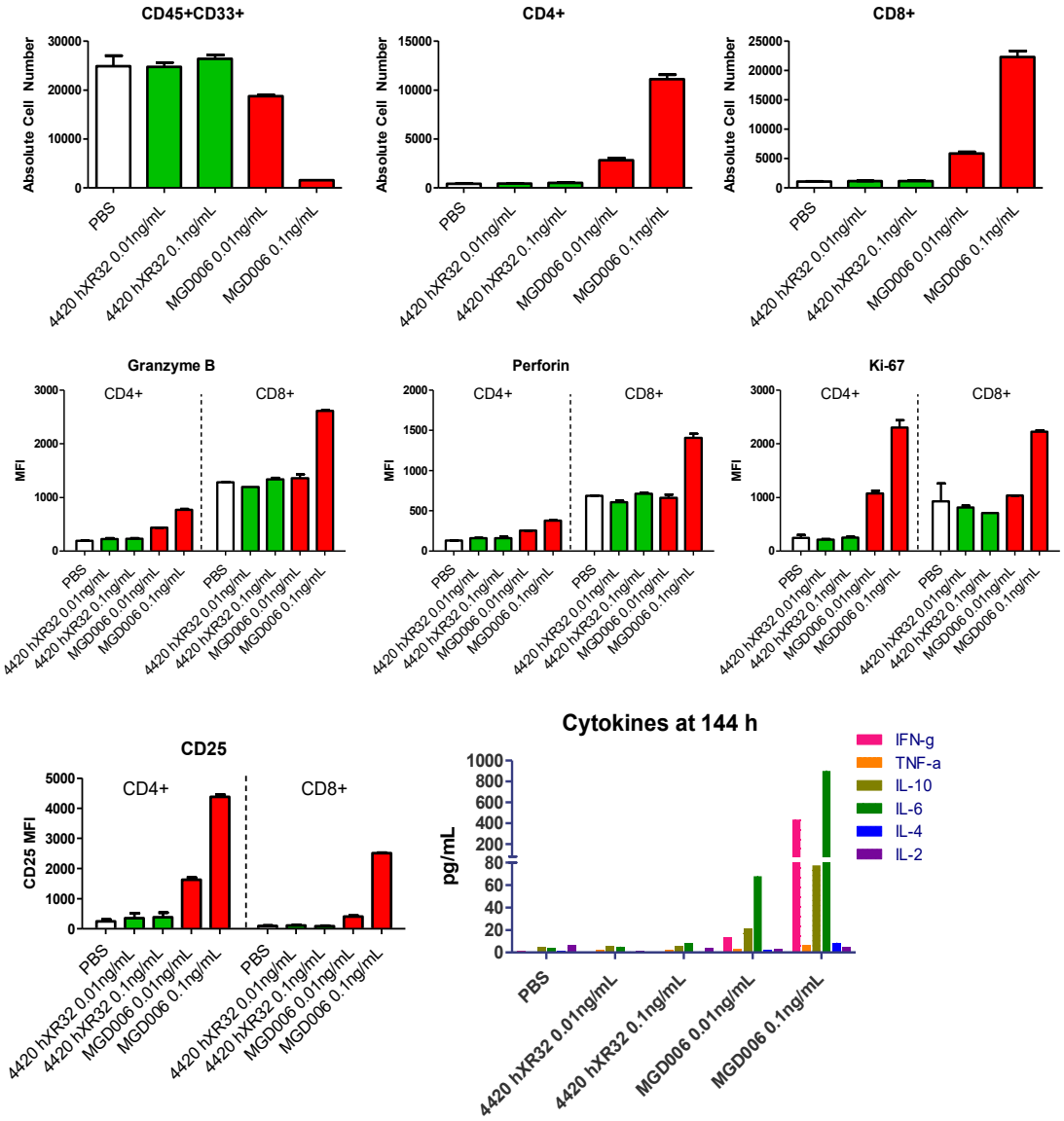
The DART technology is a novel, bispecific antibody platform designed to eradicate AML (or other tumor) cells through co-engagement of a leukemia- or tumor-specific cell surface marker (e.g., CD123) and the T-cell receptor (TCR)/CD3 complex on T cells as effector cells (**23**,**31**). Cell-association studies indicated that the DART protein architecture is well suited for maintaining cell-to-cell contact, apparently contributing to the high level of target cell killing. CD19 x TCR and CD19 x CD3 DART proteins have demonstrated in vitro killing of B-cell lymphomas by human T cells or peripheral blood mononuclear cells (PBMCs) (**23**) that exceeds the killing associated with a similar bispecific antibody construct, BiTE (**2**,**31**). The CD19 x TCR DART protein has also been used in in vivo tumor models and has demonstrated inhibition of B-cell lymphoma in NOD/SCID mice when co-administered with human PBMCs (**31**).

Flotetuzumab, also known as MGD006 and S80880, is a novel CD123 x CD3 DART protein developed by MacroGenics, Inc. Flotetuzumab is designed to target CD123-positive cells (including AML cells) for recognition and elimination by CD3-expressing T lymphocytes as effector cells (**7**,**23**,**31**).

In nonclinical studies, the ability of flotetuzumab to mediate redirected T cell killing was demonstrated across multiple CD123-expressing target tumor cell lines in vitro with efficacy generally correlating with the level of CD123 cell surface expression. Data from multiple experiments with T cells from different human donors have indicated that flotetuzumab-mediated redirected cell killing is potent. Flotetuzumab-mediated redirected killing was associated with a concomitant dose-dependent activation of T cells evidenced by upregulation of the activation markers CD25 and CD69 on CD4 and CD8 T cells. Dose-dependent upregulation of granzyme B and perforin levels in both CD8 and CD4 T cells also was observed. Upregulation of granzyme B and perforin was almost two-fold higher in CD8 T cells compared with CD4 T cells, suggesting that flotetuzumab-mediated target cell killing may be preferentially mediated through the granzyme B and perforin pathway by CD8+ T cells. Similarly, treatment of normal PBMC from healthy donors in vitro with flotetuzumab resulted in depletion of the CD123 expressing cells [i.e., plasmacytoid dendritic cells (pDCs) and monocytes in healthy PBMC] that was accompanied by a concomitant T cell activation markers (CD25 and CD69), upregulation of granzyme B and perforin, and secretion of cytokines.

In AML patient primary PBMC samples with very low E:T ratio (~1:300), flotetuzumab treatment resulted in near complete depletion of blast cells with concomitant expansion of residual CD8+ and CD4+ T cells. Expanded T cells exhibited greater Ki67 and CD25 expression suggesting T cell activation and proliferation. Additional evaluations performed to elucidate mechanism showed that expression of granzyme B and perforin was elevated in CD8+ T cells following flotetuzumab treatment, while a more modest elevation was observed on CD4+ T cells. Secretion of cytokines, predominantly interferon gamma (IFN-γ) and IL-6, were also observed following treatment with flotetuzumab (**Figure 1**). Additional in vitro data demonstrated productive T cell activation and re-directed killing by flotetuzumab is dependent upon co-engagement of both the T cell and the CD123-expressing target cell.

Figure 1 Flotetuzumab Activity in AML Patient PBMCs

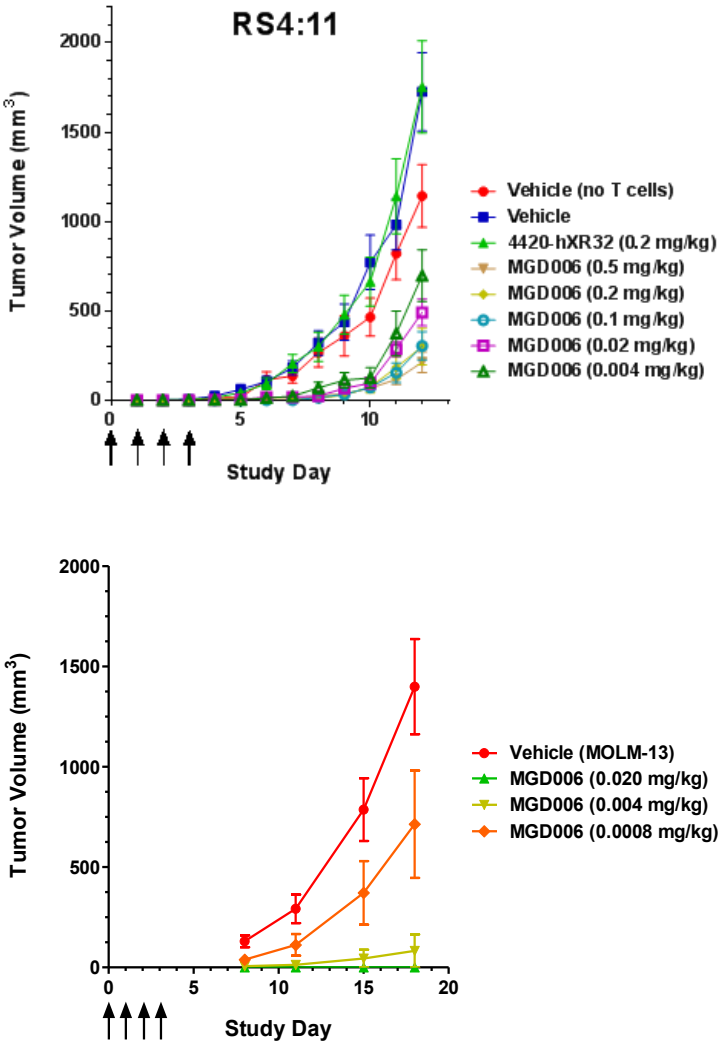


AML patient primary PBMCs were treated with flotetuzumab (MGD006), phosphate-buffered saline, or a control DART protein with an irrelevant anti-fluorescein arm and the same anti-CD3 arm as in flotetuzumab (4420-hXR32) for 144 hours. Absolute numbers of leukemic blast cells (CD45+/CD33+) and T cells (top panel) and mean fluorescence intensity of granzyme B, perforin, Ki67 (middle row), and CD25 (bottom left), cytokines measured in the culture supernatants after indicated treatments (bottom right) are shown.

Flotetuzumab also showed potent anti-tumor activity against human tumor cell lines implanted in mice. Flotetuzumab administered intravenously (IV) to NOD/SCID mice implanted with RS4:11 acute lymphoblastic leukemia (ALL) or MOLM-13 AML leukemia cells in the presence of human T lymphocytes resulted in dose-dependent inhibition of tumor

growth. The different dose sensitivity correlated with the relative level of CD123 expression, which was low on RS4:11 cells and intermediate on MOLM-13 cells (see **Figure 2**).

Figure 2 **Inhibition of Tumor Growth by Flotetuzumab in NOD/SCID Mice Implanted with Human AML Tumor Cells (RS4:11 and MOLM-13)**



Female NOD/SCID mice (n = 8/group) were implanted subcutaneous (SC) with 1 x 10^6 human T cells + 5 x 10^6 RS4:11 tumor cells on Day 0 followed by treatment with vehicle control, negative control DART protein or flotetuzumab (MGD006) on Days 0-3 (indicated by arrows on the x-axis) for a total of 4 doses administered IV. The negative control DART protein (4420-hXR32) has an irrelevant anti-fluorescein binding arm and the same anti-CD3 arm as in flotetuzumab. RS4:11 tumor volume was measured daily through Day 12 and MOLM-13 tumor volume was measured on Days 8, 11, 15, and 18 (mean ± SEM is shown). Results of the two-way ANOVA test showed that treatment with flotetuzumab significantly inhibited tumor growth ($p < 0.0001$).

Production of cytokines from T cells stimulated through their CD3 receptor is an anticipated side effect of this molecule. In the toxicology studies, cytokine release was predominantly a

first-dose effect that was mitigated by using a within-subject dose escalation strategy such that flotetuzumab was administered at an initial low dose and escalated stepwise on a weekly basis. A series of pilot toxicology studies were performed in which small groups of 1-4 animals were treated with doses of flotetuzumab ranging from 0.1 ng/kg/day to 5 µg/kg/day as either continuous 4-day on/3-day off infusions, or back-to-back, continuous 7-day infusions. In these studies, a starting dose of 5 µg/kg/day, the highest starting dose tested, was associated with severe acute cytokine release causing pallor, weakness, and depressed body temperature eventually leading to the death or unscheduled termination of the animals. A dose of 5 µg/kg/day, however, could be reached without cytokine storm through stepwise within-subject dose escalation. The pilot toxicology studies established the highest safe starting dose (100 ng/kg/day) and weekly escalation intervals (up to 1000 ng/kg/day in 3 steps) with manageable cytokine release.

The information gained from these studies led to the design of a Good Laboratory Practice (GLP) toxicology study, in which groups of 8 animals (4 male/4 female monkeys) were treated for 4 weeks with vehicle or flotetuzumab at doses of 100, 300, 600, or 1000 ng/kg/day using within group dose-escalation such that all flotetuzumab-treated groups received an initial dose of 100 ng/kg/day but the maximum dose in a group was escalated stepwise to 300, 600 or 1000 ng/kg/day. The GLP toxicology study included evaluation of both the 4-day on, 3-day off infusion schedule as well as the continuous 7-day infusion. At the dose regimen implemented in the GLP toxicology study, production of measured cytokines IFN-γ, TNF-α, IL-2, IL-4, IL-5 or IL-6 was generally not seen or minimal; most often IL-6, most commonly on the first dose, and rarely exceeding 100 pg/mL, returning to baseline levels prior to the subsequent dose (by 72 hours post start of infusion in the GLP toxicology study).

Anemia and thrombocytopenia appeared to be proportional to total exposure and was observed later during the dosing cycle. At the highest flotetuzumab exposure achieved in the GLP toxicology study (continuous 7-day infusion starting at 100 ng/kg/day and escalated weekly stepwise to 300, 600 or 1000 ng/kg/day without interruption), the degree of hematological toxicity observed was tolerable in this species without supporting measures (e.g., blood or platelet transfusions). The observed toxicity appeared reversible during the 4-week recovery period allowed in the study.

A dose-dependent, decrease in red cell mass parameters [mean hemoglobin (Hgb), hematocrit (Hct), and red blood cells (RBC)] was observed following administration of flotetuzumab at the 100 ng/kg/day or higher dose levels. As shown in the GLP study, the decrease persisted throughout the dosing phase and its magnitude increased with each escalating dose, except where the uniform presence of ADA in a group of animals may have mitigated the effect of flotetuzumab. The effect was generally modest, with the mean hemoglobin at nadir across the flotetuzumab-treated groups ranging from 9.6 to 11.6 g/dL. Two animals in had adverse drops in hemoglobin (below 7 g/dL) at dose levels ≥ 600 ng/kg/day. The effect on red cell mass was reversible, with parameters recovering in affected animals following cessation of dosing. The erythropoietic reticulocyte response appeared to function at all dose levels of flotetuzumab evaluated as a 4-day-on/3-day-off infusion, as reticulocytes increased with respect to both vehicle-treated control animals or compared to prestudy values. The reticulocyte response in animals with the greatest exposure to flotetuzumab (e.g., 7-day continuous infusion in Group

6 in the GLP study) was lower and not commensurate with the observed drops in red cell parameters.

Substantive platelet count variations were observed across all studies; generally, however, platelet levels remained higher than baseline levels and within the expected ranges for cynomolgus monkeys throughout the treatment period in animals that received flotetuzumab on a 4-day-on/3-day-off schedule (doses up to 1000 ng/kg/day). Some animals in the GLP study that received flotetuzumab as 7-day, escalating-dose infusions to up to 1000 ng/kg/day showed mild reversible, dose-dependent thrombocytopenia (lowest individual value 69,000/mm^3), although the group average never dropped below 200,000/mm^3. There was no evidence of petechiae or suffusions for any treated monkeys at any time during the course of the studies.

CD123 is not expressed (or expressed at very low level) by normal hematopoietic stem cells (HSCs); however, expression, albeit at low levels, occurs on committed hematopoietic precursors, such as common myeloid precursors (CMPs). The anemia observed following treatment with flotetuzumab at the doses and regimen employed in the GLP study is best explained by the cumulative effects of blood sampling and the flotetuzumab-mediated cytotoxic activity against hematopoietic precursors. Flow cytometric analysis of bone marrow samples from flotetuzumab-treated animals demonstrated a decrease in the frequency of CD123+ cells among the Lineage-negative immature bone marrow cells (~ 20% reduction to a near complete depletion in individual flotetuzumab-treated animals and mean group reductions of 64-80% in Groups 2-6), an effect that was reversible following cessation of treatment. This effect did not translate into morphological or myeloid/erythroid (M/E) ratio changes in bone marrow smears during the time frame of these studies, probably because the population of CD123-positive precursors is small. The blunted reticulocyte response observed in animals subjected to the greatest flotetuzumab exposure suggests a cytotoxic activity on a precursor (e.g., CMP) from which reticulocytes originate.

In all these studies, there was consistent evidence of the pharmacodynamic activity of flotetuzumab demonstrated by the full depletion of CD123-expressing pDCs at doses as low as 10 ng/kg/d; importantly, this effect was also reversible as pDCs were observed in peripheral blood a few weeks following cessation of dosing.

In active treatment groups (Groups 2-6), 28 out of 40 monkeys treated with flotetuzumab (Groups 2-6) developed anti-drug antibodies (ADA) against flotetuzumab by Day 22 (n = 16) or Day 29 (n = 12). The range of ADA titers was broad and the impact on flotetuzumab variable across individual animals. All animals were exposed to flotetuzumab through at least Study Day 19 (i.e., through at least 2 infusion cycles) and 24 of 40 animals were exposed to flotetuzumab during all four infusion cycles (including 12 ADA-positive animals). Moreover, pharmacodynamic activity was detectable even in the presence of ADA in some animals. The presence of ADA can also interfere with the detection of flotetuzumab in the PK assay, resulting in falsely low or undetectable flotetuzumab levels. Assessment of the specificity of the ADA in these animals indicated that ADA reactivity is predominantly directed to the humanized Fv sequences of flotetuzumab as opposed to other sequences (i.e., the linker sequence) in the DART molecule. Most ADA-positive animals (19/24, 79%) showed no or minimal reactivity to the linker sequences; of the 5 remaining animals, the reactivity to the

linker sequences was several-fold lower than the reactivity to the humanized Fv sequences. Consistent with its reactivity against the antibody paratopes, presence of ADA was not associated with exaggerated pharmacology, adverse clinical observations, increased cytokine release, or other safety signal. Immunogenic responses to human therapeutic proteins in cynomolgus monkeys do not predict immunogenicity in humans; in whom the antibody sequences would not be foreign. Therefore, these data suggest immunogenicity may be anticipated to be much less prevalent in humans.

In conclusion, flotetuzumab shows potent activity to redirect T cell killing against CD123-expressing cell lines and primary AML blasts in vitro. Flotetuzumab also demonstrated inhibition of growth of leukemic cell lines in mice and depletion of the CD123-positive pDC cells in the repeat-dose toxicology studies. The data from the nonclinical studies provide strong scientific rationale that an evaluation of the safety and potential activity of the CD123 x CD3 DART protein, flotetuzumab, in patients with AML whose disease is not expected to benefit from additional cytotoxic chemotherapy (as specified below), is warranted.

The trial will be conducted in accordance with this protocol, International Conference on Harmonization Guideline on Good Clinical Practice (ICH E6), and applicable regulatory requirements.

2.3 Rationale for Study

2.3.1 First-in-Human Dose Selection

Because the target of one arm of the flotetuzumab CD123 x CD3 DART protein is a component of the T cell antigen receptor/CD3 complex with the potential to promote T cell activation and potentially clinically significant cytokine production, careful consideration was given to the determination of the first-in-human (FIH) dose for this molecule.

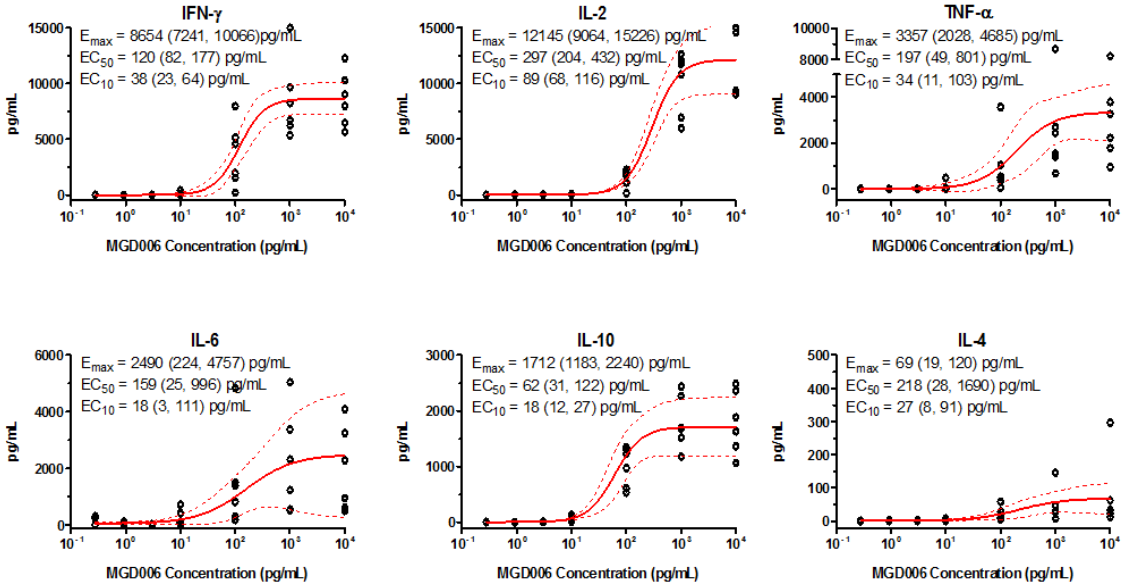
In an exploratory, non-GLP repeat-dose toxicology study in cynomolgus monkeys, significant cytokine production was observed only at the highest studied dose (5 µg/kg/day). This dose produced cytokine-related moribundity and mortality in the small number of animals employed in the study. Although the cynomolgus monkey is considered a relevant toxicological species on the basis of similar CD3 and CD123 antigen expression to which flotetuzumab binds with similar high affinities as it does the human antigens, it was considered prudent to also perform in vitro studies with human PBMCs, to ensure the potential of flotetuzumab to induce cytokine production from human cells was thoroughly evaluated prior to dosing in humans.

The determination of the starting dose for this study was based not only on the selection of a dose that is at least 10-fold less than the no observable adverse effect level (NOAEL) (which was determined to be 300 ng/kg/day in cynomolgus monkeys) from the toxicology studies, but also based on consideration of the minimal anticipated biological effect level (MABEL) of flotetuzumab.

The ability of normal human PBMCs to produce cytokines when placed in culture alone or with target cells expressing high density of CD123 in the presence of flotetuzumab was

examined experimentally. In vitro cytokine release assays were performed under two conditions: the initial set of experiments examined flotetuzumab-mediated cytokine release from human PBMCs from healthy donors. The number of PBMCs utilized in these experiments (200,000 cells/200 µL) was selected to represent the approximate density of cells in peripheral blood (about 1 x 10^6/mL), and the range of concentrations of flotetuzumab was selected to be representative of the steady state concentration level that would be predicted to exist in human serum after treatment with flotetuzumab at the planned dose range. A second set of experiments, shown in **Figure 3** below, was then performed using normal human PBMCs mixed with an equal number of Kasumi-3 target cells, an AML cell line with high CD123 surface expression. The PBMC + Kasumi-3 experiments utilized an excess of leukemic blasts (represented by Kasumi-3 cells) in combination with a physiologic number of normal T cells. This scenario simulates the situation that could be encountered in the AML patient in whom the presence of frequent target cells expressing a high antigen density is combined with a normal number of fully functional circulating T cells – presumably a "worst-case scenario" for cytokine production (highest likelihood of cytokine induction). In these experiments, IFN-γ, IL-2, TNF-α, and IL-6 were the most prominent cytokines produced (maximum plateau level of cytokine released, E_{max}) upon treatment of normal human donor PBMCs alone with flotetuzumab. Production of IL-10 and IL-4 was more modest. The addition of Kasumi-3 target cells to the PBMCs enhanced the production (E_{max}) of all cytokines tested, except for IL-4. Under these conditions, the enhancement of IL-2 and IFN-γ production was more pronounced compared to that of TNF-α, IL-6, and IL-10. Flotetuzumab EC_{50} and EC_{10} values were generally similar under both conditions tested for all cytokines evaluated.

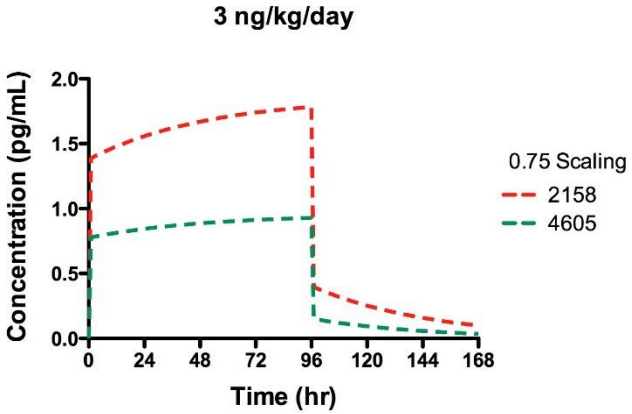
Figure 3 **Flotetuzumab-Mediated Cytokine Induction in Normal Human Donor PBMCs Co-cultured with Kasumi-3 Cells**



Dose-response curves for IFN-γ (top left), IL-2 (top middle), TNF-α (top right), IL-6 (bottom left), IL-10 (bottom middle), and IL-4 (bottom right) measured in the culture supernatants of normal human donor PBMCs mixed with Kasumi-3 cells (n=6) following incubation with flotetuzumab (MGD006) for 24 hours. Pooled data from 6 independent donors were curve fitted to a sigmoidal, 4-parameter dose response function, except for IL-6 that was fitted to a 3-parameter sigmoidal dose-response function (GraphPad Prism 5 software). Solid red lines represent the fitted curve; dotted red lines show the 95% confidence interval. For each cytokine, E_{max}, EC_{50}, and EC_{10} are reported as means and 95% confidence intervals (insets). Note different y-axis scales.

Both estimates place the dose resulting in 10% of the maximum effect (EC_{10}) for the most robust production of any cytokine at 14-18 pg/mL. Using a very conservative approach, taking the lower 95% confidence bound of the EC_{10}, a projected MABEL was estimated at 3 to 4 pg/mL. Allometric scaling from the non-GLP and GLP toxicology studies led to estimates of C_{max} in humans of 0.9-1.8 pg/mL for a 3 ng/kg/day dose of flotetuzumab (see **Figure 4**). Thus, with respect to cytokine production, the use of a dose of 3 ng/kg/day in humans would represent an approximate 2- to 3-fold safety factor from the MABEL.

Figure 4 **Projected Serum Concentration-time Profile of Flotetuzumab in Humans at a Dose of 3 ng/kg/day for 4 days Scaled from Monkey PK Parameters Obtained in Studies 4605 and Study 2158**



Two other estimates of the MABEL derived from in vitro redirected target cell killing assays and in vivo observations in cynomolgus monkeys treated with flotetuzumab yielded results similar to the MABEL estimate for cytokine production. First, in vitro redirected target cell killing assays yielded an estimate of an EC_{10} of 1 pg/mL, but this estimate of the MABEL resulted only with target cells expressing high density of CD123 (Kasumi-3) and T cells with a highly artificial, non-physiologic, effector target ratio of 10:1. Use of targets with lower CD123 density or more physiologic E:T ratios of 5:1 or 1:1 (which still represent high E:T ratios relative to the situation in the disease target) yielded EC_{10} values approximately 10 to 100 times greater than obtained with the 10:1 E:T ratio. Thus, the 1 pg/mL MABEL estimate based on redirected killing assays with a 10:1 E:T ratio is considered unrealistically conservative. Second, treatment of cynomolgus monkeys with flotetuzumab, resulted in depletion of peripheral CD123+ cells at a dose of 3 ng/kg/day (at an estimated, not measured, C_{max} of 0.43 – 0.84 pg/mL). However, in these in vivo experiments, CD123+ cell depletion would have been accomplished at an E:T ratio of ≈100:1 (assuming 6 CD123+ cells/μL and 600 T cells/μL). Reduction of CD123+ cells is a surrogate for a positive, rather than negative, effect in the patient.

Therefore, given the severity and lethality of the underlying disease being studied in the FIH trial and wishing to treat a minimal number of patients with potentially ineffective doses of flotetuzumab, we conservatively propose to use a starting dose of 3 ng/kg/day in the Single Patient Dose Escalation Segment of the study. The dose of 3 ng/kg/day in humans has a predicted serum C_{max} of 0.9-1.8 pg/mL based on allometric scaling (see again **Figure 4**) which is 2 to 4-fold less that the estimated MABEL concentration of 3 to 4 pg/mL based on flotetuzumab-mediated cytokine release in PBMCs, the major anticipated safety concern in humans.

2.3.2 Rationale for Overall Study Design and Summary of Prior Study Development

This study is designed in three segments: the Single Patient Dose Escalation Segment (completed), followed by the Multi-patient Dose Escalation Segment (completed) and the Maximum Tolerated Dose and Schedule (MTDS) Expansion Cohort segment (ongoing). In all segments of the study, flotetuzumab is administered using a series of continuous IV infusions administered on a weekly basis. Continuous infusion was chosen as the mode of administration for flotetuzumab because of its short half-life and the relative safety provided by the ability to reduce, interrupt or terminate the infusion with rapid elimination of serum concentrations in the event that cytokine-related adverse events (AEs) are encountered in the clinic, and experience of other experimental drugs in the class (**2**). A 4 days on/3 days off schedule was initially chosen over the 7-day-on schedule because it is the schedule with which the Sponsor has the most nonclinical experience, the schedule with the more limited apparent acute phase reaction, and the schedule which produced less severe and less prolonged anemia in the GLP toxicology study. Both the 4 days on/3 days off and the continuous 7-day infusion schedule are supported by pre-clinical toxicology data.

The primary objective of the dose escalation segments was:

- To characterize the dose-limiting toxicities (DLTs), and determine the maximum tolerated dose and schedule (MTDS) for the administration of flotetuzumab when given by continuous intravenous (IV) infusion for seven days in the first week, followed by infusion either for 4 days on/3 days off or 7 days continuously during the first of sequential 4-week cycles in patients with relapsed or refractory AML or intermediate-2/high risk MDS.

Secondary objectives of the dose escalation segments were:

- To describe, in preliminary fashion, the safety profile of flotetuzumab when administered by continuous IV infusion in 4-week cycles over a broad dose range.
- To characterize the pharmacokinetics (PK) and immunogenicity of flotetuzumab when given by continuous IV infusion over a broad dose range.
- To describe any evidence of anti-neoplastic activity in AML and MDS.
- To evaluate early mortality rates from any cause and overall survival.

The primary objective of the study going forward is to assess anti-neoplastic activity of flotetuzumab in AML, as determined by the proportion of patients who achieve CR/CRh (see **Section 3** for a full list of current objectives).

A summary of prior study development is provided in **Sections 2.3.2.1** to **2.3.2.3** below.

2.3.2.1 Single Patient Dose Escalation Segment (Completed)

In the initial Single Patient Dose Escalation Segment, four 1-patient mini-cohorts (constituting, in aggregate, Cohort 0) were used to examine the effects of one 4-week cycle of

very low doses of flotetuzumab, at doses of 3, 10, 30 and 100 ng/kg/day, administered on a 4 days on/3 days off schedule. This initial phase of the study was implemented to cautiously establish an acceptable starting dose for the second segment of the study, in view of the potential for systemic cytokine production with this T-cell activating molecule.

Each mini-cohort consisted of one patient unless that patient experienced a ≥ Grade 2 AE not clearly related to the underlying disease or ≥ Grade 3 infusion-related reaction (IRR, including cytokine release syndrome [CRS]) in the first 28 days of treatment. The appearance and severity of IRRs has been greatly reduced with the introduction of steroid medication prior to flotetuzumab administration **(Section 6.4.1.1)**; Grade 2 IRRs should be considered an acceptable toxicity in the context of flotetuzumab administration in this patient population. Any other AE ≥ Grade 2, or ≥ Grade 3 IRR or CRS necessitated expansion of the dose level with enrollment of an additional 3 patients at the same dose level. Use of the next dosing level occurred only if < 2 patients in the resulting 4-patient cohort experienced a DLT and all patients on that dosing sequence had completed the first 21 days of treatment (i.e., received at least 75% of planned dosing). Transition to the Multi-patient Dose Escalation Segment of the study was based on review of the aggregate safety data on all patients participating in Cohort 0.

2.3.2.2 Multi-Patient Dose Escalation Segment and Determination of MTDS (Completed)

The Multi-patient Dose Escalation Segment employed a classical 3 + 3 dose escalation study design including cohorts of patients with either AML or MDS. Prior to Amendment 5, all patients in this segment were to be treated with 100 ng/kg/day for 4 days on/3 days off in Week 1, followed by a step-up dose (to 300, 500, 700, 900, or 1000 ng/kg/day in Cohorts 1, 2, 3, 4, and 5, respectively) for 4 days on/3 days off in Week 2 and beyond. This step-up dosing followed the GLP toxicology study in which intra-monkey dose escalation appeared to enhance tolerability of flotetuzumab, apparently through reduction in cytokine release. Starting with Amendment 5, two modifications to the dosing scheme were made. The first was to include an additional step-up dose (two-step lead-in dose: 30 ng/kg/day for 3 days followed by 100 ng/kg/day for 4 days) during the first week of dosing to minimize the extent of IRR/CRS (cytokine release syndrome). The second was to evaluate, in parallel, a 7-day continuous infusion schedule in Weeks 2-4 during Cycle 1, to ameliorate IRR/CRS and AML circulating blast rebound noted with intermittent (4 days on/3 days off) dosing. The patients on the continuous infusion schedule were to receive the two-step lead-dose in Week 1, followed by 300, 500, 700, 900, and 1000 ng/kg/day in Cohorts 6, 7, 8, 9, and 10, respectively, in Week 2 and beyond.

The introduction of the two-step lead-in dose during Week 1 of Cycle 1 was justified by both the pre-clinical toxicology studies and the data observed during the evaluation of Cohort 0 and Cohorts 1 and 2 in the Multi-patient Dose Escalation segment. Nine of 10 of these patients treated with 100 ng/kg/day in the first week had Grade 1-3 IRR/CRS. Patients who went on to receive higher doses (300 ng/kg/day in Cohort 1 and 500 ng/kg/day in Cohort 2) still experienced IRR/CRS upon reintroduction of flotetuzumab in Week 2, but the severity was consistently the same or less than in week one despite the increase in dose. To fully

evaluate the effect of the two-step lead-in dose on the incidence and severity of IRR/CRS in Weeks 1 and 2, the Cohort 2 dose level (500 ng/kg/day in Weeks 2 and beyond) was re-evaluated using the two-step lead-in approach.

Evaluation of treatment by 7-day continuous infusion commenced at the 300 ng/kg/day dose level as this dose level was determined to be safe during dose escalation under Amendment 4 and was predicted to have approximately the same exposure and total dose administered as in Cohort 2 (500 ng/kg/day 4 days on/3 days off starting in Week 2), which had demonstrated acceptable tolerability. This was done in parallel with determination of the maximum tolerated dose (MTD) in the 4 days on/3 days off schedule. Dose escalation in the two schedules proceeded independently, although data from all treated patients was used for dose escalation decision making. With continuous 7-day infusion, the total dose administered was higher at any given dose level (**Table 1**). This correlates with the observed toxicity to date, IRR/CRS, which had been related to C_{max} (initially occurring within the first 24 hours) and not to total exposure.

Table 1 Planned Total Dose Administered in Cycle 1

Schedule	Dose Levels: Weeks 1/Weeks 2-4				
	Cohort 1 or 6 30 [1]-100/300	**Cohort 2 or 7 30 [1]-100/500**	**Cohort 3 or 8 30-100/700**	**Cohort 4 or 9 30-100/900**	**Cohort 5 or 10 30-100/1000**
Cohorts 1-10 (total dose Week 1)	Cohort 1: 400 ng [1] Cohort 6: 490 ng	Cohort 2: 400 and 490 ng [1] Cohort 7: 490 ng	490 ng	490 ng	490 ng
Cohorts 1-5 (total dose Weeks 2-4): 4 days on/3 days off	3600 ng	6000 ng	8400 ng	10800 ng	12000 ng
Cohorts 6-10 (total dose Weeks 2-4): 7-Day infusion	6300 ng	10500 ng	14700 ng	18900 ng	21000 ng

At the 300 ng/kg/day level (Cohort 1), no 30 ng/kg/day lead-in dose was studied for the 4 days on/ 3 days off schedule; at the 500 ng/kg/day level for the 4 days on/3 days off schedule (Cohort 2), Week 1 was studied ± the 30 ng/kg/day lead-in dose.

As of 28 July 2017, by agreement of the Sponsor's Medical Monitor, the Sponsor's Pharmacovigilance Physician, the independent Data Safety Monitor (DSM), and participating Investigators, the MTD in this study has been established as 500 ng/kg/day across both dosing schedules. The 7-day on schedule was selected to open the Cohort Expansion phase of the study, because it was tolerated and potentially offers better anti-leukemic activity. The recommended MTDS was therefore defined as initial two-step lead-in dose on Week 1 followed by 500 ng/kg/day administered by continuous intravenous infusion.

2.3.2.3 Cohort Expansion Segment (Ongoing)

Dosing Modifications

Implementation of the two-step lead-in dose with Amendment 5 decreased IRR/CRS severity compared to the single-step lead-in dose (**20**). However, data from a cynomolgus monkey toxicology study revealed that large step-up dose intervals were less well-tolerated than smaller increments (data presented in the flotetuzumab **Investigator's Brochure**). Hence, as of Amendment 8, the lead-in dose was modified from a two-step lead-in dose to a multi-step lead-in dose, comprised of sequential smaller and more frequent dose increments (30 to 60 to 100 to 200 to 300 to 400 to 500 every 24 hours) until reaching the MTD of 500 ng/kg/day. Although more involved, it was anticipated that this more conservative approach to intrapatient dose escalation may further attenuate IRR/CRS.

Prior to Amendment 9, all patients eligible to receive flotetuzumab beyond Cycle 1 received drug on a 4 days on/3 days off schedule in Cycle 2 and beyond. With Amendment 9, dosing was modified to specify 2 treatment phases, "Induction" (up to six 28-day cycles of continuous IV infusion [with multi-step lead-in dosing during Week 1 of Cycle 1] until response criteria were met), followed by "Consolidation" (up to two 28-day cycles of 4 days on/3 days off each week). With Amendment 11 all cycles of flotetuzumab, whether induction or consolidation, will be administered as a continuous infusion in 28 day cycles (see **Figure 5** and **Section 4.1**).

In summary, flotetuzumab is dosed as a continuous infusion based on data from the current trial, and centered around two basic premises. Firstly, in some patients that have not achieved full remission and are treated on intermittent dosing, rebound of peripheral blood AML blasts during the period off flotetuzumab has been observed; secondly, elimination of on-off interruptions in treatment with flotetuzumab may help further limit IRR/CRS.

Selection of Target Patient Population

Both relapsed or refractory AML and Intermediate-2/High Risk MDS patients in whom methylating agents have failed were evaluated in the initial phases of the study. CD123 is expressed on stem cells in both diseases, and both are treated similarly in clinical practice. As such, there is rationale for also evaluating treatment of MDS with flotetuzumab. Allowing both MDS and AML during dose escalation hastened identification of a safe dose to be evaluated in expansion. Given the similarity between MDS and AML in both clinical presentation and pathologic progression, it was expected that the two would behave similarly in response to flotetuzumab. As of Amendment 7, the Sponsor stopped enrolling patients with MDS into this study.

Published translational data (**44**) suggested that AML patients resistant to chemotherapy displayed an immune infiltrated bone marrow tumor microenvironment, leading to the hypothesis that immunotherapy could be effective in this population. The protocol therefore aimed to enrich the expansion cohort for patients with primary refractory disease (defined as, prior to Amendment 9, leukemia refractory to ≥ 2 induction attempts (primary induction failure; PIF), leukemia in first relapse with initial CR (CR1) duration < 6 months (early

relapse; ER), or prior treatment failure with at least four cycles of a hypomethylating agent). The initial 24 patients enrolled under Amendment 7 were restricted to 75% (i.e., 18/24) with primary refractory AML. Guided by response data from the first 24 patients, the Sponsor further restricted enrollment to PIF and ER (CR1 < 6 months) patients. Under Amendment 9, enrollment was restricted to patients with PIF AML (defined as leukemia refractory to ≥ 2 [or ≥ 1 for patients > 65 years of age] induction attempts, or leukemia in first relapse with initial CR1 duration < 6 months). Under Amendment 10, PIF AML was further defined as leukemia refractory to induction attempts, including but not limited to (1) leukemia refractory to ≥ 1 intensive induction attempt(s), per institution, or (2) for adults who are age 75 years or older, or who have comorbidities that preclude use of intensive induction chemotherapy, leukemia refractory to ≥ 2 lower intensity induction attempts; a maximum of up to 2 prior salvage attempts was allowed. With Amendment 11, primary refractory AML was redefined as patients with PIF or ER (see **Inclusion Criterion 2**). Patients treated solely with hypomethylating agents (HMA) have been excluded from the trial. Greater than 4 cycles of HMA are required to define HMA failure. Previously published data show that HMA treatment leads to upregulation of checkpoint molecules, including PD-L1 (**49**), and our clinical data suggest that PD-L1 expression decreases flotetuzumab activity (**37**).

Primary Efficacy Analysis

Based on study design and conduct, the MTD was defined at 500 ng/kg/day after noting DLT at 700 ng/kg/day. The MTD was also defined as the effective dose, as no complete responses (CR, CRh, CRi [CRn, CRp], or MLFS) were noted at doses below 500 ng/kg/day. The lead-in dose or 'priming' dose is designed to safely reach the MTD. While it is best achieved with a multi-step lead-in dose, patients treated with the two-step lead-in dose had still been able to achieve the dose of 500 ng/kg/day safely. Hence, patients treated at 500 ng/kg/day as a continuous infusion, preceded by either a two-step or multi-step lead-dose, may be included in the primary efficacy population as described below.

The sample size for the primary efficacy analysis is 170 patients, and will include all patients, irrespective of when they were enrolled in the study, that 1) were treated with flotetuzumab at the MTD of 500 ng/kg/day as a continuous IV infusion during Cycle 1, irrespective of two-step or multi-step lead-in dose; and 2) meet the definition of PIF/ER AML based on inclusion criteria as revised in Amendment 11 (see **Inclusion Criterion 2**).

2.4 Clinical Experience with Flotetuzumab

Details of the clinical experience with flotetuzumab may be found in the current version of the **Investigator's Brochure**.

2.5 Risk and Benefit Assessment

The patients expected to enroll in the CP-MGD006-01 study have primary induction failure or early relapsed AML. The administration of study drugs to patients with AML in the CP-MGD006-01 trial could potentially result in clinical benefit such as prevention of disease progression and longer survival.

In reference to safety concerns for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there is currently no known evidence of increased risk for SARS-CoV-2 infection or its exacerbation during treatment with flotetuzumab. No increased risk to patients is anticipated as a result of receiving SARS-CoV-2 vaccines, which are permitted during the study. MacroGenics' medical team will continue to review and monitor patient data and ensure patient safety at all times.

The Sponsor and the principal investigators will provide updates on the impact of prior or concurrent SARS-CoV-2 and the impact, if any, on patient safety as new information becomes available, and implement strategies to ensure patient safety and the validity of the trial.

3 STUDY PURPOSE AND OBJECTIVES

3.1 Primary Objective

To assess the anti-neoplastic activity of flotetuzumab in patients with PIF/ER AML, as determined by the proportion of patients who achieve CR/CRh.

3.2 Secondary Objectives

- Assessment of CR rate, CRh rate, overall complete response rate (OCRR; CR, CRh, CRi [CRn, CRp], or MLFS), objective response rate (CR, CRh, CRi [CRn, CRp], MLFS, or PR), time to response and duration of response (DOR).
- To measure early mortality rates, overall survival (OS) and event-free survival (EFS).
- To determine the rate of eligible patients, per institution criteria, that transition to successful stem cell transplant after achieving overall complete response (CR, CRh, CRi [CRn, CRp], or MLFS).
- To assess rate of conversion to and maintenance of transfusion independence.
- To evaluate duration of initial hospitalization for flotetuzumab administration.
- To evaluate incidence and duration of hospitalizations subsequent to initial discharge.
- To monitor the safety and tolerability of flotetuzumab.
- To characterize the PK and immunogenicity of flotetuzumab.
- To determine safety and efficacy of tocilizumab in the treatment of IRR/CRS.

3.3 Exploratory Objectives

- To evaluate CD123 expression on blast cells
- To evaluate circulating cytokine levels at baseline and over time
- To evaluate circulating leukemic and normal cells at baseline and over time
- To evaluate circulating T lymphocyte populations and activation markers at baseline and over time
- To evaluate leukemic cells, leukemic stem cells, normal progenitor cells and T lymphocytes at baseline and over time in the bone marrow
- To evaluate molecular markers of minimal residual disease (MRD)
- To examine changes in T lymphocyte repertoire
- To evaluate the correlation between cytogenetic abnormalities with responses to flotetuzumab immunotherapy.

- To study adaptive immune changes during flotetuzumab treatment.
- To study the tumor microenvironment (TME) immune contexture.
- **Ruxolitinib Cohort Objectives:**
  - To characterize the onset, duration, and severity of IRR/CRS on an exploratory, pilot basis in flotetuzumab-treated patients receiving ruxolitinib and compare that to historical experience in patients not receiving ruxolitinib.
  - To measure and compare cytokine profile and T-lymphocyte populations in patients receiving the combination of ruxolitinib and flotetuzumab vs flotetuzumab alone.
  - To determine the safety and tolerability of the combination of ruxolitinib and flotetuzumab.

4 TRIAL DESIGN

4.1 Overall Study Design and Plan

This is an open-label, multi-dose, single-arm, multi-center, Phase 1/2, dose-escalation and expansion study to define an MTDS, describe preliminarily safety, and to assess PK, immunogenicity, immunomodulatory activity, and potential anti-neoplastic activity of flotetuzumab in patients with AML and MDS whose disease is not expected to benefit from cytotoxic chemotherapy.

This study is designed in three segments: The Single Patient Dose Escalation Segment (completed), followed by the Multi-patient Dose Escalation Segment (completed), and the MTDS Expansion Cohort Segment (ongoing). Two expansion cohorts were planned, one in AML and one in MDS. As of Amendment 7, the Sponsor stopped enrolling patients with MDS into this study. The primary objective of the study going forward is to assess anti-neoplastic activity of flotetuzumab in AML, as determined by the proportion of patients who achieve CR/CRh. Trial design aspects relating to continuation of the ongoing Cohort Expansion Segment, as of Amendment 11, are presented in this section. For a summary of prior study development see **Section 2.3.2**.

A total of approximately 283 patients will ultimately be enrolled in the Cohort Expansion segment, including:

- approximately 271 patients treated with flotetuzumab at 500 ng/kg/day;
- up to 12 patients in an exploratory mini-cohort treated with flotetuzumab at 500 ng/kg/day plus ruxolitinib.

As previously stated (**Section 2.3.2.3**), during Cohort Expansion, flotetuzumab is dosed as a continuous infusion following a multi-step lead-in dose in Cycle 1 Week 1. With Amendment 11 all cycles of flotetuzumab, whether induction or consolidation, will be administered as a continuous infusion in 28 day cycles (see **Figure 5**).

Starting with Cycle 2, patients who are benefiting from flotetuzumab treatment may continue to receive additional cycles of treatment with flotetuzumab, up to a maximum of 8 cycles in total. Benefit will be defined as a response of CR, CRh, CRi (CRn, CRp), morphologic leukemia-free state (MLFS), and PR. Patients with evidence of PD at the end of Cycle 1 may receive one further cycle to establish evidence of response (treat one cycle beyond PD), if tolerated and at the discretion of the investigator, prior to determining treatment failure. Continuation beyond Cycle 1 in these patients is justified given the mechanism of action of flotetuzumab. As is known with other immunotherapies, responses may be delayed compared to those seen with cytotoxic chemotherapy. In this study, 3/21 (14%) patients who achieved a CR did so after an initial increase (PD) in BM blast counts following Cycle 1.

Flotetuzumab dosing during the Cohort Expansion Segment consists of 2 treatment phases, “Induction” and “Consolidation” (see **Figure 5**).

Induction is comprised of Week 1 multi-step lead-in dosing of 30, 60, 100, 200, 300, 400, and 500 ng/kg/day flotetuzumab for 24 hours each for Days 1 through 7, followed by 500 ng/kg/day continuous IV infusion from Days 8 to 28 (Cycle 1). Patients who do not achieve a CR or CRh after the initial induction cycle (Cycle 1) may receive additional cycles (Cycles 2 – 6) of induction therapy. Further induction therapy consists of flotetuzumab at a dose of 500 ng/kg/day as a 7-day continuous IV infusion each week in each 28-day cycle, which does not include the lead-in dosing as in Cycle 1. Treatment may continue in 4-week cycles until:

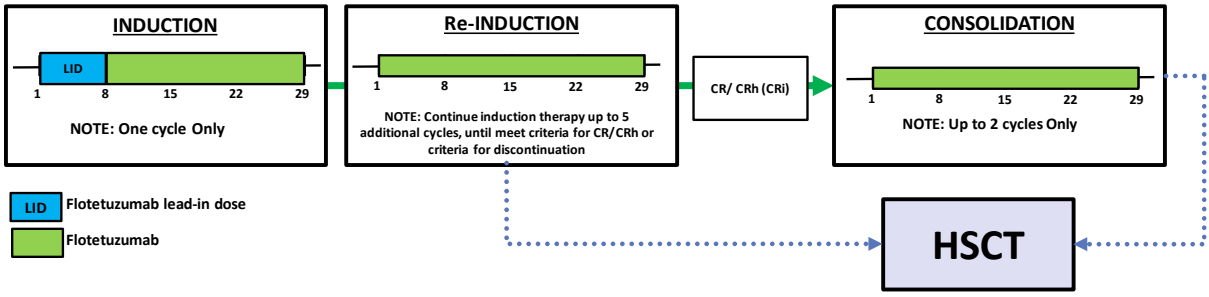
- attainment of a CR or CRh,
- no evidence of anti-leukemic activity (PR or better, defined as 50% decrease in BM blasts or a decrease to between 5 and 25% blasts in the BM) after at least 2 successive cycles,
- evidence of progressive disease after one subsequent cycle of flotetuzumab following disease progression at the end of Cycle 1 (one cycle beyond progression),
- evidence of disease progression after achieving a response of PR,
- transition to stem cell transplant,
- the occurrence of DLT,
- death,
- exercise of Investigator discretion, or
- withdrawal of consent by the patient.

Consolidation: Patients who achieve a complete remission with complete or partial hematologic recovery (CR or CRh) after the induction cycle(s) may then receive up to 2 cycles of consolidation treatment consisting of flotetuzumab at 500 ng/kg/day dosed as a 7-day continuous IV infusion each week of a 28-day cycle.

Based on the judgment of the investigator, patients who achieve a CRi may receive one additional cycle of induction (not to exceed a total of 6 induction cycles), and then be considered for transition to consolidation.

At any point during induction or consolidation therapy a patient may transition to receive a stem cell transplant, if eligible per individual institution criteria. Patients who have completed treatment and are scheduled for a stem cell transplant may continue to receive flotetuzumab treatment until the time of the transplant.

Figure 5 Induction and Consolidation Treatment in the Expansion Cohort



Treatment after Cycle 1 Day 8 (and after a minimum of 24 hours at the 500 ng/kg/day maximum dose) may take place in the outpatient setting based on the patient's tolerance of therapy.

Safety evaluations will be conducted on an ongoing basis during the Cohort Expansion Segment of the study. Review by an Independent Data Monitoring Committee (IDMC, replacing the DSM previously involved in safety reviews), consisting of two physicians and one biostatistician, will be conducted in regular intervals. The interval analysis will utilize data from all cycles of treatment. If during the conduct of the interval cohort analysis, the Bayesian posterior probability is greater than 80% that the DLT (see **Section 4.2**) event rate is more than 20%, enrollment will be paused and an ad hoc meeting of the IDMC will be held to consider dose reduction or study termination. The IDMC will also review aggregate efficacy data for two planned interim analyses to determine enrollment continuation according to futility and efficacy boundaries described in **Section 9.1**.

4.1.1 Cohort Expansion Dosing

The highest incidence and severity of IRR/CRS is usually noted in the first week of dosing, with peak incidence around 48 to 72 hours post start of dosing; IRR/CRS was not typically observed beyond the first week of administration. In view of this, patients who experienced Grade 1 or 2 IRR/CRS that required minimal intervention, i.e., only anti-pyretics or antihistamines and after consultation between the treating Investigator and the Sponsor, may receive flotetuzumab as outpatients after Cycle 1 Day 8 (and after a minimum of 24 hours at the 500 ng/kg/day maximum dose). Following a dosing interruption for > 24 hours for any reason, patients are required to be hospitalized for the first 24-48 hours of dose re-initiation for observation and management of IRR/CRS. Once no IRR/CRS ≥ Grade 2 is observed in a given patient, and they have otherwise demonstrated acceptable tolerability of the flotetuzumab regimen in the judgment of the Investigator, they may be considered for outpatient treatment without hospitalization for observation.

Flotetuzumab will be dosed using a multi-step lead-in dose approach for intrapatient dose escalation using multiple-step increments in dosing as follows: 30, 60, 100, 200, 300, 400 ng/kg/day each for 24 hours (± 2 hours). On Day 7, the dose will be increased to 500 ng/kg/day and administered as a continuous infusion for the remainder of Cycle 1 (**Table 2**). Cycle 1 Week 2 and beyond will follow the **Table 2** dosing schedule.

Patients that have a dosing interruption > 72 hours will reinitiate dosing with a modified multi-step lead-in dose starting at 30 ng/kg/day (followed by steps up to 60, 100, 200, 300, 400, and 500 ng/kg/day) with dose changes every 4-6 hours as tolerated.

Table 2 Cohort Expansion: Induction and Consolidation Dosing Schedule (Beginning with Amendment 11)

Cycle	Week	Days	ng/kg/day
Induction			
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
Additional Induction Cycles, if appropriate			
up to 5 cycles	1	Days 1 – 7	500
	2	Days 8 – 14	500
	3	Days 15 – 21	500
	4	Days 22 – 28	500
Additional Consolidation Cycles, if appropriate			
up to 2 cycles	1	Days 1 – 7	500
	2	Days 8 – 14	500
	3	Days 15 – 21	500
	4	Days 22 – 28	500

4.1.2 Concurrent Ruxolitinib Cohort within Cohort Expansion

Cytokine release syndrome (CRS) is a potentially life-threatening toxicity that has been observed following administration of natural and bispecific antibodies and adoptive T-cell therapies for cancer. CRS is believed to be the direct result of excess of inflammatory cytokines and is associated with elevated IFN-γ, IL-6, and TNFα levels with increases in IL-2, granulocyte macrophage–colony-stimulating factor (GM-CSF), IL-10, IL-8, IL-5, and fracktalkine/CX3CL1. Antibodies directed against IL-6, such as tocilizumab, have reduced the severity of CRS in patients receiving bispecific antibodies and CAR-T cell therapy, including patients treated on the current trial, where incorporation of two-step lead-in dosing and early intervention with tocilizumab has helped to ameliorate CRS in patients treated with

flotetuzumab. However, CRS may still occur despite the use of corticosteroids and anti-IL-6 therapy, which may limit the therapeutic window of novel immunotherapeutic agents. Disruption of cytokine signaling via Janus Kinase (JAK) pathway may represent a complementary approach to blocking CRS. JAK signaling involves recruitment of signal transducers and activators of transcription (STATs) to cytokine receptors, activation, and subsequent localization of STATs to the nucleus, leading to modulation of gene expression. Dysregulation of the JAK/STAT pathway is observed in patients with myeloproliferative neoplasms (**51**,**40**,**8**,**10**).

Ruxolitinib (INCB018424 phosphate, INC424, ruxolitinib phosphate) is a potent and selective inhibitor of JAK1 (inhibition concentration 50% [IC_{50}] = 3.3 ± 1.2 nM) and JAK2 (IC_{50} = 2.8 ± 1.2 nM) with modest to marked selectivity against TYK2 (tyrosine kinase 2) (IC_{50} = 19 ± 3.2 nM) and JAK3 (IC_{50} = 428 ± 243 nM), respectively, and is approved for the treatment of myelofibrosis and polycythemia vera. Ruxolitinib interferes with the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function. Data from preclinical murine models suggests that JAK inhibition blocks IFN-γ and IL-6 signaling, thus blocking the development of graft versus host disease while preserving the graft versus leukemia effect (**8**,**9**,**10**).

We hypothesize that disruption of IFN-γ and IL-6 signaling via the JAK/STAT pathway with ruxolitinib may reduce the frequency and severity of cytokine release syndrome in patients undergoing treatment with flotetuzumab. We propose to test this hypothesis by investigating a regimen consisting of prophylactic oral ruxolitinib in an exploratory cohort of patients with relapsed or refractory AML treated with flotetuzumab, to assess whether this can further ameliorate the occurrence and/or severity of CRS.

Overall, this cohort will enroll up to 12 patients at a single site (Washington University, St. Louis, MO) to test if the use of ruxolitinib mitigates IRR/CRS. Patients treated with strong CYP3A4 inhibitors or fluconazole will be excluded. An interim assessment by the medical monitor and investigators after 6 patients will be conducted to determine safety as well as effect on severity and incidence of IRR/CRS. Based on the toxicity profile, the dose of ruxolitinib may be increased to 20 mg twice daily (max recommended dose) (FDA package insert). The most common toxicities encountered with ruxolitinib include anemia and thrombocytopenia; hence, an increase of ≥ 2 CTCAE v4.0 grades in either hemoglobin or platelet count while on ruxolitinib will be considered a DLT in addition to the DLTs defined in **Section 4.2**. Initially, 6 patients will be treated with ruxolitinib at a dose of 10 mg twice daily on days 0-14 of Cycle 1. The dose of ruxolitinib used in the initial 6 patients will be 50% of the maximum recommended dose of ruxolitinib (package insert), as a precautionary measure. If < 2 out of 6 patients experience a DLT, as defined above, the dose will be escalated to 20 mg twice daily upon interim assessment and discussion between medical monitor and principal investigator. Six more patients will be treated at 20 mg twice daily on days 0-14 of Cycle 1. The cohort will be stopped and dose deescalated to 10 mg if 2 or more patients experience a DLT, as defined above, at 20 mg twice daily. If at any point during the ruxolitinib cohort, 4 or more patients experience a DLT, as defined above, the cohort will be stopped. Based on the mechanism of action of ruxolitinib and flotetuzumab and the observed and reported toxicities, it is not anticipated that the side effects will be additive. Most

toxicities observed with ruxolitinib are a consequence of chronic usage. Patients on the ruxolitinib cohort will only be treated for 15 days of Cycle 1.

4.2 Dose Limiting Toxicity Definitions

DLT will be defined employing the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 4.0 (CTCAE v4.0), except IRR/CRS which will be defined by the modified criteria proposed by Lee et al. (**26**) and described in **Section 6.4.1.2** and in **Table 5**. Note: IRR/CRS will be assessed by the grading of the overall IRR/CRS event, and not the grade of component signs or symptoms.

DLT is defined as:

- Grade 4 neutropenia lasting ≥ 42 days from start of cycle in absence of evidence of active AML (< 5% blasts).
- Grade 3−5 nonhematologic toxicity not clearly resulting from the underlying disease and at least possibly related to protocol indicated treatment, EXCEPT:
    - Grade 3 fatigue, asthenia, fever, anorexia, or constipation;
    - Grade 3 nausea, vomiting or diarrhea not requiring tube feeding, total parenteral nutrition, or requiring or prolonging hospitalization, and resolve, with or without intervention, to ≤ Grade 2 levels in < 7 days will not be considered DLT;
    - Infection, bleeding, or other expected direct complication of cytopenias due to active underlying disease;
    - Grade 3 or 4 tumor lysis syndrome (TLS) if it is successfully managed clinically and resolves within 7 days without end-organ damage;
    - Grade 3 or 4 isolated biochemical laboratory abnormalities (i.e., those occurring without clinical consequence) that resolve, with or without intervention, to ≤ Grade 2 levels in < 72 hours will not be considered DLT.
    - Grade 3 rashes, myalgias or arthralgias that resolve within 96 hours and respond to medical intervention.
    - Grade 3 infusion-related reaction or CRS that lasts < 72 hours and responds to medical intervention.
- For patients in the ruxolitinib cohort, an increase of ≥ 2 CTCAE v4.0 grades in either hemoglobin or platelet count in patients while on ruxolitinib will also be considered a DLT.
- Any treatment-related death.

4.3 Study Duration and Dates

The Single Patient Dose Escalation Segment of the study occurred over an 18-month period and the Multi-patient Dose Escalation Segment of the study occurred over a 20-month period.

The Cohort Expansion Segment of the study is expected to occur over an approximately 5-year period.

Total time for conduct of the trial is expected to be approximately 8 years. These estimates of timing for study conduct may vary from that observed in the actual conduct of the trial.

4.4 Definition of End of Trial

End of trial will occur after the last patient has received the last dose of study drug and has been followed until disease progression, stem cell transplant, withdrawal of consent, or until death, whichever occurs first, and the data collection process is complete (time of study database lock).

5 SELECTION AND WITHDRAWAL OF PATIENTS

5.1 Inclusion Criteria

Each patient must meet all the following criteria to be enrolled in this study. No exceptions to these criteria can be granted by the Sponsor.

1. Patients must have a confirmed diagnosis of primary or secondary AML (any subtype except acute promyelocytic leukemia [APL]) according to World Health Organization (WHO) classification.
2. Patients with AML must meet one of the following criteria, a or b:
    a. **Primary Induction Failure (PIF)** AML, defined as disease refractory to one of the following, i or ii:
        i. An intensive induction attempt, per institution.

            Induction attempts include high-dose and/or standard-dose cytarabine ± an anthracyclines/anthracenedione ± an anti-metabolite, with or without growth factor or targeted therapy containing regimens.

            Examples include but are not limited to:

            1. One cycle of high dose cytarabine (HiDAC) containing regimen
            2. One cycle of liposomal cytarabine and daunorubicin
            3. Two cycles of standard dose cytarabine containing regimen

        ii. For adults who are age 75 years or older, or who have comorbidities that preclude use of intensive induction chemotherapy; PIF is defined as AML refractory to one of the following less intensive regimens, 1 or 2:

            1. ≥ 2 but ≤ 4 cycles of Bcl-2 inhibitors in combination with azacitidine, decitabine, or low dose cytarabine
            2. ≥ 2 but ≤ 4 cycles of gemtuzumab ozogamicin monotherapy

    b. **Early relapse (ER)** AML, defined as AML in first relapse with initial CR1 duration < 6 months
3. Limit of 3 prior lines of therapy (excluding focal radiation therapy for palliative purposes): up to 2 induction (induction, re-induction) or 1 induction plus/minus 1 consolidation attempt, followed by a maximum of 1 salvage/re-induction attempt.
4. Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2.
5. Life expectancy of at least 4 weeks.
6. Peripheral blast count ≤ 20,000/mm^3 at the time of first dose of study treatment (see related **Exclusion Criterion 3**).
7. Adequate hepatic and renal function:

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. serum creatinine level ≤ 1.5 times the ULN or a calculated or measured creatinine clearance of ≥ 50 ml/min.

8. Adequate organ reserve including cardiovascular (left ventricular ejection fraction [LVEF] within institutional normal limits, if patient has a history or signs and symptoms of cardiac disease), 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.

    - During the coronavirus disease-2019 (COVID-19) pandemic, except in Germany, if PFT equipment is not available, a correlative stair-climbing test may be conducted instead, together with lung CT and echocardiogram irrespective of cardiac history. The patient must pass the stair-climbing test, with O_2 saturation ≥ 95%, lung CT with normal appearing lung parenchyma, and normal echocardiogram [see **Section 8.1.1**].

9. Normal thyroid function or stable thyroid tests on supplementation, except euthyroid sick syndrome.

10. 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).

11. 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.

12. Eighteen (18) years of age or older.

13. Ability to comply with study procedures, including hospitalization and protocol-specified acquisition of blood and/or bone marrow specimens.
14. Provide informed consent as documented by signing an IRB/IEC-approved informed consent document prior to execution of any study procedures not considered to be standard of care.

5.2 Exclusion Criteria

Patients who meet any of the following criteria will be excluded from the study. No exceptions to these criteria can be granted by the Sponsor.

1. Prior history of allogeneic stem cell transplantation.
2. Prior treatment with an anti-CD123-directed agent.
3. Need for concurrent other cytoreductive chemotherapy (use of hydroxyurea or other cytoreductive agents should have ceased by the time the study medication is begun (Cycle 1 Day 1) – however, hydroxyurea may be reinstituted at the discretion of the Investigator for the prevention or treatment of signs or symptoms of leukostasis, see **Section 6.6**), i.e., the target peripheral blast count for inclusion in the study should be ≤ 20,000/mm^3 at the time of first dose of study treatment.
4. 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).
5. Second primary malignancy that requires active therapy. Adjuvant hormonal therapy is allowed.
6. Antitumor therapy (chemotherapy, radiotherapy, antibody therapy, molecular-targeted therapy, retinoid therapy, or investigational agent) within 14 days or 5 half-lives of Cycle 1 Day 1, whichever is longer. Ruxolitinib is permitted for patients in the ruxolitinib mini-cohort.
7. Requirement, at the time of study entry, for concurrent steroids > 10 mg/day of oral prednisone or the equivalent, except steroid inhaler, otic preparations, nasal spray or ophthalmic solution.
8. Use of immunosuppressant medications (other than steroids as noted) in the 2 weeks prior to study drug administration (Cycle 1 Day 1).
9. Use of granulocyte colony stimulating or granulocyte-macrophage colony stimulating factor in the 2 weeks prior to study drug administration (Cycle 1 Day 1).
10. 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.

11. Any medical or psychiatric condition limiting full compliance or increasing the safety risk, such as:
    a. active uncontrolled infection (including, but not limited to viral, bacterial, fungal, or mycobacterial infection),
    b. known human immunodeficiency virus infection, unless all of the following criteria are met:
        i. CD4+ count ≥ 350 cells/μL,
        ii. undetectable viral load, and
        iii. receiving highly active antiretroviral therapy.
    c. known history of or current acute or chronic hepatitis B virus (HBV) infection (as evidenced by detectable HBV surface antigen and HBV DNA ≥ 500 IU/mL),
    d. history of hepatitis C virus (HCV) infection, unless the infection has been treated and cured,
    e. active SARS-CoV-2 infection. While SARS-CoV-2 testing is not mandatory for study entry, testing for ongoing infection should follow local clinical practice guidelines/standards. Participants with a positive test result for ongoing SARS-CoV-2 infection, known ongoing asymptomatic infection, or suspected ongoing infection are excluded from enrolling in the study. Such patients who are asymptomatic, or become asymptomatic, and who have a subsequent negative SARS-CoV-2 laboratory test may be enrolled.
    f. Grade 3 or 4 bleeding,
    g. significant pulmonary compromise including the requirement for supplemental oxygen, history of non-infectious pneumonitis (including radiation pneumonitis), pulmonary fibrosis, or severe chronic obstructive pulmonary disease (COPD),
    h. uncontrolled (persistent) hypertension (systolic pressure > 180 mm Hg or diastolic pressure > 100 mm Hg),
    i. clinically significant arrhythmia, or QTcF > 480 msec (ECG to be obtained in triplicate, patient to be excluded if average of 3 readings is > 480 msec),
    j. unstable angina,
    k. recent myocardial infarction within 6 months prior to study drug administration (Cycle 1 Day 1),
    l. clinically significant heart disease, such as, congestive heart failure, history of pericarditis, myocarditis,
    m. history of stroke or transient ischemic event within 3 months prior to study drug administration (Cycle 1 Day 1),

n. untreated pulmonary embolism, or non-catheter-related deep-vein thrombosis in the 3 months prior to study drug administration (Cycle 1 Day 1),

o. known adrenal insufficiency,

p. pregnancy, or breast feeding,

q. major surgery or trauma within 4 weeks before enrollment.

12. Known hypersensitivity to murine, yeast, or recombinant proteins; [redacted] recombinant human serum albumin; benzyl alcohol; or any excipient contained in the flotetuzumab drug formulation.
13. Dementia or altered mental status that would preclude sufficient understanding to provide informed consent.
14. Currently abuses drugs or alcohol that, in the Investigator's opinion, would cause the individual to be noncompliant.
15. Any investigative site personnel directly affiliated with this study.
16. Employees of MacroGenics, Inc.
17. Prisoners or others who are compulsorily detained.
18. Any reason that, in the opinion of the Investigator, would contraindicate the patient's participation in the trial or confound the results of the study.
19. Treatment with strong CYP3A4 inhibitors or fluconazole (ruxolitinib exploratory mini-cohort only).

5.3 Rationale for Exclusion of Certain Study Candidates

The Exclusion Criteria above include clinical situations that may prevent patients from completing the protocol, have an influence or effect on tolerance of study drug thus confounding the data analysis, or are serious conditions that pose an increased risk for morbidity and mortality while the patient would be participating in the study.

5.4 Withdrawal of Patient from the Trial or Study Drug

The Investigator may withdraw a patient from the study and/or from dosing for any of the following reasons. The Sponsor or its designee must be notified within 24 hours using the procedures outlined in **Section 7.12**:

- A violation of enrollment criteria or other significant protocol violation is discovered after a patient has enrolled and been treated,
- A serious, intolerable, or dose-limiting AE (as described in the DLT definitions above, **Section 4.2**) occurs,
- Patients who are symptomatic from, or require treatment for, SARS-CoV-2 active infection should be withdrawn from study drug. The Sponsor, in consultation with the investigator, may grant exceptions to this rule if the benefit:risk ratio of

continuing therapy with study drug outweighs the benefit:risk ratio of withdrawal from study drug (see **Section 6.3**),

- The Sponsor, Investigator, or regulatory agency terminates the study,
- The patient requests to be discontinued from the study,
- The Investigator, in his/her best medical judgment, decides that the patient should be withdrawn from the study or from dosing,
- If the patient becomes pregnant during the study, the patient must discontinue the study drug immediately. The health status of mother and child should be followed and reported after delivery,
- Patient has not achieved at least a PR (defined as decrease of bone marrow blast percentage to 5% to 25%, or decrease of pretreatment bone marrow blast percentage by at least 50%) following 2 cycles of flotetuzumab therapy,
- Patient with evidence of disease progression after achieving at least PR,
- Patient transitions to stem cell transplant.

6 STUDY TREATMENTS

6.1 Description of Treatment(s)

Flotetuzumab is the only study-specific investigational drug product to be administered in this study. Flotetuzumab will be administered as described in **Section 4.1.1**.

6.2 Treatments Administered

6.2.1 Flotetuzumab

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.

6.2.1.1 Drug Supply

6.2.1.1.1 Flotetuzumab

Flotetuzumab drug product (DP) is provided as a sterile aqueous solution

Drug product is supplied as a sterile aqueous solution packaged in a United States Pharmacopeia (USP) and Ph. Eur. conforming Type I borosilicate, 5 cc clear glass vial with a 20 mm FluroTec® and B2-40-coated 4432/50 gray butyl rubber serum stopper. The vial is sealed with a 20 mm TruEdge® aluminum closure with a plastic overseal.

6.2.1.1.2 IV Solution Stabilizer

Intravenous (IV) Solution Stabilizer is provided with flotetuzumab and used during dose solution preparation to coat the IV bag prior to the addition of flotetuzumab. This step helps ensure that the active drug product does not adhere to the IV bag and IV tubing. There are two custom IV Solution Stabilizers; which one of MGV002 or MGV004 IV Solution Stabilizer will be used for dose solution preparation and continuous infusion depends on country-specific Regulatory Authority requirements and approvals.

6.2.1.2 Drug Dose Solution Preparation and Administration

Flotetuzumab DP, and MGV002 IV Solution Stabilizer or MGV004 IV Solution Stabilizer will be supplied by the Sponsor for use in this study as appropriate to accommodate the method of study drug dose preparation and duration of continuous IV administration.

Parenteral drug products should be inspected visually prior to use. If discoloration or foreign particulates are observed, drug should not be administered. Some proteinaceous particles may be present; thus, a low protein binding 0.2 μm in-line filter must be used for administration of flotetuzumab.

Recommended safety measures for handling and preparation include masks, protective clothing, gloves, and vertical laminar airflow safety cabinet.

Flotetuzumab is administered to patients at exceedingly low doses.
All doses employed in this study are described in units of ng/kg/day.
Nanogram measurement is infrequently used in clinical practice.
[A nanogram (ng) is
1 billionth ($1/10^9$) of a gram (g),
1 millionth ($1/10^6$) of a milligram (mg); and
1 thousandth ($1/10^3$) of a microgram (μg)]
Errors in dilution could result in fatal Cytokine Release Syndrome (CRS).
Every reasonable precaution should be exercised
in the preparation, verification, and administration of the flotetuzumab dose.

Independent verification of patient weight and of the calculated dose must be carried out and documented by a second individual.
Similarly, the administration pump settings should be independently reviewed and documented by a second individual
before study drug administration commences.

Due to the low concentration of flotetuzumab in prepared doses, MGV002 IV Solution Stabilizer or MGV004 IV Solution Stabilizer is used during dose solution preparation to coat the IV bag and reduce adsorption and minimize losses of active drug during administration.

Flotetuzumab is administered by IV infusion using a single ambulatory pump, or standard IV infusion pump. Instructions for the dose solution preparation and administration of flotetuzumab are detailed in the Pharmacy Manual.

General Administration Information:

Flotetuzumab must not be administered as an IV push or bolus. During the Cohort Expansion Phase, at least for the first 8 days of Cycle 1 (and after a minimum of 24 hours at the 500 ng/kg/day maximum dose), the drug will be administered in an inpatient hospital setting; but afterwards may be administered in an outpatient setting as described in **Section 4.1.1**. The type of pump used for administration of flotetuzumab may differ by country or region. It is important that at any point, THE INFUSION BAG CONTAINING FLOTETUZUMAB MUST NOT BE SHAKEN; excessive agitation may cause aggregate formation. Subsequent to Cycle 1 Day 8 (and after a minimum of 24 hours at the 500 ng/kg/day maximum dose), patients may, as appropriate (see **Section 6.3**), receive flotetuzumab as a continuous infusion in an outpatient setting using an ambulatory pump configuration. Specific instructions for dose preparation and administration in both the inpatient and outpatient settings are provided in the Pharmacy Manual.

Sites must ensure patients who receive flotetuzumab in the outpatient setting will be provided instructions and trained appropriately and monitored for compliance and safety.

Flotetuzumab should not be admixed or diluted with other drugs. Any partially used vials or diluted dosing solutions should be discarded using appropriate drug disposal procedures.

6.3 Modification (Including Discontinuation) of Flotetuzumab Dose or Schedule

Interruption or discontinuation of dose or schedule may be necessitated by the observation of suspected drug-associated or drug-related adverse events (AEs). The Investigator should use his/her best medical judgment to intervene in order to safeguard the patient's welfare. The following guidelines should be observed:

In general, observation of any ≥ Grade 3 AE (by CTCAE v4.0), except IRR/CRS which will be by the modified criteria proposed by Lee et al. (**26**) considered related to flotetuzumab should result in suspension of flotetuzumab study drug administration; for patients in the mini-cohort, ruxolitinib administration should also be suspended. Reinstitution of assigned

flotetuzumab study drug administration, and ruxolitinib administration for patients in the mini-cohort, should occur if the AE meets one of the exceptions to the definition of DLT (see **Section 4.2**). Otherwise, the patient should be withdrawn from further study drug administration unless there are extenuating circumstances, and the Investigator concludes that the patient is continuing to derive clinical benefit.

If after reinstituting flotetuzumab for a first occurrence of a ≥ Grade 3 AE (as exempted by the DLT definition), the patient experiences a second (either similar or different) ≥ Grade 3 AE, study drug administration should once again be suspended. As before, reinstitution of any study drug administration should be delayed to see if the AE meets one of the exceptions to the DLT definitions. If so, the Investigator may elect to reinstitute flotetuzumab; patients that have a dosing interruption > 72 hours will reinitiate dosing with a modified multi-step lead-in dose starting at 30 ng/kg/day (followed by steps up to 60, 100, 200, 300, 400, and 500 ng/kg/day) with dose changes every 4-6 hours as tolerated. If the observed AE is associated with autoimmunity, the study drug should be discontinued.

If the second ≥ Grade 3 AE meets the definition of DLT, or a third instance of a ≥ Grade 3 AE occurs, the Investigator should consider the patient to have experienced DLT and the patient should be withdrawn from further study drug administration (see **Section 5.4**).

Signs and symptoms of active viral infections may overlap with the signs and symptoms of CRS (e.g., fever, hypoxemia). The management of CRS in the setting of active coronavirus infections could theoretically potentially confound the course of the viral infection. The following guidelines apply to participants with confirmed (by a laboratory test) or presumed (test pending/clinical suspicion) SARS-CoV-2 infection:

- Study drug should be stopped in patients who are symptomatic from, or require treatment for, SARS-CoV-2 active infection. The Sponsor, in consultation with the investigator, may permit study drug to be restarted, on a case-by-case basis, if the benefit:risk ratio of continuing therapy with study drug outweighs the benefit:risk ratio of withdrawal from study drug.
- Prior to restarting study drug, when stopped for SARS-CoV-2 infection, participants should be afebrile for at least 72 hours, and SARS-CoV-2–related symptoms should have recovered to ≤ Grade 1 or baseline for a minimum of 48 hours.
- The Sponsor should be informed when restarting flotetuzumab.

When dosing is delayed for ≥ 24 hours, continue to follow the Schedule of Events in Appendix 1 and Blood Sampling Schedule in Appendix 2, including on days when no study drug is administered (with the exception of samples for PK, which should not be collected on days when no study drug is administered) or when lead-in dosing escalation is needed to restart flotetuzumab. If a delay of ≥ 24 hours during any dosing period occurs, or if less than 75% of the intended dose will be administered if the original schedule is followed, the MacroGenics Medical Monitor should be consulted as to whether the total infusion time should be extended to allow sufficient study drug to be administered.

6.4 Special Situations and Supportive Care Measures

6.4.1 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 which may have anaphylactoid features.

Patients should be monitored closely for the development of IRRs during flotetuzumab infusion. 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 (including SARS-CoV-2), 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 7.11.2**) to the infusion of study drug, the study drug may be reinstituted at the

same rate of administration after administration of ibuprofen or acetaminophen and diphenhydramine hydrochloride if not administered previously for premedication. More complex symptom composites may require additional intervention. Please refer to **Section 6.4.1.2** for management of infusion reactions if the event is considered related to flotetuzumab. If during assessment of IRR/CRS a patient undergoes imaging study and/or sample collection, e.g., pleural fluid, intra-articular joint aspiration, or ascitic fluid, a sample may be provided to the Sponsor to help elucidate etiology.

6.4.1.1 Premedications and Prophylaxis

Based on the clinical experience to date **(Section 2.4**), similar findings from other bispecific molecules, such as blinatumomab, reported clinical experience with CAR-T therapies, and after consultation with the study investigators and the prior independent DSM (now replaced by the IDMC), all patients treated in clinical protocol CP-MGD006-01 will receive premedication to prevent or mitigate potential infusion-related reactions. Specific pre-treatment regimens are described below. **Table 4** presents the premedication and prophylaxis regimens.

Prior to the first dose and during Cycle 1 Week 1, all patients are to be treated as described in **Table 4** below. Patients should receive adequate hydration during study therapy, preferentially oral if tolerated. 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.

For infusion-related reactions during the course of treatment, supportive care measures should be implemented as outlined in **Section 6.4.1**.

Dose interruption:

If dosing is interrupted for > 24 hours, the premedication schedule for Week 1 Day 1 should be followed (**Table 4**). If dosing is interrupted twice for > 24 hours, and the patient has no IRR/CRS upon each resumption of dosing, then no premedication is required after the third and subsequent interruptions. Patients that have a dosing interruption > 72 hours will reinitiate dosing with a modified multi-step lead-in dose starting at 30 ng/kg/day (followed by steps up to 60, 100, 200, 300, 400, and 500 ng/kg/day) with dose changes every 4-6 hours as tolerated.

Ruxolitinib Cohort (Single Site [Washington University, St. Louis, MO]; up to 12 patients)

Treatment Plan:

Up to 12 patients will be treated with prophylactic ruxolitinib and flotetuzumab as follows:

1. Ruxolitinib 10 mg PO BID will be administered on Days 0-14 of Cycle 1 of flotetuzumab beginning the day prior to initiation of flotetuzumab.
    a. Patients in the ruxolitinib cohort should receive all other prophylaxis as indicated in **Table 4**.

b. Based on cohort interim safety assessment, outlined above, ruxolitinib may be increased to 20 mg twice daily (max recommended dose) (**8**).

2. Flotetuzumab will be administered per the Cycle 1 schedule provided in **Table 2**.

Dose modifications:

Ruxolitinib may be interrupted or reduced to 5 mg BID for suspected ruxolitinib-related adverse events (AEs), i.e., anemia or thrombocytopenia decrease of > 2 Grade decrease while on ruxolitinib.

Table 4 Premedication and Prophylaxis for Infusion-related Reactions

Medication	7-day Continuous Infusion Schedule, Cycle 1 Week 1 [1]	
	Day 1	Day 7
Acetaminophen (1000 mg PO) *Or* **Paracetamol** (1000 mg PO) *Or* **Ibuprofen** (400 mg PO)	30 minutes prior to dosing Then q 8 hours for 48 hours	30 minutes prior to dose change Then q 8 hours for 48 hours
Diphenhydramine (25-50 mg IV or PO) or equivalent	30 minutes prior to dosing Then q 8 hours for 48 hours	30 minutes prior to dose change Then q 8 hours for 48 hours
Famotidine (20 mg IV or PO) or equivalent	30 minutes prior to dosing Then q 8 hours for 48 hours	30 minutes prior to dose change Then q 8 hours for 48 hours
Dexamethasone (or equivalent)	10-20 mg IV up to 30 minutes prior to dosing Then 4 mg IV at 12 hours after dosing Then 4–10 mg up to every 8 hours [2]	10 mg IV up to 30 minutes prior to dose change Then 4 mg 12 hours later Then 4–10 mg up to every 8 hours [2]

1 If dosing is interrupted for > 24 hours during continuous infusion, the premedication schedule for Week 1 should be followed.

2 Low dose steroids may be administered every 8 hours as clinically indicated to facilitate achievement of the target dose 500 mg/kg.

6.4.1.2 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.

Through Amendment 4, IRR/CRS has been graded using CTCAE v4.0. 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. An alternative grading scale for CRS proposed by Lee et al. (**26**) allows for more intensive supportive care and treatment of the underlying cause of the CRS (excessive cytokine production) 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) as implemented in Amendment 5 and further modified in Amendment 13, 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.

Infusion related reaction including CRS is a common toxicity with flotetuzumab, especially when the drug is initiated and dose escalated during lead-in dosing. Evaluate for and treat other causes of fever, hypoxia, and hypotension according to institutional best practices or guidelines. Initial IRR/CRS management with fluids and antipyretics is allowed as clinically indicated, per local institutional practices. Celecoxib (**14**), if platelet counts allow, may be considered for myalgia and arthralgia. If celecoxib is contraindicated consider the use of corticosteroids per **Table 5**, Grade 1 CRS. If CRS is suspected, refer to **Table 5** for management guidelines or treat according to local institutional guidance. Every attempt should be made to continue the infusion during management of the reaction. The definitions for high-dose vasopressors are shown in **Table 6**.

Table 5 CRS Grading and Recommended Management Guidance

CRS Grade (26)	Dose Modification	Tocilizumab	Corticosteroids
Grade 1 Symptoms are not life threatening and require symptomatic treatment only, e.g., fever, nausea, fatigue, headache, myalgias, malaise	Slow infusion rate by 10-20%. After stabilization/resolution increase infusion rate as clinically indicated every 4-6 hours.	Administer tocilizumab (14) 8 mg/kg IV over 1 hour (not to exceed 800 mg/dose). If no clinical improvement in the signs and symptoms of CRS after the first dose, repeat tocilizumab every 8 hours as needed. Limit to a maximum of 3 doses in a 24-hour period; maximum total of 4 doses.	Administer dexamethasone (e.g., 4 to 6 mg IV or oral every 8 hours) for 24-48 hours.
Grade 2 Symptoms require and respond to moderate intervention Oxygen requirement < 40% or Hypotension responsive to fluids or low-dose of one vasopressor (Table 6) or Grade 2 organ toxicity	Slow infusion rate by 25-50%. After stabilization/resolution increase infusion rate as clinically indicated every 4-6 hours.	Per Grade 1	Manage per Grade 3 if no improvement or worsening within 24 hours after starting tocilizumab.
Grade 3 Symptoms require and respond to aggressive intervention Oxygen requirement ≥ 40% or Hypotension requiring high-dose vasopressors or multiple vasopressors (Table 6) or Grade 3 organ toxicity (except transaminitis) or Grade 4 transaminitis	Interrupt infusion, DO NOT FLUSH line. Aspirate residual drug from line. After resolution or stabilization to Grade 1 resume infusion at previous tolerated dose, and increase rate as clinically indicated every 4-6 hours.	Per Grade 1	Administer dexamethasone (e.g., 8 to 10 mg intravenously every 8 hours) or equivalent. Continue corticosteroid use until the event is Grade 1 or less, then taper over 3 days.

Table 5 CRS Grading and Recommended Management Guidance

CRS Grade (26)	Dose Modification	Tocilizumab	Corticosteroids
Grade 4 Life-threatening symptoms Requirement for ventilator support or Grade 4 organ toxicity (excluding transaminitis)	Discontinue flotetuzumab permanently.	Per Grade 1	Per Grade 3
Grade 5	• Notify the Sponsor's Medical Monitor or designee immediately. • Report the event as an SAE.		

Table 6 Definition of High Dose Vasopressor

Pressor	High Dose (doses less than these would be considered Low Dose)
Norepinephrine monotherapy	≥ 20 µg/min
Dopamine monotherapy	≥ 10 µg/kg/min
Phenylephrine monotherapy	≥ 200 µg/min
Epinephrine monotherapy	≥ 10 µg/min
If on vasopressin	Vasopressin + norepinephrine equivalent of ≥ 10 µg/min*
If on combination vasopressors (not vasopressin)	Norepinephrine equivalent of ≥ 20 µg/min*

* VASST Trial vasopressor equivalent equation: norepinephrine equivalent dose = [norepinephrine (µg/min)] + [dopamine (µg/kg/min) ÷ 2] + [epinephrine (µg/min)] + [phenylephrine (µg/min) ÷10].
Source: Lee, et al. (**26**)

The above grading scale 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. If these events are considered part of an IRR/CRS, however, they should be captured within the Adverse Event of Special Interest (AESI) reporting criteria as described in **Section 7.11.3** and graded according to the criteria in **Table 5**. The Sponsor's Medical Monitor or designee should be contacted if questions arise concerning the grade of the reaction.

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. Consultation with the study Medical Monitor is encouraged.

6.4.2 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 (**19**). 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.

6.4.3 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 in these patients. Early initiation of empirical broad-spectrum antibacterial antibiotics 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. Again, the Investigator should employ best medical judgment and institutional guidelines. 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.

6.4.4 Immune-related Adverse Experiences

Immune checkpoint blockade has been associated with several syndromes resulting from the breaking of immunological tolerance (**42**). 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. Immune related adverse experiences will be considered AESIs **(Section 7.11.3**). Their occurrence dictates interruption, and potentially discontinuation, of study drug administration pending further evaluation.

6.4.5 Neurotoxicity

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.

6.4.6 Capillary Leak Syndrome

Risk of capillary leak syndrome (CLS) has been associated with T-cell redirecting therapy, including chimeric antigen receptor (CAR-T)-cell and CD3-engaging bispecific antibody-based molecules, and reported for the CD123-directed cytotoxin, tagraxofusp-erzs (Elzonris®). While CLS has not been observed with frequency in patients treated with flotetuzumab, a detailed review of recent clinical data across all flotetuzumab studies identified 3 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. Please be aware that CLS may or may not be associated with hemoconcentration in this patient population.

Healthcare professionals should monitor for weight gain of greater than 2.0 kg compared to previous day's weight, fluid status, new onset or worsening edema, and decreased albumin. Patient's fluid status should be managed as clinically indicated with intravenous fluids and vasopressors if hypotensive, and with diuretics if normotensive or hypertensive until the patient's body weight normalizes. Flotetuzumab dose interruption should be considered. Further recommendations include administration of 10 mg dexamethasone or equivalent for patients with weight gain of greater than 2.0 kg, fluid overload, and edema, non-responsive to diuretics and/or fluid management.

6.4.7 Epstein-Barr Virus Reactivation

Epstein-Barr virus (EBV) belongs to the herpes virus family. After primary EBV infection, the virus quickly enters the latent phase and is characterized by a lifelong presence in B-cells. EBV-reactivation may occur in immune-compromised patients, and patients may experience signs or symptoms of mononucleosis, such as fatigue, weakness, fever, sore throat, and swollen lymph nodes. In immunosuppressed patients, including those who have received flotetuzumab, viral reactivation, specifically, EBV reactivation, has been reported. To date, on the CP-MGD006-01 study, four patients have experienced EBV reactivation. Reported symptoms included non-CRS related fever and fatigue. Rituximab (anti-CD20 antibody) is an effective treatment option in patients with EBV-reactivation and should be considered per institutional guidelines.

6.5 Blinding

No blinding will be employed.

6.6 Concomitant Therapy

All concomitant medications and blood products administered during the patient's participation in the study must be recorded in the source document and on the electronic Case Report Form (eCRF). Patients' blood product administered up to 28 days before enrollment should also be specified in the eCRF. Specifically, concomitant medications must be recorded through 7 days after the last administration of study drug or until the End-of-Treatment visit, whichever is later. Any concomitant medication associated with an AE or SAE that is

considered related to study drug must be recorded through 28 days after the last administration of study drug or End-of-Treatment visit, whichever is later.

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 ≤ 20,000/mm^3 should have ceased by the time of first dose of study treatment (Cycle 1 Day 1). 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.
- Previous 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) is prohibited. Ruxolitinib is permitted for patients in the ruxolitinib mini-cohort.
- 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 to the extent possible. Chronic doses of corticosteroids in excess of 10 mg daily of prednisone or equivalent are prohibited other than for the management of drug-related adverse experiences. Corticosteroid pretreatment should follow the recommended dose and schedule. Steroids may be employed in the treatment of suspected flotetuzumab-associated immunoinflammatory or autoimmune AEs or leukemia-associated AEs in consultation with the Sponsor.
- Use of granulocyte colony stimulating factor or granulocyte-macrophage colony stimulating factor is prohibited during Cycle 1.
- Vaccinations (with the exception of the annual inactivated influenza vaccine and SARS-CoV-2 vaccines) are prohibited during the study. SARS-CoV-2 vaccine may be administered during screening and up to 3 days prior to the first dose or after reaching the MTD of 500 ng/kg/day flotetuzumab.

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 other medications intended to treat symptoms or signs of disease.
- Transfusions such as red blood cells and platelets are permitted to treat symptoms or signs of anemia or thrombocytopenia and should be documented on the transfusions eCRF.

- The use of hematopoietic growth factors is discouraged. However, granulocyte colony stimulating factor or Granulocyte-macrophage colony stimulating factor may be used in accordance with the practice of medicine and institutional norms or guidelines outside of Cycle 1.

6.7 Restrictions

6.7.1 Prior Therapy

Prior therapy restrictions are described in the inclusion/exclusion criteria specified in **Sections 5.1** and **5.2**.

6.7.2 Fluid and Food Intake

There are no requirements for fasting and no restrictions for fluid and food intake by the patients during the study, although it is recommended that, to the extent possible, patients have a fluid intake of ≥ 2 liters on days associated with PK sampling, and that electrocardiograms (ECGs) be obtained pre-meal. Potassium and phosphorus intake should be limited during the risk period for TLS according to institutional practices and guidelines.

6.7.3 Patient Activity Restrictions

Patients who have received flotetuzumab must refrain from driving a motor vehicle or operating heavy machinery while receiving flotetuzumab, and for 30 days from the date of last study drug administration.

6.8 Treatment Compliance

Flotetuzumab will be administered by healthcare professionals under the supervision of the Investigators. Records of flotetuzumab dose calculation, administration, and dosing regimen will be accurately maintained by site staff. The monitor will review medication accountability during investigational site visits and at the completion of the study.

6.9 Packaging and Labeling

All investigational product will be labeled, at a minimum, according to local regulatory requirements. Please see the Pharmacy Manual for detailed information about the packaging and labeling of the flotetuzumab study drug, MGV002 IV Solution Stabilizer, and MGV004 IV Solution Stabilizer.

6.10 Storage and Accountability

6.10.1 Flotetuzumab and IV Solution Stabilizer Storage

Vials containing flotetuzumab, MGV002 IV Solution Stabilizer, or MGV004 IV Solution Stabilizer 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, MGV002 IV Solution Stabilizer, and MGV004 IV Solution Stabilizer 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 of the drug product and preparation of the dose solution 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.

6.10.2 Accounting for the Materials

Accurate accounting of all study medication must be maintained. The Investigator agrees to keep an inventory of study drugs using the institution's drug accountability logs or logs provided by MacroGenics. Drug disposition records must be kept in compliance with applicable guidelines and regulations.

A Pharmacy Manual will be provided to the Investigator or designee. When the study is completed, copies of all study drug accountability records must be provided to the Sponsor. Original drug accountability records must be maintained with the rest of the documentation in accordance with **Sections 10** and **11** for inspection by the study monitors.

6.10.3 Return or Destruction of Investigational Product

All vials of flotetuzumab study medication and all vials of, MGV002 IV Solution Stabilizer and MGV004 IV Solution Stabilizer must be returned to MacroGenics or its representative, unless the site has received written authorization from MacroGenics to destroy vials at the site. All drug or stabilizer solution returns to MacroGenics must be accompanied by the appropriate documentation and be clearly identified by protocol number and study site number on the outermost shipping container. If MacroGenics approves the destruction of drug and/or stabilizer solution at the site, the Investigator must ensure arrangements are made for proper disposal and that appropriate records of disposal are documented and maintained and copies provided to the Sponsor.

7 STUDY PROCEDURES

This section provides a detailed description of the procedures and assessments associated with the study. A tabular schedule of events is shown in **Appendix 1** for Cohort Expansion patients.

7.1 Informed Consent

The Investigator is responsible for ensuring that the patient or his/her legal representative provides informed consent prior to performing any study related assessments, evaluations, or procedures. This event should be documented in the patient's medical records. Informed consent for this study may be provided by signing an IRB/IEC-approved informed consent document (Consent for Study Participation). A copy of the relevant signed informed consent document must be provided to the patient, another copy kept with the study records, and the original maintained according to institutional procedures. In addition, the medical record will include documentation of the informed consent process (see **Section 11.4**).

7.2 Medical History

A complete medical history should be obtained during the screening visit. All concurrent medical conditions in the last 60 days and any significant medical conditions (e.g., hospitalizations, surgeries, prior cancer history) should be collected. During the screening period (prior to first dose of flotetuzumab), any untoward event that occurs should be collected as a concurrent medical history and not as an adverse event, unless it is due to a protocol-related procedure. Thereafter (i.e., after the time of drug administration), any untoward event should be collected as an AE. Details of the disease (sub-classification for AML and risk stratification (e.g., cytogenetics) for AML) will be collected on the eCRF.

7.3 Prior and Concomitant Medications

All concomitant medications, including blood products, administered during the patient's participation in the study must be recorded in the source document and on the eCRF. Historical data regarding blood products administered up to 28 days prior to enrollment will also be collected in the eCRF. Specifically, concomitant medications must be recorded through 7 days after the last administration of study drug or End-of-Treatment visit, whichever is later. Any concomitant medication associated with an AE or SAE that is considered related to study drug must be recorded through 28 days after the last administration of study drug or End-of-Treatment visit, whichever is later.

Prior courses of chemotherapy will be documented on the eCRF.

7.4 Physical Examination

The Investigator will perform physical examination of all patients. Physical examination will include height (screening only), weight, and examination of skin, HEENT (head, eyes, ears, nose, and throat), lymph nodes, heart, chest, lungs, abdomen, extremities, and

neurologic system as specified in the Schedule of Events for Cohort Expansion (**Appendix 1**).

7.5 Vital Signs

Vital signs including blood pressure, heart rate, respirations, pulse oximetry, and temperature will be taken immediately prior to the start of flotetuzumab infusion, and repeated as outlined in **Appendix 1**. Vital signs may also be captured as necessary to elucidate the course of any untoward event or AE.

7.6 Clinical Laboratory Tests

Blood and urine specimens will be collected at the times specified in **Appendix 1**. Hematology, chemistry, coagulation, pregnancy, and urinalysis testing will be performed locally. **Table 7** below lists the specific tests that will be performed locally for this study. Investigators must document their review of each laboratory result.

Table 7 Local Routine Laboratory Tests

Hematology:

- Hematocrit (Hct)
- Hemoglobin (Hgb)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular Hgb concentration (MCHC)
- Erythrocytes distribution width (RDW)
- Red blood cell count (RBC)
- White blood cell (WBC) count with differential including blast count
- Platelet count
- Mean platelet volume (MPV)
- Reticulocytes

Urinalysis:

- glucose (by standard dipstick)
- bilirubin (by standard dipstick)
- ketones (by standard dipstick)
- specific gravity (by standard dipstick)
- blood (by standard dipstick)
- pH (by standard dipstick)
- protein (by standard dipstick)
- urobilinogen (by standard dipstick)
- nitrite (by standard dipstick)
- leukocytes (by standard dipstick)
- microscopic evaluation of urinary sediment

Urine or serum human chorionic gonadotropin (hCG) (only for females who are not postmenopausal or sterile as the result of surgical procedure)

Serum Chemistry:

- Albumin (ALB)
- Alkaline phosphatase (ALK-P)
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Blood urea nitrogen (BUN)
- Bicarbonate (HCO_3)
- C-reactive protein (CRP)
- Calcium (Ca)
- Chloride (Cl)
- Creatinine
- Glucose
- Potassium (K)
- Sodium (Na)
- Total bilirubin
- Total protein

Special Chemistry:

- Uric Acid
- Phosphorus
- Creatine kinase (CK)

Coagulation:

- Prothrombin time (PT)
- Activated partial thromboplastin time (APTT)

Bone marrow aspiration and biopsy specimens will be processed and evaluated locally at the investigational institution. Material may be requested for central review during or following completion of the study. A portion of the bone marrow aspirate and biopsy specimen will be collected for research purposes at specific time points (see **Appendix 1**).

Certain other testing of blood samples and bone marrow aspirate and biopsy specimens (PK, ADA, PBMC subsets and activation markers) will be carried out at Sponsor-specified central laboratories (refer to the Laboratory Manual).

7.7 Electrocardiography and Continuous Telemetry Monitoring

Twelve-lead ECGs will be obtained according to **Appendix 1** to evaluate the potential cardiac effect, including QTc interval prolongation. There are no requirements for fasting and no restrictions for fluid and food intake by the patients during the study, although it is recommended that, to the extent possible, ECGs be obtained pre-meal.

To account for intrinsic variability, screening and Cycle 1 Day 15 (or when patient has reached 500 ng/kg/day dose level, if later than Cycle 1 Day 15) ECGs should be obtained in triplicate (3 ECGs per time point at approximately 1-minute intervals). Subsequent ECGs will be performed when clinically indicated, and will be single tracing unless clinical circumstances, and at the discretion of the investigator, indicate otherwise. Central interpretation of ECGs will be used for data analysis purposes.

7.8 Left Ventricular Ejection Fraction

In the Cohort Expansion Segment, if the patient has a history or signs and symptoms of cardiac disease, an echocardiogram (or, except in Germany, multiple-gated acquisition scan [MUGA] if indicated) will be obtained during the screening period, if one was not performed within 30 days before the start of treatment (Cycle 1 Day 1). An echocardiogram (or, except in Germany, MUGA if indicated) may also be conducted during the study if clinically indicated.

7.9 Pulmonary Function Testing

In the Cohort Expansion Segment, PFT, including DLCO and FEV1, will be conducted during the screening period if testing was not performed within 30 days before the start of treatment (Cycle 1 Day 1). PFT may also be conducted during the study if clinically indicated.

- During the COVID-19 pandemic, except in Germany, if PFT equipment is not available, a correlative stair-climbing test may be conducted instead, together with lung CT and echocardiogram irrespective of cardiac history. The patient must pass the stair-climbing test, with O_2 saturation $\geq$ 95%, lung CT with normal appearing lung parenchyma, and normal echocardiogram (see **Section 8.1.1**).

7.10 Efficacy Assessments

Response Assessments: evidence of clinical activity in these patients will be assessed by the study investigators by using CBC and peripheral blood cell morphological examination, examination of bone marrow aspiration (or biopsy if required), and physical exam according to the rules modified from the Revised Recommendations of the International Working Group

(IWG) for Diagnosis Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia (**Appendix 3**), with modifications as described below. Response assessment will be based on bone marrow aspirate/biopsy and the best CBC up to 14 days post bone marrow aspirate/biopsy. Responses will be categorized as:

- CR: mCR (morphologic CR), CRc (cytogenic CR), or CRm (molecular CR);
- CRh (CR with partial hematologic recovery);
- CRi (CR with incomplete blood count recovery): CRn (CR with incomplete neutrophil recovery) or CRp (CR with incomplete platelet recovery);
- MLFS (morphologic leukemia free state);
- PR (partial response);
- OB (other benefit);
- SD (stable disease);
- PD (progressive disease).

Bone marrow assessment should be performed at the end of each cycle whenever possible. However, at the end of Cycle 2, and at the end of each subsequent even cycle, in consultation with the Medical Monitor, the bone marrow assessment may be missed if the patient has clearly not achieved CR or CRh using the following criteria:

- The patient has not achieved CR/CRh because the peripheral blood neutrophils are < 500/µL or platelets are < 50,000/µL, based on Study Day 22 CBC results for the cycle in question, and
- The results of the bone marrow assessment will not influence the decision to continue study treatment (i.e., assessment of risks and benefits).

The overall complete response rate (BM blasts < 5%) will be the proportion of patients with AML achieving any CR, CRh, CRi, [CRn, CRp], or MLFS when such responses are documented at any point of any cycle of treatment. The objective response rate will be the proportion of patients with AML achieving CR, CRh, CRi, [CRn, CRp], MLFS, or PR when such responses are documented at any point of any cycle of treatment. To support the composite endpoint CR/CRh, the duration of initial hospitalization for flotetuzumab administration, the incidence and duration of subsequent hospitalizations, and transfusion independence rate, as well as rate of conversion to transfusion independence and rate of maintenance of transfusion independence, will be assessed. The timing of the AML response evaluation, i.e., the allowance for response at a time later than Day 25 from the start of treatment, differs from those modified from the IWG guidelines for AML (which are based on responses to cytotoxic chemotherapy) to allow sufficient time for an immune-mediated response to be observed. Patients who do not demonstrate an objective response at early evaluations, may be subsequently categorized as responders at later evaluations. Response duration will be calculated from the time of initial response to the time of relapse or death, whichever occurs first. Patients who complete the study while still in response will be

censored at their last disease assessment. Time to response will be calculated from the time of first dose of study drug to the time of initial response.

Event-free survival (EFS) will be calculated as the time from the first dose of study drug until date of relapse from CR, CRh, or CRi (CRn, CRp), or death from any cause, whichever occurs first.

Overall survival (OS) will be calculated as the time from the first dose of study drug until the occurrence of death from any cause. Mortality rates from any cause within 30, 60, 90, and 180 days from the first dose of study drug will be calculated. Six-month and one-year survival rates will be calculated.

7.11 Safety Assessments

7.11.1 Adverse Event: Definitions

Adverse Event

An adverse event (AE) is any untoward medical occurrence in a clinical investigation patient to whom a pharmaceutical product is administered. The event may or may not have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE will also be considered to be any untoward effect of a study-related procedure, which may be conducted after signed informed consent and prior to study drug administration.

Adverse Drug Reaction

An adverse drug reaction (ADR) is any noxious and unintended response to the medicinal product related to any dose. As used herein, the phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, (i.e., the relationship cannot be ruled out).

Adverse Event of Special Interest

An AESI is an event of scientific and medical interest or concern to the Sponsor’s product or program, for which ongoing monitoring and rapid communication to the Sponsor could be appropriate. It may be a serious or non-serious AE, which may require further investigation in order to characterize and understand it.

Treatment-Emergent Adverse Event

An event that is temporally associated with administration of study drug is defined as a treatment-emergent adverse event (TEAE). Events meeting this definition will be those occurring during study drug administration and up to and including at least terminal elimination of the drug (roughly 5 half-lives; for flotetuzumab the estimated elimination half-life is a few hours). Events that existed before the first administration of study drug and then

increased in severity during or after the first administration of study drug will also be considered treatment emergent. Such events will be captured on the eCRF as new events, with the onset date as the date of the increase in severity.

Serious Adverse Events

A serious adverse event (SAE) is any AE that results in any of the following outcomes:

- Death
- Life-threatening (immediate risk of death)
- Inpatient hospitalization or prolongation of existing hospitalization (even if the event is Grade 1)
- Persistent or significant disability or incapacity
- Congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization or the development of drug dependency or drug abuse.

Immediately Reportable Event

Immediately reportable events (IREs) are events that must be reported immediately to MacroGenics Product Safety or designee within 24 hours of being identified (see **Section 7.12**). IREs include but are not limited to:

- SAEs
- AEs leading to permanent discontinuation of study drug in an individual patient
- 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.
- Grade 2 or higher infusion-related reactions including CRS
- TLS as defined in **Section 6.4.2**
- Neutropenic sepsis or fungemia as defined in **Section 6.4.3**

- Immune related adverse events as defined in **Section 6.4.4**
- Grade 2 or higher neurological events as described in **Section 6.4.5**
- Capillary leak syndrome as described in **Section 6.4.6**
- EBV reactivation as described in **Section 6.4.7**
- Confirmed or presumed (test pending/clinical suspicion) SARS-CoV-2 infection.

Withdrawal of patient from study drug administration due to toxicity (e.g., AE, SAE, AESI) or pregnancy.

In those cases in which the IRE is considered related to study drug and the event is of sufficient clinical concern, the study drug may be discontinued and the patient will continue participation in the study for observational safety and analysis (except for cases where the patient is withdrawn from the study by the Investigator). At any time after completion of the study, if an Investigator becomes aware of a SAE that s/he suspects is related to study drug, the Investigator should report the event to MacroGenics Product Safety or designee.

7.11.2 Performing Adverse Event Assessments

Medical evaluation and classification of the AE must be performed by the Investigator who is qualified to review AE information. The determination of seriousness, severity and causality must be made according to the following criteria:

Assessment of Seriousness: Event *seriousness* will be determined according to the definition of an SAE in **Section 7.11.1**. Seriousness serves as a guide for defining regulatory reporting obligations for AEs.

Assessment of Severity: Event *severity* will be assigned according to the Investigator's assessment using the following:

CTCAE v4.0 (for described events and syndromes), except IRR/CRS which will be defined by the modified criteria proposed by Lee et al. (**26**) as described in **Section 6.4.1.2**, or *for events not specifically defined in the CTCAE,* using the following scale:

Grade 1= Mild AE;

Grade 2 = Moderate AE;

Grade 3 = Severe AE;

Grade 4 = Life-threatening or disabling AE; and

Grade 5 = Death related to AE

Any event or laboratory value judged as Grade 4 severity should be separately evaluated to determine whether it also meets the serious criterion of "immediately life threatening."

Note: Severity is not synonymous with seriousness. The term "severe" is often used to describe the intensity (severity) of a specific event. The event itself, however, may be of

relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning.

Assessment of Causality: The Investigator is required to provide an assessment of causality or relationship of AEs to the study drug based on: 1) temporal relationship of the event to the administration of study drug, 2) whether an alternative etiology has been identified, and 3) biological plausibility. The causality assessment categories that will be used for this study are described below.

Causality assessments that are considered **Not Related** to study drug:

None: The event is related to an etiology other than the study drug (the alternative etiology must be documented in the patient's medical record).

Unlikely: The event is unlikely to be related to the study drug and likely to be related to factors other than study drug.

If an SAE is considered "unlikely" or "unrelated" to study drug, the Investigator should offer his/her clinical opinion as to what factor(s), agent(s), or process(es) were the likely causative mechanism for the event.

Causality assessments that are considered **Related** to study drug:

Possible: There is an association between the event and the administration of the study drug and there is a plausible mechanism for the event to be related to study drug; but there may also be alternative etiology, such as characteristics of the patient's clinical status or underlying disease.

Probable: There is an association between the event and the administration of study drug, a plausible mechanism for the event to be related to the study drug and the event could not be reasonably explained by known characteristics of the patient's clinical status or an alternative etiology is not apparent.

Definite: There is an association between the event and the administration of study drug; a plausible mechanism for the event to be related to the study drug and causes other than the study drug have been ruled out and/or the event re-appeared on re-exposure to the study drug.

Assessment of Expectedness: As part of the regulatory reporting requirements, the Sponsor must perform an assessment of expectedness (expected/unexpected from the perspective of previously observed, not on the basis of what might be anticipated from the pharmacological properties of a medicinal product) for AEs. Adverse reactions will be considered unexpected if the nature, seriousness, severity or outcome of the reaction(s) is not consistent with the reference information (e.g., the Adverse Drug Reaction section of the Investigator's Brochure) for the study product.

7.11.3 Adverse Events of Special Interest

In this study, TLS (as defined in **Section 6.4.2**), neutropenic sepsis/fungemia (**Section 6.4.3**), immune-related events (**Section 6.4.4**), treatment-related ≥ Grade 2 neurological events (**Section 6.4.5**), capillary leak syndrome (**Section 6.4.6**), and EBV reactivation (**Section 6.4.7**) are considered AESIs and should be reported as IREs and documented on the appropriate eCRF irrespective of attribution to flotetuzumab.

All infusion related reactions including CRS are considered AESIs and should be documented on the appropriate eCRF; only ≥ Grade 2 events need to be reported to the Sponsor as IREs.

7.11.4 Reporting of Adverse Events to the Sponsor

Adverse events and SAEs will be collected and followed from the time the patient provides informed consent for the study (see **Section 8.1**) through 28 days after the last administration of study drug or until the start of a subsequent systemic anticancer therapy, if earlier. If a patient experiences an AE after the informed consent document is signed and prior to treatment with study drug, it should be captured as medical history; however, if the Investigator believes the AE may have been caused by a protocol procedure, or related to ruxolitinib, it will be reported to the Sponsor or its designee and/or entered on the eCRF.

Adverse events occurring after the patient undergoes early termination or completes the trial (attains 28 days after last administration of study drug or starts a subsequent systemic anticancer therapy, if earlier) need not be reported unless the event is serious and the Investigator believes that the event may have been caused by the study drug or a protocol procedure.

To identify the occurrence of any new medical complaints or worsening of previous complaints, non-leading questioning should be posed to the patient.

Events related to leukemia progression/worsening of underlying disease (including those with a fatal outcome) will be collected as efficacy endpoints, and not documented as AEs/SAEs. However, if an SAE occurs in a patient and it is unclear if the event is due to progressive disease, the SAE should be reported.

If a patient reports signs and symptoms that represent a single medical syndrome, diagnosis, or concept, the syndrome/diagnosis/concept should be documented as an AE (e.g., cough, runny nose, fever = Upper Respiratory Tract Infection) in the eCRF. One exception is for IRR/CRS, for which the Sponsor would like to collect specific information on signs and symptoms. For IRR/CRS, report just the IRR/CRS event as an AE; signs and symptoms associated with IRR/CRS should only be reported on the IRR/CRS eCRF page and not as independent AEs.

The Investigator must follow all SAEs until resolution. Resolution of an event is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

Clinical Laboratory Changes: Safety laboratory assessments will be carried out locally and evaluated by the Investigator to ensure patient safety. The Investigator is responsible for reviewing the results of all laboratory tests as they become available. Laboratory tests will be graded according to CTCAE v4.0. Laboratory values that fall outside of a clinically accepted reference range or values that differ significantly from previous values must be evaluated by the Investigator for clinical significance. The Investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests, in which case the repeat assessment will be considered the true value. If the Investigator determines the laboratory value is an abnormal change from baseline and is of clinical significance for that patient, it is considered an AE. Generally, Grade 1 laboratory findings need not be reported as AEs unless clinically significant. The Investigator will evaluate laboratory findings of ≥ Grade 2 classification to determine their clinical significance and if an AE has occurred. Consistent with the CTCAE designation of Grade 3 events as severe or medically significant and Grade 4 events as life-threatening, all such events should be reported as AEs or SAEs, as appropriate, unless these are considered an expected consequence of the underlying disease. Grade 2 events may be reported as AEs if, in the opinion of the Investigator, the event exhibits clinical significance. If clinically relevant abnormal laboratory values are associated with clinical symptom(s), or consistent with a diagnosis, the diagnosis should be reported as the AE (e.g., hemoglobin 9 g/dl in an adult female = anemia). If these clinically relevant abnormal laboratory values do not result in a diagnosis, the test result or finding should be reported as the AE assuming that it does not represent a laboratory error. Repeat testing may be indicated. Such laboratory values should generally be recorded as “increased” or “decreased” (e.g., change from baseline hemoglobin of 13 g/dl to 11 g/dl = hemoglobin decreased).

7.12 Notification to the Sponsor of Events Requiring Immediate Reporting

Throughout the study, the Investigator must document all AEs on the eCRF in a timely manner. IREs, as defined in **Section 7.11.1**, are events that must be reported immediately to MacroGenics Product Safety within 24 hours of being identified. The Investigator must immediately complete and email or fax the *Immediately Reportable Event (IRE)* Report Form within 24 hours of identifying the event to MacroGenics Product Safety/designee (see the IRE Report Form and IRE Report Form Completion Guidelines for details).

For pregnancy, the MacroGenics Pregnancy Exposure Form must also be completed and faxed/emailed to MacroGenics Product Safety. The Investigator must attempt to follow the pregnancy to term or termination in order to report on outcome and health status of mother and child.

Contact information for MacroGenics Product Safety is shown below.

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

7.13 Emergency Unblinding

Not applicable.

7.14 Other Study Procedures

7.14.1 Pharmacokinetics, Biomarkers, and ADA

MacroGenics or its designee will provide the following blood sampling supplies: polypropylene transport tubes, labels, and log sheets. Serum tubes should be labeled with the patient's unique study number, date, and time of sampling. The Investigator will maintain a log with the same data.

Specimens must be appropriately prepared, divided if appropriate, frozen, and shipped (while often retaining certain replicate samples at the site) according to the instructions in the Laboratory Manual.

7.14.1.1 Pharmacokinetics

It is expected that the sensitivity of the PK assay will be sufficient to detect concentrations of study drug in samples taken from patients receiving doses ≥ 100 ng/mg/day. Serum concentrations of flotetuzumab will be monitored using an electrochemiluminescence-based sandwich assay. PK analyses will be performed using industry standard software. Population PK modeling will be performed and an appropriate model will be selected to describe the data.

Venous blood samples for PK analyses for flotetuzumab will be processed as instructed in the Laboratory Manual.

7.14.1.2 ADA (Anti-flotetuzumab Antibodies)

Venous blood samples for ADA will be processed as instructed in the Laboratory Manual.

Serum samples from all patients may be retained as described in **Section 7.14.9** for detection of ADA and evaluation of neutralizing antibodies if there are any ADA- positive samples.

7.14.2 Flow Cytometric Analyses on Bone Marrow and Peripheral Blood Samples

Multiparameter flow cytometric determination of the absolute number and phenotype of monocytes, NK cells, pDCs, and B and T lymphocyte subsets (including Treg, naïve/central memory/effector memory and Th1/Th2/Th17 subsets) will be performed based on the flow cytometry panels recently described by the Human Immunophenotyping Consortium (**27**). Other cell types based on the Human Immunophenotyping Consortium (**27**) flow cytometry panels may also be evaluated. Quantification of the number and phenotype of AML blast cells in the peripheral blood and bone marrow will be determined on fresh samples at each time point as described previously (**43**). Since AML is a heterogeneous and molecularly

complex group of diseases with variable hematologic phenotypes (**14**,**33**) selection of antigens for the identification and immunophenotypic characterization of AML blast cells will be determined from each patient's prior flow cytometric testing. Antigenic expression patterns of CD123 on all normal peripheral blood cell subsets as well as malignant blasts will be evaluated. The activation state of CD3+CD4+ and CD3+CD8+ T cells could be assessed using monoclonal antibodies to Lag-3, PD-1, CD25 and CD69. The percentage of positive cells and median fluorescence intensity of each antigen will be determined.

7.14.3 Other Assessments

Transcriptome in bone marrow aspirate samples will be analyzed for expression patterns reflective of immune cell infiltration to determine the effect of flotetuzumab on the tumor microenvironment.

Generation of adaptive immune responses during flotetuzumab treatment will be evaluated in vitro cytotoxicity assays. T-cells will be interrogated for activation patterns, subgroup phenotyping, T cell receptor rearrangements, and complementarity determining region 3 (CDR3) sequencing (see **Section 7.14.7**).

7.14.4 Bone Marrow Assessments

Bone marrow aspirate and trephine biopsy will be obtained and analyzed locally per institutional guidelines during screening and upon completion of every treatment cycle. Marrow smears will be morphologically examined using a May-Grünwald-Giemsa or a Wright-Giemsa stain with a nucleated differential count from at least 200 cells performed. Percent marrow blasts should be recorded at both baseline and on study. Conventional cytogenetic analysis with a minimum of 20 metaphase cells analyzed from bone marrow should be performed at each assessment. For patients with pretreatment cytogenetic abnormalities, additional fluorescence in situ hybridization (FISH) may be performed at the time of baseline evaluation and again at the time of response evaluation.

7.14.5 Cytokine Analyses

Cytokines will be centrally evaluated in serum using an immunoassay.

7.14.6 Minimal Residual Disease

Change in tumor burden as measured by next generation sequencing with digital sequencing read counts of commonly recurring mutations in AML may predict clinical benefit in patients treated with flotetuzumab. Targeted resequencing of DNA obtained from serial bone marrow samples will be performed.

In selected patients in the United States (only Washington University site in St. Louis, MO), sequencing of DNA obtained from buccal mucosa swab or saliva samples will be compared to baseline tumor samples to help define which nucleotide variants in these genes that are acquired in the malignant sample.

The change in mutation burden will be then tracked over the course of the patient's treatment on the clinical trial. This analysis will also help identify and characterize individual subclones of tumor cells, which may demonstrate differential sensitivity to treatment. In addition, tracking the change of tumor burden may serve as a more sensitive early indicator or biomarker of response to treatment.

7.14.7 T Lymphocyte Repertoire

To assess changes in T cell clonality and diversity in response to flotetuzumab, IgH and TCR from genomic DNA will be amplified using universal primer sets and quantify the frequency of clones using next-generation sequencing. CDR3 sequences will also be analyzed for clonotype across patients.

7.14.8 Sample Collection, Storage, and Shipping

For samples to be analyzed at a central laboratory, sample collection, handling, labeling, and shipping procedures are described in the Laboratory Manual.

7.14.9 Sample Retention and Further Testing

Samples acquired for protocol specified assays will be retained for study purposes (analysis/re-analysis) and may be retained up to 2 years after last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since formal discontinuation of clinical development of the investigational product. If patients consent to the use of their study samples for non-study research purposes, these samples may also be used for exploratory testing (including assay development/optimization) and may be retained up to 15 years from the end of study.

7.15 Appropriateness of Measurements

Routine laboratory evaluations including hematology, chemistry, special chemistry, coagulation, and urinalysis will be carried out in local institutional laboratories. Additional local safety laboratory assessments may be used to supplement the protocol-prescribed assessments and may be used to elucidate certain AEs. If during assessment of AEs a patient undergoes imaging study and/or sample collection, e.g., pleural fluid, intra-articular joint aspiration, or ascitic fluid, a sample may be provided to the Sponsor to help elucidate etiology.

Serum concentrations of flotetuzumab will be monitored. Standard bridging enzyme-linked immunosorbent assays (ELISAs) will be carried out in the Sponsor's designated central laboratory to characterize the immunogenicity of flotetuzumab. Analysis of PK data will be carried out using industry standard software. The impact of AML on PK parameters will be evaluated.

Baseline, on-treatment, and follow-up bone marrow aspiration and/or biopsy specimens will be used for the assessment of disease status and effects of flotetuzumab treatment. These

assessments will be carried out in routine fashion in the investigational institutions' facilities. Material may be requested for central review during or following completion of the study.

8 STUDY ACTIVITIES

A table of study activities by study day is presented in the Schedule of Events for Cohort Expansion (**Appendix 1**).

8.1 Pre-treatment Evaluations

Patients will be evaluated before registration. At this screening visit, patients will enter the study upon signing the study-specific informed consent document. Only those patients who meet all inclusion/exclusion criteria specified in **Sections 5.1** and **5.2** will be enrolled. **Informed consent must be obtained prior to performing any screening procedures and may be obtained prior to the start of the 14 day screening period.**

8.1.1 Within 14 Days Before Treatment

The screening window will start with the conduct of the first pre-treatment evaluation.

Pre-treatment evaluations and activities include:

- Obtain bone marrow aspirate and/or biopsy specimen, if not performed within 30 days before the start of treatment (Cycle 1 Day 1), for local histopathological and immunohistochemical evaluation, central flow cytometric evaluation, and minimal residual disease analysis.
- Obtain buccal mucosal sample (only Washington University Site in St. Louis, MO).
- If patient has a history or signs and symptoms of cardiac disease, obtain echocardiogram (or, except in Germany, MUGA if indicated) if one was not performed within 30 days before the start of treatment (Cycle 1 Day 1). An echocardiogram (or, except in Germany, MUGA if indicated) may also be conducted during the study if clinically indicated.
- Conduct PFT, including DLCO and FEV1, if testing was not performed within 30 days before the start of treatment (Cycle 1 Day 1). PFT may also be conducted during the study if clinically indicated.
    - During the COVID-19 pandemic, except in Germany, if PFT equipment is not available to perform the required screening procedure, the recommended alternative is to perform a correlative stair-climbing test of 5 flights (127 steps), per Bolton (**3**). The patient is to walk up several flights of stairs at his/her own moderate pace (timed with a stopwatch) and encouraged to proceed as far as possible without stopping. The patient is considered passing the stair climb screening test if the patient can complete all 5 flights (127 steps) without complications, such as prolonged recovery time, severe air hunger, or stopping the climb due to non-pulmonary complaints. The site will be expected to provide baseline data (including pulse rate, blood pressure, and respiratory rate) obtained prior to the stair climb, documentation of the number of stairs the patient

was able to climb, time in seconds, reason for stopping, and the O_2 saturation the patient achieved as described in Bolton et al. In addition, the pulse rate, blood pressure and respiratory rate should be recorded immediately post climb, at 2 minutes after stopping, at 5 minutes after stopping and at 10 minutes after stopping if the patient did not fully recover within 5 minutes. If the patient passes the stair climbing test, CT of the lung and echocardiogram, irrespective of cardiac history, must be performed.

8.1.2 Within 5 Days Before Treatment

- Conduct medical history (including collection of AE reporting) and review of concomitant medications (including all transfusion requirements up to 28 days prior to dosing)
- Report human leukocyte antigen (HLA) typing if available
- Evaluate ECOG performance status
- Conduct physical examination (examination of skin, HEENT [head, eyes, ears, nose, throat], lymph nodes, heart, chest, lungs, abdomen, extremities, and neurologic system), including height, weight, and disease assessment
- Obtain ECG, standard 12-lead
- Take vital signs (including blood pressure, heart rate, respirations, pulse oximetry, and temperature)
- Obtain specimen for CBC with differential and platelet count
- Obtain specimen for chemistry panel (ALB, ALK-P, ALT, AST, BUN, HCO3, Ca, Cl, creatinine, glucose, K, Na, total bilirubin, total protein) and CRP
- Obtain specimen for Special Chemistry (phosphorus, uric acid, CK)
- Obtain specimen for coagulation studies (PT, aPTT)
- Obtain specimen for urinalysis and examination of urinary sediment
- Perform urine or serum β-hCG pregnancy test for women of childbearing potential
- Obtain peripheral blood for flow cytometric evaluation

8.1.2.1 Patient Registration

Each patient must be registered with MacroGenics before study entry. The following information should be provided during registration:

- AML diagnosis, including disease risk classification and cytogenetics

- Prior AML treatment history, including dates of treatment, outcome of treatment, best response, and treatment failure, as well as number of treatment cycles received and duration of prior treatment outcome.
- Date of signed informed consent
- Planned date of first drug administration
- Confirmation of conformity to the entry criteria

8.1.3 Within 1 Day Before Treatment

Initiate TLS prophylaxis for patients with AML employing hydration, allopurinol, and oral phosphate binders as defined by institutional practices and standard of care.

8.2 Study Evaluations Applicable During Cohort Expansion

Patients may be treated on an outpatient basis after Cycle 1 Day 8 (and after a minimum of 24 hours at the 500 ng/kg/day maximum dose) of study treatment guided by tolerability of the regimen during the first 8 days of dosing, and at the discretion of the Investigator. Cycles 2 and greater may be administered as an outpatient if the previous cycle was well-tolerated.

During Cycle 1, laboratory assessment samples are to be drawn around the same time each day, and prior to start of infusion or dose change as applicable and after end of infusion on days when infusions end unless otherwise indicated.

8.3 End of Treatment Visit/Follow-up

8.3.1 End of Treatment Visit: 28-Days (4 Weeks) After Last Dose of Study Drug or at the Time of Treatment Failure

It is recognized that certain patients (such as those experiencing progression of disease) may be cared for in facilities other than the participating investigational site, may proceed to receive other anti-leukemic therapy, and/or may elect not to return to the investigational site. Therefore, this visit is considered optional, but should be carried out whenever possible.

8.3.2 Follow-up Period

Follow-up information will be requested by the Sponsor after end of treatment until death or withdrawal of consent, but not beyond 2 years after the last patient is enrolled. All patients will have clinical information including safety, transfusion requirements, disease and vital status information collected during the follow-up period. Depending on treatment response, patients may be required to continue follow-up at the study site for the first 6 months post treatment. After these first 6 months, follow-up can be conducted outside of study site, but follow-up information must still be provided to Sponsor as outlined.

Follow-up Frequency

- **Responders (CR, CRh, CRi [CRn, CRp], or MLFS):**

  Patients who respond to treatment should have monthly visits at the study site for 6 months post end of treatment. After 6 months post treatment, patients may be followed up at the study site or by a local physician, and follow-up data will be collected at 3-month intervals.

- **Non-Responders:**

  Patients who do not respond to treatment may be followed up at the study site or by a local physician, and follow-up data will be collected at 3-month intervals post treatment.

Bone Marrow Aspirate and Biopsy

- **Responders (CR, CRh, CRi [CRn, CRp], or MLFS):**

  Patients who respond to treatment should have a bone marrow aspirate and biopsy collection at 3 and 6 months post treatment at the study site. After 6 months post treatment, bone marrow aspirate and/or biopsy will be performed per standard of care.

- **Non-Responders:**

  Patients who do not respond to treatment will have bone marrow aspirate and/or biopsy performed per standard of care.

- **All patients:**

  If patients have a bone marrow aspirate and/or biopsy done as part of their standard of care, they have the option to consent to donate aspirate or biopsy specimens for assessment of disease status and further research to help elucidate immune mechanisms involved in flotetuzumab function. As with all other biopsies collected for this study, these samples may be used for histopathological and immunohistochemical evaluation, flow cytometric evaluation, and minimal residual disease analysis.

Disease and Vital Status

The Sponsor will request the following clinical data that supports or gives context to the patient's reported disease status. This information is to be collected at all follow-ups for all patients.

- hematology data (i.e., CBC results per standard of care)
- applicable concomitant medications
- further treatments, including transplant status
- transfusion information - date of transfusions, transfusion frequency, units, type (red blood cells, platelets), and reason for transfusion
- date of disease progression (if applicable)

- death (if applicable) - appropriately redacted documentation should be collected for source document verification (i.e. a redacted death certificate)

Safety Follow-up

Ongoing AEs at the time of study drug discontinuation will be followed until either 28 days after last administration of study drug or until the start of a subsequent systemic anticancer therapy, if earlier. SAEs will be followed to resolution or stabilization (see **Section 7.11.4**).

9 PLANNED STATISTICAL METHODS

Summary statistics will consist of absolute and relative frequencies of each category of discrete variables as well as of means, standard deviations, medians, minimum and maximum values of continuous variables. These summaries will be applied by dose cohort and by dose schedule in each phase of the study. Statistical methods will be applied primarily to the expansion cohorts. AML and MDS patients will be summarized together for safety and the AML efficacy population separately for efficacy analysis.

9.1 Determination of Sample Size for Primary Efficacy Analysis

The planned sample size for the pivotal portion of the study under Protocol Amendment 11 onwards is 170 patients, based on a three-stage design.

To determine the sample size under Amendment 11 inclusion criteria, historical reference response rates to standard treatments were ascertained through a meta-analysis based on 5 published studies that included PIF/ER AML patients selected from 963 publications identified through database search (**11**,**12**,**13**,**30**,**36**). Based on this analysis, the estimated combined CR/CRh rate was 11.8%, with a 95% confidence interval of 9.4% to 14.7%. The upper 95% CI of 14.7% was chosen as the rate for the null hypothesis (H0) and a target of 23% CR/CRh was established as a clinically meaningful endpoint in this patient population.

A three-stage design with two interim analyses will be employed to test the null hypothesis (H0) of CR/CRh rate = 14.7% against the alternative hypothesis (H1) of CR/CRh rate = 23%. Demonstration of CR/CRh rate improvement (H1) by rejecting H0 at a 1-sided type I error of 0.025 with adequate power (80%) in a sequential design requires 170 patients. Specifically, the planned number of patients and the futility and efficacy boundaries at each stage are as follows:

a. **First interim analysis:** The first interim analysis will be performed after 40 patients have been enrolled and either have had their responses evaluated or were discontinued for any reason from study treatment prior to their first response assessments. This interim analysis is for futility only, that is, the study enrollment will continue if at least 5 (12.5%) out of 40 patients have response of CR/CRh.

b. **Second interim analysis:** If the enrollment continues after the first interim analysis, a second interim analysis will be performed after additional 60 patients have been enrolled (100 patients in total) and either have had their response evaluated or were discontinued for any reason from study treatment prior to their first response assessment. This interim analysis is for both futility and efficacy. Specifically,

    i. If the number of CR/CRh is less than 15 (15%) out of 100 patients, then the study is considered futile and enrollment will be stopped.

    ii. If the number of CR/CRh is 15 (15%) or greater out of 100 patients, then enrollment will continue.

iii. If the number of CR/CRh is 25 (25%) or greater out of 100 patients, then H0 is rejected and the study is considered positive. The enrollment will still continue.

c. **Final Analysis:** If enrollment continues after the second interim, then the final analysis will be performed after additional 70 patients have been enrolled (170 patients in total) and either have had their response evaluated or were discontinued for any reason from study treatment prior to their first response assessments. If the number of CR/CRh is at least 35 (20.6%) out of the total of 170 patients, then H0 is rejected and the study is considered positive.

The above design yields a 1-sided alpha of 0.005 for the efficacy boundary at the second interim analysis and an overall 1-sided alpha of 0.024 with approximately 80% power at the final analysis. The probability of early termination under H0 (futility) in the first two stages is 55%.

9.2 Analysis Populations

Three populations will be used for analysis, as defined below:

- **Efficacy population** – All patients that have been enrolled under Protocol Amendment 11 or later and treated with flotetuzumab at the MTD of 500 ng/kg/day as a continuous 7-day per week IV infusion during Cycle 1, have received any portion of one dose of flotetuzumab, and met the definition of PIF/ER AML based on inclusion criteria as revised in Amendment 11 (see **Section 5.1**). This population will be used for the primary analysis of efficacy endpoints.
- **Safety population** – All patients who received any portion of any dose of flotetuzumab. AML and MDS will be analyzed as pooled and separate populations for safety and PK. The analysis of all safety endpoints will be based on the safety population.
- **Response evaluable population** – All patients in the efficacy population that have a baseline bone marrow assessment, have at least one post-infusion assessment of their disease status or were removed from the study for reason of documented evidence of disease progression, death or treatment-related adverse event. This population will be used in the summary of response rate as a secondary analysis.

9.3 Demographics and Baseline Characteristics

Patient disposition, demographics and baseline characteristics, AML status (PIF/ER) and prior therapy, AML subclassifications and cytogenetics, and medical history will be summarized.

9.4 Study Drug Exposures and Concomitant Medications

Study drug exposures and concomitant medications will be summarized by descriptive statistics. Dose intensity will be calculated as the percentage of dose actually administrated divided by the intended dose during the treatment period and up to the last dose on study drug. Dose intensity will be summarized by cycle.

9.5 Safety Endpoint(s) and Analyses

9.5.1 Adverse Events

Adverse events will be coded to the Medical Dictionary for Regulatory Activities (MedDRA). Only TEAEs, as defined in **Section 7.11.1** will be summarized in tables. Events prior to treatment (e.g., due to study-related procedure) will be listed in an appendix to the final study report. DLTs will be listed.

The following tables of AE data will be created to summarize the number and percent of patients who experience at least one event of each of the following types:

- All AEs
- Drug-related AEs
- AEs by CTCAE Grade
- Drug related AEs by CTCAE Grade
- AEs with CTCAE Grade severity ≥ Grade 3
- Drug-related AEs with CTCAE Grade severity ≥ Grade 3
- SAEs (this may be a listing if there are few events)
- Drug-related SAEs
- Fatal AEs (this may be a listing if there are few events)
- AEs that result in study discontinuation
- AEs that lead to withdrawal of study drug
- AEs categorized as AESI and/or IREs

Tabulated data will display the number and percent of patients that experience individual events by System Organ Class (SOC) and Preferred Term (PT). Events will be displayed alphabetically for SOC and in descending order of overall PT incidence within each SOC.

An overall summary of AEs will display the number and percent of patients who experience at least one event of each of the following types:

- Any AE
- Any drug-related AE

- Any AE with CTCAE Grade severity ≥ Grade 3
- Any drug-related AE with CTCAE Grade severity ≥ Grade 3
- Any SAE
- Any drug-related SAE
- Any AE that results in study or study drug discontinuation
- Any fatal AE
- Any AESI
- Any IREs

9.5.2 Laboratory Values

Summaries of laboratory values will display descriptive statistics for numerically quantified labs. Summaries will be grouped by lab panel (e.g., hematology, blood chemistry, and urinalysis) and will be displayed by visit for each lab parameter. Graphs of mean values over time or individual values at each time point may be used.

Shift tables will be used to display the percent of patients who have a shift in their lab values from normal at baseline to each post-baseline visit by CTCAE v4.0 severity Grade.

9.5.3 Other Safety Endpoints

Electrocardiograms will be collected and analyzed for evidence of cardiac toxicity, especially prolongation of QT interval. Vital signs will be summarized with descriptive statistics at each visit and time point where they are collected.

9.6 Efficacy Endpoints and Analyses

Disease activity will be assessed by the study investigators, by using CBC and peripheral blood cell morphological examination, examination of bone marrow aspiration (or biopsy if required), and physical examination. 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 (**Appendix 3**). Response assessment will be based on bone marrow aspirate/biopsy and the best CBC up to 14 days post bone marrow aspirate/biopsy. Responses will be categorized as:

- CR (mCR [morphologic CR], CRc [cytogenic CR], or CRm [molecular CR]);
- CRh;
- CRi (CRn [CR with incomplete neutrophil recovery]; CRp [CR with incomplete platelet recovery]);
- MLFS;

- PR;
- OB;
- SD;
- PD.

The number and percent of PIF/ER AML patients will be summarized by best overall response. Efficacy analyses will be based on the PIF/ER AML population (see **Inclusion Criterion 2**). All efficacy results will be summarized by PIF, ER and the total combined PIF/ER populations.

9.6.1 Primary Efficacy Analysis

The primary efficacy endpoint for the AML expansion cohort is the CR/CRh rate, calculated as the proportion of patients in the efficacy population that has achieved a best response of CR (mCR, CRc, or CRm) or CRh at any point during treatment by investigator's assessment, according to IWG AML response criteria (**Appendix 3**). CR/CRh rate will be summarized for the total combined PIF/ER patients as the primary analysis, as well as for PIF and ER patients separately, and for PIF/ER patients in the response-evaluable population as a sensitivity analyses. A two-sided exact 95% confidence interval will be calculated around response rates.

For the second interim analysis of 100 patients, as well as for the final analysis of 170 patients, all treated patients will be followed for at least 6 months or until death from any cause, whichever occurs first, prior to data cutoff for the analysis. If the trial passes the pre-specified efficacy boundary (25% observed CR/CRh rate) at the second interim analysis, a two-sided exact 95% confidence interval as described by Jennison and Turnbull (**21**) will be produced to account for the bias in the conventional exact confidence interval for fixed sample test.

9.6.2 Secondary Efficacy Analyses

Counts, percentages, and two-sided exact 95% confidence intervals will be used to describe categorical secondary variables. Kaplan-Meier (KM) methods will be used to estimate time-to-event endpoints, unless otherwise specified. All secondary efficacy endpoints will be summarized in both efficacy and response evaluable population by PIF, ER, and total.

9.6.2.1 Key Secondary Efficacy Analyses

The key secondary efficacy endpoints include CR rate, CRh rate, duration of response, post-baseline transfusion independence rate, HSCT rate, and incidence and duration of hospitalization.

Duration of response (DOR) will be calculated from the time of initial documentation of response to the time of disease relapse or death due to any cause, whichever occurs first. Patients who are still in response at study completion without alternative therapies will be censored at the time of their last disease assessment. Duration of response will be calculated

for patients who achieve the first evidence of CR or CRh as primary analysis. A separate exploratory analysis will be conducted for patients who achieve the first evidence of overall complete response (CR, CRh, CRi [CRn, CRp], or MLFS).

Post-baseline transfusion independence is defined as no transfusion during any consecutive 56-day period on-study. Post-baseline transfusion independence will be summarized for RBC and platelet transfusion separately and in total. Transfusion dependence at baseline is defined as any red blood cell or platelet transfusions during the 28 days prior to the first dose of study drug. The rate of conversion from transfusion dependence to transfusion independence will be calculated. The rate of patients who are transfusion independent at baseline and remain independent during any 56-day post-baseline period will also be calculated.

HSCT rate is defined as rate of successful HSCT through study treatment. Patients who received other therapies (except for conditioning therapy) for AML after discontinuation of study drug and later undergo a subsequent HSCT will not be included. Number and percentage of patients with HSCT will be summarized.

Incidence and Duration of Hospitalization, summaries will be provided for the efficacy population, as well as independently for patients who achieved CR or CRh. Summaries will include duration of initial hospitalization as well as incidence, number and duration of subsequent hospitalizations following the initial discharge, number of days hospitalized, and the reasons for hospitalization.

9.6.2.2 Additional Secondary Efficacy Analyses

Additional secondary efficacy endpoints include overall complete response rate, objective response rate, subgroup analyses of CR/CRh, CR, CRh and overall complete response rates, overall survival, event-free survival, time to response, mortality rate at 30, 60, 90, and 180 days, six-month and one-year survival.

Overall complete response rate (OCRR) is defined as the rate of CR+CRh+CRi [CRn, CRp]+MLFS according to IWG AML response criteria (**Appendix 3**), and includes any response which achieves < 5% BM blasts by morphology.

Objective response rate is defined as the rate of CR+CRh+CRi [CRn, CRp]+MLFS+PR according to IWG AML response criteria (**Appendix 3**).

Subgroup analyses of CR/CRh, CR, CRh, and overall complete response rates will be performed by baseline disease characteristic (e.g., age group [< 70, ≥ 70], gender, cytogenetic risk status [high, intermediate, low], prior line of therapy [1, 2, 3], TP53 gene mutations).

Overall survival is defined as the time between the date of the first dose of study drug and death from any cause. Patients who are alive or whose death status is unknown will be censored at the last date known to be alive on or before the data cutoff date.

Event-free survival is defined as the time from the first dose of study drug until date of relapse from CR, CRh, or CRi (CRn, CRp), or death from any cause, whichever occurs first.

Patients who are not known to have any of these events at study completion will be censored at the date of the last disease assessment.

Time to response (TTR) is defined as time from first dose of study drug to first CR, CRh, CRi (CRn, CRp), or MLFS, and will be summarized with descriptive statistics. Time to CR/CRh and time to best overall response will be calculated.

Mortality rate is defined as rate of death from any cause within 30, 60, 90, or 180 days of first dose of study drug.

Six-month survival rate is defined as the probability of survival at 6 months from first dose of study drug.

One-year survival rate is defined as the probability of survival at 1 year from first dose of study drug.

9.7 Other Assessments or Analyses

9.7.1 Immunogenicity

Anti-drug antibody production will be monitored using a bridging ELISA method. The proportion of patients who become positive in this assay will be reported.

9.7.2 Pharmacokinetics

Serum concentrations of flotetuzumab will be monitored using an electrochemiluminescence-based sandwich assay. PK analyses will be performed using industry standard software. Population PK modeling will be performed and an appropriate model and model parameters will be described. This analysis will be performed by an outside consultant.

9.7.3 Safety and Efficacy of Tocilizumab in Treatment of IRR/CRS

IRR/CRS events are captured as AESIs (see **Section 7.11.3**). The incidence, severity and duration of IRR/CRS events will be summarized in tables. Furthermore, safety with the use of tocilizumab will also be assessed.

9.7.4 Other Studies

The following exploratory studies may be performed in all or a subset of patients:

- CD123 expression on leukemic blasts in bone marrow and peripheral blood at baseline and over time
- Flow cytometric analysis of bone marrow and peripheral blood cells to describe the status of the leukemia and monocytes, natural killer (NK) cells, plasmacytoid dendritic cells (pDC), lymphocytes, and lymphocyte subsets and activation markers at baseline and over time
- Minimal residual disease (MRD)

- T-cell immune function (TCR repertoire, in vitro immune assessments)
- Transcriptomic bone marrow tumor microenvironment immune contexture
- Cytokine production at baseline and over time.

These data will be summarized by using descriptive statistics and, where appropriate, graphed over time.

9.8 Final Analysis at End of Trial

If there are patients continuing on study at the time of the primary analysis, a final analysis of all data will be carried out at the End of the Trial (as defined in **Section 4.4**).

10 QUALITY CONTROL AND ASSURANCE

Quality review activities will be undertaken to ensure accurate, complete, and reliable data. MacroGenics, Inc. and/or its representatives will do the following:

- Provide instructional material to the study sites, as appropriate.
- Sponsor a start-up training session (Investigator Meeting or Study Initiation Visit) to instruct the Investigators and study coordinators. This session will give instruction on the protocol, the completion of the eCRFs, and study procedures.
- Make periodic visits to the study site.
- Be available for consultation and stay in contact with the study site personnel by mail, e-mail, telephone, and/or fax.
- Review and evaluate eCRF data and use standard computer checks to detect and query errors in data collection.
- Conduct a quality review of the database.

10.1 Investigational Site Training

MacroGenics, Inc. will provide quality investigational staff training prior to study initiation. Training topics will include but are not limited to: GCP, AE reporting, study details and procedures, eCRFs, study documentation, informed consent, and enrollment of women of child-bearing potential.

10.2 Monitoring, Auditing and Inspections

To ensure the safety of participants in the study, compliance with applicable regulations, and to ensure accurate, complete, and reliable data, the Investigator will keep records of laboratory tests, clinical notes, and patient medical records in the patient files as source documents for the study (refer to **Section 11.7** for additional information on source documents).

MacroGenics, Inc. or its designee will monitor the study on a regular basis throughout the study period according to the study monitoring plan. The Investigator will allocate adequate time for such monitoring activities. The study monitor periodically will conduct a cross-check of a sample of the patient data recorded on eCRFs against source documents at the study site. The Investigator will also ensure that the monitor is given access to all the above noted study-related documents, source documents (regardless of media) and study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit. Queries may be raised if any datum is unclear or contradictory. The Investigator must address all queries.

Participation as an Investigator in this study implies acceptance of the potential for inspection by the study Sponsor/representatives, US or non-US government regulatory authorities, IRB/IEC and applicable compliance and quality assurance offices. The Investigator will

permit study-related audits and inspections and will provide access to all study-related documents (e.g., source documents, regulatory documents, data collection instruments, study data, etc.). The Investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

10.3 Data Entry and Computerized Systems

An electronic data capture system will be used in this trial. Other data assessments, such as ECG data, will be managed by central vendors for transfer to MacroGenics, Inc. or representative for use in the study analysis database.

11 ADMINISTRATIVE CONSIDERATIONS

11.1 Institutional Review Board (IRB/IEC) or Independent Ethics Committee (IEC) Approval

The Investigator should provide the Sponsor with a statement of compliance from the IRB/IEC indicating compliance with the applicable regulations in the region and ICH. Any documents that the IRB/IEC may need to fulfill its responsibilities, such as the protocol and any amendments, Investigator's Brochure, and information concerning patient recruitment, payment or compensation procedures, or information from the Sponsor will be submitted to the IRB/IEC. The IRB/IEC's written unconditional approval of the study protocol and the informed consent forms will be in the possession of the Investigator and the Sponsor before shipment of study drug to the Investigator's site. The Investigator will transmit the IRB/IEC's unconditional approval statement to the Sponsor. This approval must include the date of review, and refer to the study by protocol title and/or study number and version number and refer to the informed consent forms by version number or date. If the IRB/IEC or institution uses its own unique number for the protocol instead of the Sponsor's number, that unique number should be noted on the approval statement. If approval of the informed consent forms is stamped on the forms (instead of documented in the IRB/IEC approval statement) the date of approval and/or expiration must be included.

Protocol modifications or changes may not be initiated without approval from the Sponsor and, when required, prior written IRB/IEC approval, except when necessary to eliminate immediate hazards to the patients. Such modifications will be submitted to the IRB/IEC; written verification that the modification was submitted should be obtained.

The Investigator must submit to the IRB/IEC:

- The protocol and the Investigator's Brochure (IB) and any amendments or updates
- The informed consent form and any amendments or changes
- Any documents given to patients or potential patients (e.g., recruitment materials, diary cards) and the plan for distribution/use
- Revisions of other documents originally submitted for review or for notification
- Serious and/or unexpected AEs occurring during the study, according to local regulations/procedures
- New information that may adversely affect the safety of patients or conduct of the study
- At minimum, an annual update and/or request for re-approval of study, unless otherwise specified by IRB/IEC
- Protocol violations or deviations, as required by local regulations
- Notification when the study has been completed
- Proof of indemnity/liability insurance

11.2 Data Safety Monitor and Independent Data Monitoring Committee

An independent DSM (with disease-specific and/or immunotherapy expertise) was involved in all decisions regarding transition from the Single Patient Dose Escalation Segment to the Multi-patient Dose Escalation Segment and regarding the definition of MTDS and transition to the MTDS Cohort Expansion Segment of the study. In the Single Patient Dose Escalation Segment, safety data from all patients followed through Study Day 28 (Cycle 2 Day 1) was reviewed. Safety data from each dose escalation cohort in the Multi-patient Dose Escalation Segment was made available to the DSM after each cohort of patients had been followed for a minimum of 28 Study Days.

Safety evaluations will be conducted on an ongoing basis during the Cohort Expansion Segment of the study. Review by an IDMC (replacing the DSM previously involved in safety reviews), consisting of two physicians and one biostatistician, will be conducted in regular intervals. The interval analysis will utilize data from all cycles of treatment. If during the conduct of the interval cohort analysis, the Bayesian posterior probability is greater than 80% that the DLT (see **Section 4.2**) event rate is more than 20%, enrollment will be paused and an ad hoc meeting of the IDMC will be held to consider dose reduction or study termination (see **Section 4.1**). The IDMC will also review aggregate efficacy data for two planned interim analyses to determine enrollment continuation according to futility and efficacy boundaries described in **Section 9.1**.

11.3 Ethical Conduct of the Study

The investigational study will be conducted according to the Protection of Human Subjects (21 CFR 50), IRBs (21 CFR 56), Obligations of Clinical Investigators (21 CFR 312.60 – 312.69), the current ICH Guideline for Good Clinical Practice (ICH E6), and all other applicable regulations.

The protocol and the informed consent document will be reviewed and approved by the IRB/IEC of each participating center before study initiation. SAEs regardless of causality will be reported to the Sponsor/designee and to the IRB/IEC, and the Investigator will keep the IRB/IEC informed regarding the progress of the study.

11.4 Patient Information and Consent

It is the responsibility of the Investigator to obtain and document oral or written informed consent from the patient or legally authorized representative. Informed consent in compliance with the principles of informed consent in ICH E6 and all applicable local regulations should be obtained before any protocol-specified procedures or interventions are conducted. The Sponsor reserves the right to delay initiation of the study at a site where informed consent forms do not meet the standards of applicable local regulations or ICH E6.

Information should be given to the patient in both oral and written form, and patients or their legally authorized representatives must be given ample opportunity to inquire about details of the study.

The consent form generated by the Investigator must be approved by the IRB/IEC. The Investigator will provide the Sponsor with a copy of the IRB/IEC-approved consent forms and a copy of the IRB/IEC's written approval before the start of the study.

Consent forms must be written so as to be understood by the prospective patient. Informed consent will be documented by the use of a written consent form approved by the IRB/IEC. Special attention will be given to the sections of the consent form relevant to genetic analyses (minimal residual disease and T lymphocyte repertoire). The form must be signed and dated by the patient or the patient's legally authorized representative, and by the person who conducted the discussion of the informed consent.

All versions of each patient's signed informed consent form must be kept on file by the Investigator for possible inspection by regulatory authorities and/or authorized MacroGenics' professional and regulatory compliance persons. The patient or the patient's legal representative should receive a copy of the signed and dated written informed consent form and any other written information provided to the patients.

11.5 Patient Confidentiality

To maintain confidentiality of patients, all laboratory specimens, evaluation forms, reports, and other records will be identified by a coded number and initials only. Clinical information will not be released without written permission of the patient, except as necessary for monitoring by the relevant regulatory authorities, the Sponsor of the clinical trial, or the Sponsor's representative. The Investigator must also comply with all local applicable privacy regulations [e.g., US Health Insurance Portability and Accountability Act of 1996 (HIPAA)], on protection of individuals with regard to personal data.

11.6 Case Report Forms and Study Records

Source data in a clinical trial are the original records or certified copies where clinical observations are first recorded, which may include, but are not limited to, the patient's medical file, original laboratory reports, histology, and pathology reports (as applicable). The Investigator is responsible for maintaining adequate and accurate medical records from which accurate information will be entered onto eCRFs designed to capture all observations and other data pertinent to the clinical investigation. Data should be recorded on source documents and entered onto eCRFs. Electronic CRFs should be filled out completely by the Investigator or his/her designee. Prior to eCRF database lock, the Investigator will verify the completeness and accuracy of the data and indicate that he/she has done so by providing a signature on the appropriate eCRF. The Investigator will retain a copy of all source documents.

11.7 Access to Source Documentation

The Investigator and study center will permit the Sponsor, its representatives, IRB/IEC and all relevant regulatory agencies direct access to all original source data and documents regardless of media, for study monitoring audits and inspections.

The Investigator may be subjected to a field audit by MacroGenics (or designee) and/or regulatory inspectors in order to validate the participation of patients in the study and to verify the data reported on the eCRFs on file at MacroGenics, Inc. MacroGenics should be notified immediately of any audits scheduled by any regulatory authorities. Copies of audit reports, findings and/or correspondence from regulatory authorities for audits conducted on a MacroGenics-sponsored study should be promptly forwarded to MacroGenics.

11.8 Retention of Data

Per ICH guidelines, all essential documents, including eCRFs, source documents (regardless of media), signed informed consent forms, and laboratory test results, should be retained by the Investigator for at least 2 years after last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since formal discontinuation of clinical development of the investigational product. There may be other circumstances for which MacroGenics, Inc. is required to maintain study records for longer periods (e.g., applicable local regulations); therefore, MacroGenics, Inc. should be contacted before study records are removed from the investigational site for any reason. The Investigator must obtain written permission from MacroGenics, Inc. prior to destruction of study documents.

11.9 Financial Disclosure

The Investigator and Sub-Investigators will be required to disclose any financial arrangement whereby the value of the compensation for conducting the study could be influenced by the outcome of the study. The following information will be collected: any significant payments of other sorts from MacroGenics, Inc., such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria, any proprietary interest in flotetuzumab, and any significant equity interest in MacroGenics, as defined in 21 CFR 54. Investigators are obligated to update the Sponsor with any changes in reported information up to one year following the end of the study (as defined in **Section 4.4**).

In consideration of participation in the study, MacroGenics, Inc., or its designee, will pay the Investigator or nominated payee the sums set out in the payment schedule attached to the Investigator agreement.

Financial disclosure information will be documented in writing and signed and dated by the Investigator. This information will be collected at the beginning of the study.

11.10 Publication and Disclosure Policy

Data collected in this clinical study belong to the study Sponsor, which will formulate a policy on the use of study data. This includes authorship issues, scheduling and prioritizing analyses for reports, publications, and presentations, and developing a review and approval process.

11.11 Discontinuation of the Study or Study Sites

11.11.1 Discontinuation of Study Sites

Participation may be discontinued if MacroGenics, the Investigator, a regulatory authority, or the IRB/IEC of the study sites deems it necessary for any reason.

11.11.2 Discontinuation of the Study

The study may be discontinued by a regulatory authority or at the discretion of the Sponsor.

The Investigator maintains the right to discontinue his/her participation in the study should his/her clinical judgment so dictate. The Investigator will notify the IRB/IEC of any study discontinuation. Study records must be retained as noted above.

11.12 Identification of the Coordinating Principal Investigator

Dr. John DiPersio (Washington University School of Medicine, St. Louis, MO) has been appointed as the US Coordinating Principal Investigator. For Europe, Dr. Norbert Vey (Institute Paoli-Calmettes, Marseille, France) is the European Coordinator.

As part of their responsibilities, the Coordinating Principal Investigator/European Coordinator will review the final Clinical Study Report. Agreement with the final Clinical Study Report will be documented by the dated signature of the Coordinating Principal Investigator/European Coordinator.

12 REFERENCES

Note: *Newly added literature references are in colored text. Previously cited references are in black text.*

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Appendix 1 Schedule of Events Applicable to Cohort Expansion (All Cycles)

The study procedures outlined below also apply to patients with AML who participate in the ruxolitinib cohort.

Informed consent must be obtained prior to performing any screening procedures, and may be obtained prior to the start of the 14 day screening period.

		Screening			Cycle 1 28 days					Subsequent Cycles 28 days					End of Treatment	Post-treatment Follow-up
	Day	**Up to -14 Days**	**Up to -5 Days**	**Up to -1 Days**	**1**	**8**	**15**	**22**	**25 and/or 26**	**1**	**8**	**15**	**22**	**25 and/or 26**	**28-days Following Last Treatment or At Time of Treatment Failure**	
	Study Day Window				**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+3**	
	Week				**1**	**2**	**3**	**4**		**5, 9, 13, etc.**	**6, 10, 14, etc.**	**7, 11, 15, etc.**	**8, 12, 16, etc.**			
EVALUATION OR PROCEDURE																
STUDY DRUG ADMINISTRATION																
TLS prophylaxis				X												
Flotetuzumab [3]					1→7 [1]	8→14 [1]	15→21 [1]	22→24 [1]	25→28 [1]	1→7 [2]	8→14 [2]	15→21 [2]	22→24 [2]	25→28 [2]		
*1. Cycle 1 only: flotetuzumab administration will occur from Days 1 to 28 (7-day continuous IV infusion). Week 1 only: Patients will receive step-up dosing as detailed in **Table 2**. Weeks 2-4: Dosing will follow the scheme shown in **Table 2**.* *2. All other cycles: flotetuzumab administration will occur in a 7-Day continuous infusion schedule according to **Table 2**.* *3. All cycles: Scheduled dose/bag changes have a ± 2 hour window.*																
ELIGIBILITY																
Diagnostic Bone Marrow Aspirate and core biopsy for local histopathological and immunohistochemical analysis, central flow cytometric evaluation, and next generation sequencing		X [4]														
4. Obtain if not performed within 30 days before the start of treatment (Cycle 1, Day 1).																
Buccal mucosal sampling (Only Washington U., St. Louis, MO)		X														

		Screening			Cycle 1 28 days					Subsequent Cycles 28 days					End of Treatment	Post-treatment Follow-up
	Day	**Up to -14 Days**	**Up to -5 Days**	**Up to -1 Days**	**1**	**8**	**15**	**22**	**25 and/or 26**	**1**	**8**	**15**	**22**	**25 and/or 26**	**28-days Following Last Treatment or At Time of Treatment Failure**	
	Study Day Window				**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+3**	
	Week				**1**	**2**	**3**	**4**		**5, 9, 13, etc.**	**6, 10, 14, etc.**	**7, 11, 15, etc.**	**8, 12, 16, etc.**			
Medical history			X													
Report HLA typing (if available)			X													
Review of concomitant medications, including all transfusion requirements up to 28 days prior to dosing			X		-- Continuous --										X	X [5]
5. *During follow-up, collect the following transfusion information: date of last transfusion; transfusion frequency, type (red blood cells, platelets), and units; and reason for transfusion. Also collect further treatment and transplant status. Applicable concomitant medications that support or give context to the patient's reported disease status will also be collected.*																
ECOG PS			X							X [6]						
6. *Cycle 2, Day 1 only*																
ECG [7]			X				X									
Left ventricular ejection fraction	X [8]															
7. *ECG to be performed in triplicate (at approximately 1-minute intervals) at screening and Cycle 1 Day 15 (or when patient reaches 500 ng/kg/day, if this is later than Day 15). Subsequent ECGs to be performed when clinically indicated, and will be single tracing unless clinical circumstances, and at the discretion of the investigator, indicate otherwise. Treatment with flotetuzumab should be held or delayed for a clinically significant QTcF prolongation > 480 msec.* 8. *If patient has a history or signs and symptoms of cardiac disease, obtain echocardiogram (or, except in Germany, MUGA if indicated) if one was not performed within 30 days before the start of treatment (Cycle 1, Day 1). An echocardiogram (or, except in Germany, MUGA if indicated) may also be conducted during the study if clinically indicated.*															
PFT	X [9]															
9. *PFT to include DLCO and FEV1, if testing was not performed within 30 days before the start of treatment (Cycle 1 Day 1). PFT may also be conducted during the study if clinically indicated. During the COVID-19 pandemic, except in Germany, if PFT equipment is not available, a correlative stair-climbing test may be conducted instead, together with lung CT and echocardiogram irrespective of cardiac history (see **Section 8.1.1**).*																
Serum or Urine β-hCG pregnancy test [10]			X		X					X						

		Screening			Cycle 1 28 days					Subsequent Cycles 28 days					End of Treatment	Post-treatment Follow-up
	Day	**Up to -14 Days**	**Up to -5 Days**	**Up to -1 Days**	**1**	**8**	**15**	**22**	**25 and/or 26**	**1**	**8**	**15**	**22**	**25 and/or 26**	**28-days Following Last Treatment or At Time of Treatment Failure**	
	Study Day Window				**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+3**	
	Week				**1**	**2**	**3**	**4**		**5, 9, 13, etc.**	**6, 10, 14, etc.**	**7, 11, 15, etc.**	**8, 12, 16, etc.**			
10. <u>All Cycles:</u> Day 1 only, predose or prior to bag change as applicable. Results of the pregnancy test must be negative prior to starting new cycle of flotetuzumab infusion. Women of childbearing potential only.																
Patient registration			X													
SAFETY, PK, & MECHANISTIC EVALUATION																
Physical examination [11]			X						X [12]					X [12]	X	
Weight [13]			X		X	X	X	X	X	X	X	X	X	X	X	
11. Includes height (screening only), and examination of skin, HEENT (head, eyes, ears, nose, and throat), lymph nodes, heart, chest, lungs, abdomen, extremities, and neurologic system *12. Day 25-28 (to coincide with bone marrow aspiration and biopsy)* *13. <u>All Cycles</u>: Obtain weight daily during in hospital stay and at each clinic visit while an outpatient.*																
CBC with differential, platelet count			X [14]		X [15]	X [15]	X [15]	X [15]	X [15]	X [16]	X [16]	X [16]	X [16]	X [16]	X	
14. May be used as Study Day 1 baseline if within 24 hours of drug administration start *15. <u>Cycle 1 only</u>, Days 1 -28: Study Day 1 - pre-infusion; Study Days 2 through 8 - sample may be drawn at any time relative to infusion. If inpatient, Study Days 9-28 (sample may be drawn any time relative to infusion). If outpatient, Study Days 15, 22 and 25 (sample may be drawn any time relative to infusion).* *16. <u>All other cycles</u>: Study Day 1, 8, 15 and 22 – prior to bag change as applicable; Study Days 5, 12, and 19 - sample may be drawn at any time relative to infusion (if patient returns to study site for end of infusion or bag change; collect prior to end of infusion/bag change); Study Day 26 - sample may be drawn at any time relative to infusion (when patient returns to study site for end of infusion or bag change; collect prior to end of infusion/bag change). A CBC must also be drawn on the same day of the bone marrow aspirate or biopsy.*																

		Screening			Cycle 1 28 days					Subsequent Cycles 28 days					End of Treatment	Post-treatment Follow-up
	Day	**Up to -14 Days**	**Up to -5 Days**	**Up to -1 Days**	**1**	**8**	**15**	**22**	**25 and/or 26**	**1**	**8**	**15**	**22**	**25 and/or 26**	**28-days Following Last Treatment or At Time of Treatment Failure**	
	Study Day Window				**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+3**	
	Week				**1**	**2**	**3**	**4**		**5, 9, 13, etc.**	**6, 10, 14, etc.**	**7, 11, 15, etc.**	**8, 12, 16, etc.**			
Chemistry panel and CRP			X [17]		X [18]	X [18]	X [18]	X [18]	X [18]	X [19]	X [19]	X [19]	X [19]	X [19]	X	
17. May be used as Study Day 1 baseline if within 24 hours of drug administration start *18. Cycle 1: Study Day 1 (pre-infusion), Days 2 through 8 (sample may be drawn any time relative to infusion). If inpatient, Study Days 9-28 (sample may be drawn any time relative to infusion). If outpatient, Study Days 15, 22 and 25 (sample may be drawn any time relative to infusion).* *19. All other cycles: Study Day 1, 8, 15 and 22 – pre-infusion or prior to bag change as applicable; Study Days 5, 12, and 19 - if patient returns to study site for end of infusion or bag change; collect prior to end of infusion/bag change; Study Day 26 - when patient returns to study site for end of infusion or bag change; collect prior to end of infusion/bag change.*															
Special Chemistry			X [20]		X [21]	X [21]	X [21]	X [21]		X [21]	X [21]	X [21]	X [21]		X	
20. May be used as Study Day 1 baseline if within 24 hours of drug administration start *21. Cycle 1 and all other cycles: Study Days 1, 8, 15, and 22. Samples to be obtained pre-infusion or prior to dose or bag change as applicable.*																
Prothrombin time; Activated partial thromboplastin time			X [22]		X [23]					X [23]					X	
22. May be used as Study Day 1 baseline if within 24 hours of drug administration start *23. All cycles: Study Day 1. Samples to be obtained pre-infusion or prior to bag change, as applicable*																
Urinalysis with microscopic evaluation of urinary sediment			X [24]		X [25]					X [25]					X	
24. May be used as Study Day 1 baseline if within 24 hours of drug administration start *25. All cycles: Study Day 1. Samples to be obtained pre-infusion or prior to bag change as applicable*																
PK for flotetuzumab sampling	---See **Appendix 2** for all sample times and windows---															
ADA blood sample (anti-flotetuzumab)	---See **Appendix 2** for all sample times and windows---															
Peripheral Blood Sample for Flow Cytometric Analysis	---See **Appendix 2** for all sample times and windows---															
Peripheral Blood Sample for T cell Repertoire	---See **Appendix 2** for all sample times and windows---															

		Screening			Cycle 1 28 days					Subsequent Cycles 28 days					End of Treatment	Post-treatment Follow-up
	Day	**Up to -14 Days**	**Up to -5 Days**	**Up to -1 Days**	**1**	**8**	**15**	**22**	**25 and/or 26**	**1**	**8**	**15**	**22**	**25 and/or 26**	**28-days Following Last Treatment or At Time of Treatment Failure**	
	Study Day Window				**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+3**	
	Week				**1**	**2**	**3**	**4**		**5, 9, 13, etc.**	**6, 10, 14, etc.**	**7, 11, 15, etc.**	**8, 12, 16, etc.**			
Vital signs (temperature, respiration, pulse oximetry, heart rate, blood pressure)			X		X [26]	X [26]	X [26]	X [26]	X [26]	X [27]	X [27]	X [27]	X [27]	X [27]		
26. Cycle 1 only, Days 1 - 7: Study Day 1 – pre-infusion, at 1 hour, and 4 hours after infusion start; Study Days 2 - 7– prior to dose change and at 1 hour and 4 hours after dose change; except for pre-infusion or prior to dose change, vital signs can be taken within ± 10 minutes of indicated collection time. If inpatient: Study Day 11 – approximately 96 hours after bag change; Study Days 15, 19, 22 and 26 – prior to bag changes as applicable. If outpatient: Study Days 15, 22, and 26 – prior to bag changes as applicable. *27. All other cycles: Days 1, 8, 15, and 22 – prior to bag change as applicable; Days 5, 12, and 19 – if patient returns to study site for end of infusion or bag change; collect at end of infusion/before bag change; Study Day 26 – when patient returns to study site for end of infusion or bag change; collect at end of infusion/prior to bag change.*															
Toxicity review and AE assessment [28]				--- Continuous ---											X	X
28. Including orientation to person, place, and time. Ongoing AEs at the time of study drug discontinuation will be followed until either 28 days after last administration of study drug or until the start of a subsequent systemic anticancer therapy, whichever is earlier.																
THERAPEUTIC ACTIVITY																
Re-evaluation Bone Marrow Aspirate and core biopsy for local histopathological and immunohistochemical analysis, central flow cytometric analysis, and next generation sequencing [29]								X						X	X [30]	X [31]
Survival																X [32]

		Screening			Cycle 1 28 days					Subsequent Cycles 28 days					End of Treatment	Post-treatment Follow-up
	Day	**Up to -14 Days**	**Up to -5 Days**	**Up to -1 Days**	**1**	**8**	**15**	**22**	**25 and/or 26**	**1**	**8**	**15**	**22**	**25 and/or 26**	**28-days Following Last Treatment or At Time of Treatment Failure**	
	Study Day Window				**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+ 3**	**+3**	
	Week				**1**	**2**	**3**	**4**		**5, 9, 13, etc.**	**6, 10, 14, etc.**	**7, 11, 15, etc.**	**8, 12, 16, etc.**			

29. *For Cycles > 1, bone marrow assessment should be performed at the end of every cycle whenever possible. A CBC must be drawn on the same day of the bone marrow aspirate. At the end of Cycle 2, and at the end of each subsequent even cycle, in consultation with the Medical Monitor, the bone marrow assessment may be missed if the patient has clearly not achieved CR or CRh using the following criteria: the patient has not achieved CR/CRh because the peripheral blood neutrophils are < 500/μL or platelets are < 50,000/μL), based on Study Day 22 CBC results for the cycle in question; and the results of the bone marrow assessment will not influence the decision to continue study treatment (i.e., assessment of risks and benefits).*
30. *If indicated to establish treatment failure.*
31. ***Bone Marrow Collections: Responders (CR, CRh, CRi [CRn, CRp], or MLFS):*** *bone marrow aspirate and biopsy collection at 3 and 6 months post treatment at the study site. After 6 months post treatment, bone marrow aspirate and/or biopsy will be performed per standard of care.* ***Non-responders:*** *bone marrow aspirate and/or biopsy performed per standard of care.* ***All patients:*** *During study follow-up, if patients have a bone marrow aspiration and/or biopsy done as part of their standard of care, they have the option to consent to donate aspirate or biopsy specimens for assessment of disease status and further research to help elucidate immune mechanisms involved in flotetuzumab function. As with all other biopsies collected for this study, these samples may be used for histopathological and immunohistochemical evaluation, flow cytometric evaluation, and minimal residual disease analysis.*
32. ***Follow-up Frequency: Responders (CR, CRh, CRi [CRn, CRp], or MLFS):*** *monthly visits at the study site for 6 months post end of treatment. After 6 months post treatment, patients may be followed up at the study site or by a local physician, and follow-up data will be collected at 3-month intervals.* ***Non-responders:*** *follow up at the study site or by a local physician, and follow-up data will be collected at 3-month intervals post treatment.*

Appendix 2 Pharmacokinetic, Immunogenicity, and Pharmacodynamic Biomarkers Blood Sampling Schedule

Cycle	Day	PK [1]	ADA	PBMC Flow Cytometry	PBMC T Cell Repertoire
Screening	Screen			Screening [2]	
Cycle 1	1	Pre infusion	Pre Infusion	Pre Infusion	Pre Infusion
	7-14	Only done **ONCE** on Days 7-14, the first time the patient reaches 500 ng/kg/day dose level One sample at least 1 hour after 500 ng/kg/day dose level reached		**Only done on Day 8** Sample may be drawn at any time relative to infusion	
	15	**Patient reached 500 ng/kg/day dose level before Day 15:** Sample may be drawn at any time relative to infusion **Patient reaches 500 ng/kg/day dose level on Day 15:** Sample at least 1 hour after 500 ng/kg/day dose level reached		Sample may be drawn at any time relative to infusion	
	22	**Patient reached 500 ng/kg/day dose level before Day 22:** Sample may be drawn at any time relative to infusion **Patient reaches 500 ng/kg/day dose level on Day 22:** Sample at least 1 hour after 500 ng/kg/day dose level reached		Sample may be drawn at any time relative to infusion	
	25	**Patient reached 500 ng/kg/day dose level before Day 25:** Sample may be drawn at any time relative to infusion **Patient reaches 500 ng/kg/day dose level on Day 25:** Sample at least 1 hour after 500 ng/kg/day dose level reached		Sample may be drawn at any time relative to infusion	Sample may be drawn at any time relative to infusion
	29	Immediately prior to end of infusion [3]			

Cycle	Day	PK [1]	ADA	PBMC Flow Cytometry	PBMC T Cell Repertoire
Cycles ≥ 2	1	Sample may be drawn at any time relative to infusion	Sample may be drawn at any time relative to infusion	Sample may be drawn at any time relative to infusion	Sample may be drawn at any time relative to infusion
End of Treatment	n/a	28-days following last treatment or at time of treatment failure	28-days following last treatment or at time of treatment failure	28-days following last treatment or at time of treatment failure	28-days following last treatment or at time of treatment failure

Abbreviations: ADA = anti-drug antibody; PBMC = peripheral blood mononuclear cell; PK = pharmacokinetics.

1 For patients who will discontinue therapy, a PK sample is to be collected at end of infusion. When dosing is delayed for ≥ 24 hours, PK samples should not be collected on days when no study drug is administered. After Cycle 1 Day 1, PK samples do not need to be collected if patient does not reach 500 ng/kg/day dose level.

2 Sample may be drawn up to Day -5.

3 Cycle 1 Day 29 PK samples are only to be collected if there is a > 24 hour interruption in treatment between Cycles 1 and 2.

Appendix 3 International Working Group (IWG) Response Criteria for Acute Myeloid Leukemia (AML) Modified for Assessment of AML in this Study

Response Criterion	Time of Assessment	Neutrophils* (µL)	Platelets* (µL)	Bone Marrow Blasts (%)	Other
Complete Remission (CR)	Day 25 + 3 days (each cycle)	≥ 1,000	≥ 100,000	< 5	Transfusion[#] Extramedullary disease (EMD)
• Morphologic CR (mCR)	Day 25 + 3 days (each cycle)	≥ 1,000	≥ 100,000	< 5	Transfusion[#] EMD
• Cytogenetic CR (CRc)	Day 25 + 3 days (each cycle)	≥ 1,000	≥ 100,000	< 5	Cytogenetics – normal, EMD
• Molecular CR (CRm)	Day 25 + 3 days (each cycle)	≥ 1,000	≥ 100,000	< 5	Molecular – negative, EMD
CR with partial hematologic recovery (CRh)	Day 25 + 3 days (each cycle)	≥ 500	≥ 50,000	< 5	Transfusion[$] EMD
CR with incomplete blood count recovery (CRi)	Day 25 + 3 days (each cycle)	< 1,000	< 100,000	< 5	Transfusion[$] EMD
• CR with incomplete neutrophil recovery (CRn)	Day 25 + 3 days (each cycle)	< 1,000	≥ 100,000	< 5	Transfusion[$] EMD
• CR with incomplete platelet recovery (CRp)	Day 25 + 3 days (each cycle)	≥ 1,000	< 100,000	< 5	Transfusion[$] EMD
Morphologic leukemia-free state (MLFS)	Day 25 + 3 days (each cycle)	NA	NA	< 5	EMD
Partial remission (PR)	Day 25 + 3 days (each cycle)	> 1,000	> 100,000	> 50% decrease or decrease to 5-25	Blasts < 5% if Auer rod positive
Other benefit (OB)	Day 25 + 3 days (each cycle)	NA	NA	> 30% decrease	NA
Stable disease (SD)	Day 25 + 3 days (each cycle)	NA	NA	Absence of CR, CRh, CRi, PR, MLFS, OB, and criteria for PD not met	NA

Response Criterion	Time of Assessment	Neutrophils* (µL)	Platelets* (µL)	Bone Marrow Blasts (%)	Other
Progressive disease	NA	NA	NA	Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood: • > 50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with < 30% blasts at baseline; or persistent marrow blast percentage of > 70% over at least 3 mo; without at least a 100% improvement in ANC to an absolute level (>0.5 x 10^9/L [500/mL], and/or platelet count to >50 x 10^9/L [50 000/mL] non-transfused); or • > 50% increase in peripheral blasts (WBC x % blasts) to > 25 x 10^9/L (> 25 000/mL) (in the absence of differentiation syndrome)†; or • New extramedullary disease	**In general, at least 2 cycles of flotetuzumab should be administered to determine "progressive disease".** "Progressive disease" is usually accompanied by a decline in ANC and platelets, increased transfusion requirement, and decline in performance status or increase in symptoms
Recurrence/relapse	Relapse after CR is defined as a reappearance of leukemic blasts in the peripheral blood and ≥ 5% blasts in the bone marrow not attributable to any other cause (e.g., bone marrow regeneration after consolidation therapy)				

CR = complete remission (includes mCR, CRc, CRm).
CRi = includes neutrophil count ≥ 1000 or platelet count ≥ 100,000.
*Assessment is quantitated by BM biopsy and best CBC (up to 14 days post BM biopsy).
[#] No RBC and/or platelet transfusion within 5 days prior to response assessment.
[$] No platelet transfusion within 5 days prior to response assessment

† Certain targeted therapies, for example, those inhibiting mutant IDH proteins, may cause a differentiation syndrome, that is, a transient increase in the percentage of bone marrow blasts and an absolute increase in blood blasts; in the setting of therapy with such compounds, an increase in blasts may not necessarily indicate PD.

Appendix 4 Eastern Cooperative Oncology Group (ECOG) Performance Status

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light house work or office work)
2	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	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry-on any self-care. Totally confined to bed or chair.
5	Dead

Appendix 5 Principal Investigator's Agreement

Study Title:	A Phase 1/2, First-in-Human, Dose Escalation Study of MGD006, a CD123 x CD3 Dual Affinity Re-Targeting (DART) Bi-Specific Antibody-Based Molecule, in Patients with Relapsed or Refractory Acute Myeloid Leukemia or Intermediate-2/High Risk Myelodysplastic Syndrome
Study Number:	CP-MGD006-01

I have read the protocol described above.

I have fully discussed the objectives of this trial and the contents of this protocol with the Sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution of the ethical review of the study, without written authorization from MacroGenics, Inc. It is, however, permissible to provide information to a subject in order to obtain consent.

I agree to conduct this trial according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with ICH guidelines on GCP and with the applicable regulatory requirements.

I understand that the Sponsor may decide to suspend or prematurely terminate the trial at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial, I will communicate my intention immediately in writing to the Sponsor.

Signed: _____ **Date:** _____

PI Name (printed): _____

PI Affiliation: _____

PI Address: _____

PI Phone number: _____

CP-MGD006-01 Protocol Amendment 13 (26-Apr-2021)
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