Protocol Title: A Phase Ib to Investigate the CD123-targeted DART Flotetuzumab Following Allogeneic Transplant for Patients with CD123+ Acute Myeloid Leukemia

Sponsor: Johns Hopkins Bone Marrow Transplant P01 CA015396

Principal Investigator: Jonathan A Webster, MD
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins Hospital
1650 Orleans Street, CRB1 Rm 245
Baltimore, MD 21287
410-614-9106 (p)
410-614-7279 (f)
jwebst17@jhmi.edu

Statistician: Ravi Varadhan PhD

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1. Objectives

1.1. Primary Objective

Define the maximum tolerated dose (MTD) of flotetuzumab in patients with relapsed/refractory AML following alloHSCT.

1.2. Secondary Objective(s)

1.2.1. Response-related Objectives

1) Assess response (CR, CRi, PR) to flotetuzumab in patients with relapsed AML following alloHSCT

1.2.2. Toxicity-related Objectives

2) Assess acute GVHD incidence
3) Determine chronic GVHD incidence
4) Measure non-relapse mortality

2. Background

2.1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease and treatment outcomes with initial intensive induction chemotherapy are dependent on a number of factors including patient age, performance status, cytogenetics, molecular markers, and a history of an antecedent hematologic disorder or prior chemotherapy.[1-4] In younger patients, complete remission (CR) rates of ≥80% can be achieved with intensive chemotherapy, but 5-year overall survival is ~40%.[5] Complete remissions are less frequent with advancing age with <50% of those over age 70 ever achieving a CR, and a concomitant decrease in 5-year overall survival to ~10% even among patients who are healthy enough for intensive chemotherapy.[1,2] Thus, most patients with a new diagnosis of AML who undergo intensive induction chemotherapy are able to achieve a first remission, but long-term survival remains poor due to disease relapse and treatment-related complications. This has led to the development of a number of strategies for post-remission therapy to prevent relapse including consolidation chemotherapy and allogeneic hematopoietic stem cell transplant (alloHSCT).[5] The majority of new AML patients present with disease that is classified as intermediate or poor risk by cytogenetics, and multiple large studies

have shown that overall survival in these risk groups is improved by using alloHSCT as post-remission therapy.[1,6,7]

2.2. Allogeneic Transplant in AML

While alloHSCT improves overall survival for the majority of patients with AML, mortality remains quite high due to both treatment-related mortality (TRM) and disease relapse. The initial approach to alloHSCT for AML relied on myeloablative conditioning (MAC) and yielded relapse rates of 21-24%, but this intensive conditioning regimen was not appropriate for older patients and resulted in TRM as high as 43%.[8-11] The high rates of TRM with MAC led to the development of alternative conditioning regimens that would allow allogeneic stem cell engraftment while reducing fatal complications. The initial reduced-intensity conditioning (RIC) regimens led to a significant reduction in non-relapse mortality (NRM) to 6.5-17%, but a concomitant increase in relapse incidence ranging from 28.6% to as high as 51% in patients with poor risk cytogenetics.[12-14] Further reductions in the intensity of conditioning have made alloHSCT available to older patients, but this nonmyeloablative (NMA) conditioning approach results in relapse rates of 42-54%.[15-17] Thus there is a need to develop better strategies to identify which patients are at highest risk for post-transplant relapse, develop maintenance strategies to augment the graft-versus-leukemia (GVL) effect to reduce disease relapse, and develop treatment strategies for patients who relapse post-transplant.

2.3. MRD and Post-transplant Relapse Risk

The detection of minimal residual disease (MRD) in spite of a morphologic CR is a validated prognostic marker for relapse risk in acute lymphoblastic leukemia (ALL), and it has shown promise as a marker of relapse risk for AML patients undergoing alloHSCT. In ALL, the detection of MRD prior to transplant is significantly associated with increased relapse risk and decreased survival, while the detection of MRD post-transplant has been used to guide pre-emptive donor lymphocyte infusion (DLI) to stimulate the GVL effect because it is highly predictive of relapse.[18-27] The ability to reliably detect MRD by both multiparameter flow cytometry (MFC) and PCR amplification of the Ig or TCR genes has allowed for extensive study of the prognostic significance of MRD in ALL, whereas the latter method is not applicable in AML, which has made it necessary to develop alternate markers of MRD.[28] In AML, the presence of MRD by MFC is highly correlated with the expression of leukemia-specific fusion transcripts, and patients with persistent MRD prior to and following transplant have worse overall survival.[29] For example, Zhou et al. found that patients with persistent MRD by MFC prior

to transplant had a three-year relapse-free survival (RFS) of just 17% as opposed to a three-year RFS of 70% among patients who cleared their MRD prior to a myeloablative transplant, and outcomes were even worse for those with MRD post-transplant with a three-year RFS of just 13%.[30] Subsequent studies have shown that the 3-year cumulative incidence of relapse is similar among patients with pre-transplant MRD by MFC who undergo myeloablative (63%) or nonmyeloablative conditioning (57%) with nearly all relapses occurring within the first year post-transplant.[31] As an alternative to MFC, the panleukemia marker WT1 is expressed in 90% of AML, and WT1 gene expression has been observed to increase prior to cytologic relapse in post-transplant patients with higher levels of expression correlating with increased risk of relapse.[32-34] Goswami et al. analyzed pre-transplant peripheral blood specimens for 74 patients who underwent a MAC alloHSCT for AML at the National Institutes of Health, and showed that a five-gene RQ-PCR array including WT1 was 89% sensitive and 100% specific in predicting post-transplant relapse within one year of transplant.[35] This five-gene RQ-PCR array as a measure of MRD has been tested exclusively in the setting of MAC alloHSCT where the relapse risk is lower than with NMA alloHSCT; thus this technique is likely less sensitive for predicting relapse after NMA alloHSCT but should retain its specificity.

2.4. Other Risk Factors for Post-Transplant Relapse

In addition to the presence of MRD in the peri-transplant period, a number of other factors are clearly associated with adverse outcomes following transplant for AML including: complex cytogenetics at diagnosis, an initial diagnosis of therapy-related myeloid neoplasm, AML arising from an antecedent myeloproliferative neoplasm (MPN) or myelodysplastic syndrome (MDS), and AML in 2nd remission or beyond. A study by the Center for International Blood and Marrow Transplant (CIBMTR) of 821 adult patients who underwent alloHSCT demonstrated a 5-year overall survival of just 18% among patients with a complex karyotype as compared to 64% for those with a favorable karyotype and 50% for all other karyotypes.[36] The majority of patients in this study underwent MAC alloHSCT, and the relapse-free survival (RFS) at 1-year among the patients with complex cytogenetics was <40%. A study of 545 patients with therapy-related AML (t-AML) who underwent alloHSCT demonstrated a 1-year RFS of <50% with a relapse rate of <20% for those patients in CR at the time of transplant with more dismal outcomes for patients who did not achieve a CR prior to transplant.[37] Since the majority of patients in this study also underwent a MAC alloHSCT, one might expect a relatively consistent RFS at 1-year with decreased TRM and increased relapse in patients who receive less intensive conditioning regimens prior to transplant. Meanwhile, a recent retrospective study of 802 patients who

underwent alloHSCT for AML following an antecedent hematologic disorder (MDS or MPN) demonstrated a 2-year leukemia-free survival of 45% among patients who received a MAC alloHSCT and just 37% following RIC alloHSCT with the vast majority of failures due to relapse within the first year.[38]Thus 1-year RFS appears to be convincingly <50% among patients who undergo an alloHSCT for AML with complex cytogenetics, arising after prior chemotherapy, or arising from an antecedent MPN/MDS. The 1-year RFS may be slightly better in patients who undergo alloHSCT in CR2 or greater, but the long-term RFS is just 44%.[39]

2.5. Peritransplant Maintenance Therapies

Post-transplant maintenance therapies have recently gained traction as a viable strategy for relapse prevention across a variety of diseases including AML. In Ph-positive ALL, the addition of a tyrosine kinase inhibitor following transplant has led to improved leukemia-free and overall survival.[40-42] In FLT3-mutated AML, the multi-kinase inhibitor sorafenib given to all patients after alloHSCT has led to a one-year progression-free survival of 95% for patients in CR1/CR2 at the time of transplant.[43] These examples demonstrate the potential of post-transplant maintenance therapy for patients with known mutations, but many patients lack a targetable mutation. This is often the case in non-Hodgkin lymphoma (NHL) where the addition of the CD20 monoclonal antibody rituximab in the peri-transplant setting has proven safe and may explain improved relapse-free survival in multiple trials.[44-46] The introduction of monoclonal antibodies shortly after alloHSCT raises concern about how the nascent immune system will respond, but cellular immune reconstitution in patients treated with post-transplant cyclophosphamide as part of their GVHD prophylaxis is favorable for the early integration of immunological strategies to augment anti-tumor immunity.[47] For patients with $CD19^{+}$ ALL and NHL, bispecific antibody therapy with blinatumomab has proven to be very well tolerated with 91.7% of patients enrolled in a phase I trial completing one cycle of therapy,[48] and 27 total patients now having been treated with excellent disease-specific outcomes and no non-relapse mortality. Given limited experience, it is unclear if the promising safety and efficacy results with rituximab and blinatumomab will be generalizable to other antibodies in the post-transplant setting, but there is great enthusiasm for incorporating both monoclonal and bispecific antibodies due to their generally limited side effect profile. Thus, post-transplant maintenance strategies have successfully incorporated molecularly targeted therapy and antibodies in a variety of different diseases to reduce relapse and prolong survival.

2.6. Prognosis and Treatment of Post-Transplant Relapse in AML

When patients do relapse following alloHSCT, the prognosis is quite poor, but the non-tolerized allogeneic immune system may enhance the efficacy of treatment. A study of 263 AML patients who relapsed following a RIC alloHSCT demonstrated a subsequent CR rate of just 32% with a 2-year OS of 14% from the time of relapse.[49] Notably, 2-year OS after relapse was particularly poor among patients who were treated with chemotherapy alone (4.4-6.8%), whereas a purely immunologic strategy using donor lymphocyte infusion (DLI) resulted in an improved CR rate of 36% with a subsequently better 2-year OS of 25%, although some patients did go on to receive a second alloHSCT. In relapsed FLT3-mutated AML, deeper and more durable responses have been achieved with TKIs in the post-transplant setting than in patients who have been treated with chemotherapy alone.[50] The improved outcomes in post-transplant patients suggest that the non-tolerized allogeneic immune system augments the benefits of post-transplant therapies

2.7. Leukemic Stem Cells in AML

A potential target for post-transplant therapy in AML is the leukemia stem cell (LSC), which can be differentiated from normal hematopoietic stem cells (HSCs) by the robust expression of CD123. The identity of LSCs as $CD34^+CD38^-$ cells was first confirmed by Bonnet et al., who determined that human AML cells with this phenotype were capable of differentiation, proliferation, and self-renewal when transplanted into non-obese diabetic mice with severe combined immunodeficiency disease (NOD/SCID mice).[51] However, normal HSCs share this $CD34^+CD38^-$ phenotype as demonstrated by their ability to repopulate NOD/SCID mice.[52] Thus exclusively targeting $CD34^+CD38^-$ cells to eradicate AML stem cells is impractical because it would also ablate normal hematopoiesis, so the identification of markers that differentiate LSCs from HSCs is critical before treatment can be targeted at LSCs. Recently, Jordan et al. determined that the interleukin-3 receptor alpha subunit (CD123) is expressed on >90% of $CD34^+CD38^-$ LSCs in 16/18 AML patient samples, while <1% of $CD34^+CD38^-$ HSCs express CD123 in 5 normal patient samples. As additional evidence of the importance of this cell population, $CD34^+CD123^+$ cells from three separate AML specimens were transplanted into NOD/SCID mice and engrafted, corroborating that CD123 is expressed on the leukemia stem cell.[53] The differential expression of CD123 on leukemia stem cells and normal bone marrow stem cells suggests that CD123 would be an appealing target for leukemia-directed therapy.

Beyond their potential as therapeutic targets, LSCs also serve as robust markers of MRD. This has been demonstrated using aldehyde dehydrogenase (ALDH) activity instead of CD123 to

define subpopulations of $CD34^+CD38^-$ cells that can be isolated from the bone marrow of most AML patients. In a study at Johns Hopkins, Gerber et al. demonstrated that cells with high ALDH activity ($CD34^+CD38^-ALDH^{high}$) lacked leukemia-specific cytogenetic abnormalities, cells with low ALDH ($CD34^+CD38^-ALDH^{low}$) activity were unable to engraft NOD/SCID mice, and those with intermediate ALDH ($CD34^+CD38^-ALDH^{int}$) activity were the only subpopulation capable of producing leukemic engraftment of NOD/SCID mice.[54] Thus $CD34^+CD38^-ALDH^{int}$ cells represent LSCs. Importantly, this $CD34^+CD38^-ALDH^{int}$ cell population is not present in normal bone marrow. Analysis of serial bone marrow samples from 16 AML patients following induction or consolidation chemotherapy demonstrated detectable levels of $CD34^+CD38^-ALDH^{int}$ cells by MFC in 7 patients of whom 6 suffered a subsequent morphologic relapse, while the remaining 9 patients without detectable $CD34^+CD38^-ALDH^{int}$ cells remain in complete remission.[54] These findings suggest that MFC to detect the presence of $CD34^+CD38^-ALDH^{int}$ cells identifies AML patients with persistent MRD who are at the highest risk of relapse.

More recently, the Jones Laboratory at Johns Hopkins has found that LSCs are phenotypically heterogeneous, but the heterogeneity was consistent and correlated with risk groups and outcomes.[55] About 40% exhibited an $ALDH^{high}$ phenotype indistinguishable from normal HSCs, and most (85%) harbored poor-risk cytogenetics or FLT3/ITD mutations. No $CD34^+$ AML cells could be detected in a quarter of patients, including 14/21 patients with NPM1-mutations and 6/7 acute promyelocytic leukemia (APL) patients. The patients with $CD34^+CD38^-ALDH^{high}$ leukemia cells manifested a significantly lower CR rate, as well as poorer EFS and OS. Patients with no detectable $CD34^+$ AML cells had the best EFS, and those with $CD34^+CD34^-ALDH^{int}$ AML cells an intermediate prognosis. The strong clinical associations with the LSC phenotype validated the use of this definition for the clinical study of LSCs. The heterogeneous LSC phenotypes appeared to be a function of where in hematopoietic differentiation the AML arose: unfavorable risk AMLs appear to arise from primitive LSCs with the same phenotype as HSCs ($CD34^+CD34^-ALDH^{high}$), intermediate risk from less mature LSCs ($CD34^+CD34^-ALDH^{int}$), and favorable-risk

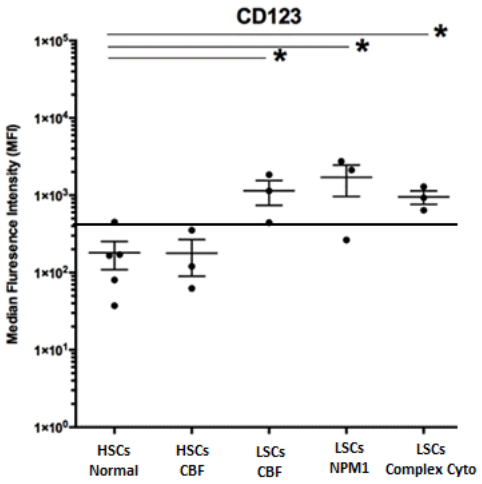

Figure 1. Expression of CD123 by primary HSCs and LSCs. Expression in 3-5 $CD34^+CD38^-ALDH^{high}$ HSCs from normals (1st column) and CBF AMLs (2nd column), CBF AML LSCs ($CD34^+CD38^-ALDH^{int}$, 3rd), NPM1 mutated LSCs ($CD34^-$, 4th), and complex cytogenetic LSCs $CD34^+CD38^-ALDH^{high}$, 5th column). Line represents Ab control. * represent p values < 0.05 compared to HSCs from normal.

from LSCs with mature hematopoietic phenotypes ($CD34^+CD38^-$ or $CD34^-$). However, despite the heterogeneity in phenotype, our group found that all the LSCs expressed CD123 regardless of their phenotype, while HSCs from normals and AML patients did not (Figure 1).

2.8. Flotetuzumab Pre-clinical Development

The identification of CD123 as a marker of LSCs has led to the development of a number of therapies targeting CD123 including both monoclonal antibodies and a dual-affinity retargeting molecule (DART), flotetuzumab, generated from antibodies to CD3 and CD123, designed to redirect T cells against acute myeloid leukemia blasts. Flotetuzumab has been shown to increase the association between CD3-expressing Jurkat cells and CD123-expressing malignancies by at least 17-fold compared to control DARTs or non-CD123-expressing malignancies.[56] Furthermore, flotetuzumab leads to T cell activation with a dramatic increase in CD25 expression on $CD4^+$ and $CD8^+$ T cells *in vitro* upon incubation with CD123-expressing malignancies, and a marked T cell proliferation when $CD4^+$ and $CD8^+$ T cells are incubated with CD123-expressing malignancies. The activation and proliferation of T cells by flotetuzumab in turn led to CD123-specific cytotoxicity *in vitro* that was dependent on both the dose of flotetuzumab and the effector T cell:target cell ratio. These findings of DART-mediated T cell activation and cytotoxicity were subsequently replicated using primary AML blasts *in vitro*, and the activity of flotetuzumab was also demonstrated when such samples were cultured in the presence of stroma.[56] Notably, these experiments also demonstrated an increase in TCR diversity when next generation sequencing was applied to T cells following exposure to flotetuzumab and primary AML blasts. Ultimately, *in vivo* experiments using sublethally irradiated NSG mice bearing CD123-expressing tumor cells demonstrated that flotetuzumab reduced leukemic cell burden by 1415-fold compared to untreated mice.[56]

To ascertain the safety and potential efficacy of flotetuzumab in primates, toxicology and pharmacodynamic studies were performed using cynomolgus macaques. In this study, flotetuzumab was administered using step-wise intra-patient dose escalation to mitigate the anticipated cytokine release syndrome (CRS). This dose escalation strategy proved effective with a minimal, transient, first dose effect that correlated with increased IL-6 levels.[57] Importantly, white blood cell count; neutrophils; and platelets remained within the normal range during treatment with flotetuzumab, except for one treatment group that had a transient decline in platelets just below the reference range. There was a dose-dependent decrease in red cell mass, but this was reversible upon cessation of the drug.[57] These findings demonstrate the

limited hematologic toxicity of flotetuzumab in primates, which might be expected from the differential expression of CD123 on leukemic stem cells and normal hematopoietic progenitors. In addition to the safety of flotetuzumab in an *in vivo* model, the macaque studies also demonstrated the extensive depletion of plasmacytoid dendritic cells, which are defined by their expression of CD123, by flotetuzumab with subsequent recovery when flotetuzumab was discontinued.[57] Thus the enumeration of plasmacytoid dendritic cells can serve as an important pharmacodynamic marker to verify the on-target effects of flotetuzumab.

2.9. CD123-Targeted Therapies in Clinical Trials

The *in vitro* efficacy of CD123-targeted therapies led to multiple trials of these therapies in patients with relapsed, refractory, and high-risk AML that demonstrated the feasibility of this approach. In a phase I trial of the monoclonal antibody CSL360, 40 patients with advanced AML received the study drug at five different dose levels weekly for twelve weeks without reaching a maximal tolerated dose. Complete, sustained saturation of CD123 by CSL360 was seen at the two highest dose levels, but only 2/27 patients (7.4%) in these cohorts achieved a complete remission.[58] This suggested that monoclonal antibodies directed against CD123 may be inadequate to overcome the significant disease burden in the study population. However, it was notable that both patients who achieved a CR had undergone a prior alloHSCT, while only 5 patients with a history of alloHSCT were treated in these cohorts.[58] Thus there was a 40% response rate for patients with prior alloHSCT who were treated at an adequate dose level, which raises the possibility that the allogeneic immune system is critical for the efficacy of this treatment. Overall, seven patients who had relapsed after alloHSCT were treated with CSL360 without any evidence that CD123-targeted therapy exacerbates transplant-related toxicities. The drug-antibody conjugate tagraxofusp has also been studied in patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN) with relapse following alloHSCT, and there has not been any evidence that treatment with this CD123-targeted therapy exacerbates transplant-related toxicities.[59] A subsequent phase I/II study of the flotetuzumab in patients with relapsed/refractory AML or intermediate/high-risk MDS demonstrated manageable toxicities of this drug at a maximum tolerated dose schedule of 500 ng/kg/day. In this study, 30% of patients treated at the recommended phase II dose (RP2D) achieved a morphologic response, and correlative studies demonstrated evidence of T cell activation.[60,61] The most significant toxicity of flotetuzumab at the RP2D was cytokine release syndrome (CRS) with 81% of patients enrolled in the Phase I/II developing CRS or an infusion-related reaction (IRR), although only 8% of patients treated at RP2D experienced grade 3 events without any grade 4/5 events.[61]

Notably, the severity of CRS with flotetuzumab correlates with the frequency of circulating $CD4^+$ T cells,[61] which are significantly reduced for at least a year post-transplant compared to patient's pre-alloHSCT baseline and healthy controls.[62] Given the demonstrated efficacy and limited toxicity of CD123-targeted therapies in relapsed and post-transplant AML patients, it seems logical to test CD123-directed therapies in post-transplant patients as a form of post-transplant maintenance and for the treatment of post-transplant relapse.

2.10. Rationale for the Proposed Study

Despite significant advances, the prognosis for patients with AML remains poor with 5-year overall survival of just ~40% in younger patients and much poorer long-term survival in older patients.[1,2,5] AlloHSCT as post-remission therapy has led to improved overall survival when compared to consolidation chemotherapy for the vast majority of AML patients who have intermediate or poor risk cytogenetics.[1,6,7] Due to significant TRM and poor outcomes in older patients with MAC transplantation, there have been many studies investigating the feasibility of less intensive conditioning regimens such as RIC and NMA, which have shown comparable overall survival with decreased TRM but an increased risk of relapse.[12-17] As these less intensive conditioning strategies become more widely adopted, the need to focus on the identification and treatment of AML patients at risk for post-transplant relapse increases. Maintenance therapy with tyrosine kinase inhibitors and monoclonal antibodies have proven safe and effective across a range of diseases including AML, ALL, and NHL.[40-46] LSCs are another potential target for post-transplant therapy, and the expression of CD123 readily discriminates AML LSCs from HSCs.[53] The anti-CD123 monoclonal antibody CSL360 has previously demonstrated efficacy in post-transplant patients with relapsed disease, while flotetuzumab has demonstrated efficacy in relapsed and refractory patients.[58,60] Given this preliminary data, we propose a trial of flotetuzumab as post-alloHSCT therapy for AML in patients with evidence of disease post-transplant including frank relapse. We believe that treatment with flotetuzumab in this setting will be well tolerated and effective. Flotetuzumab is not approved for use in people with AML. Its use has not been specifically studied in patients with AML following a bone marrow transplant and therefore its use in this study is investigational.

3. Patient Selection and Enrollment

3.1. Inclusion Criteria

1) A confirmed prior diagnosis of AML and underwent an alloHSCT as a form of consolidation in a morphologic complete remission

2) ECOG performance status 0-2

3) Ability to give informed consent

4) In agreement to use an effective barrier method of birth control to avoid pregnancy during the study and for a minimum of 30 days after study treatment, for all male and female patients who are fertile

5) Age ≥18 years

6) Prior treatment with a CD123-targeted therapy will be allowed assuming the patient did not have a grade 3 or 4 adverse reaction to prior use of this treatment

7) Normal thyroid function (defined by either a TSH within the reference range, a TSH above the reference range with a free T4 within the reference range, or a TSH below the reference range with both a free T4 and total T3 within the reference range) or normal thyroid tests on supplementation or treatment (defined as a TSH within the reference range)

8) Patients should be at least 30 days from transplant with morphologic evidence of disease progression on bone marrow biopsy

9) A population of blasts with expression of CD123 is noted.

10) Peripheral blast count ≤20,000/mm^3 at time of initiation on Cycle 1 Day 1

3.2. Exclusion Criteria

1) No evidence of donor engraftment (100% patient DNA in bone marrow or peripheral blood after alloHSCT based on either an unsorted specimen or CD3 sorted).

2) Active AML in CNS or testes

3) Patients with active, uncontrolled infection. If an infection is controlled and under treatment, then the patient may become eligible.

4) Patients with active acute or chronic GVHD requiring GVHD therapy (mycophenolate mofetil, tacrolimus, sirolimus, or steroids) within 30 days

5) Patients without active acute or chronic GVHD requiring prophylactic GVHD therapy (mycophenolate mofetil, tacrolimus, sirolimus, or steroids) within 30 days

6) Inadequate end organ function defined as:

- Hepatic-AST, ALT, and alkaline phosphatase > 3.5X ULN, bilirubin >2.5X ULN
- Renal-creatinine clearance <60 mL/min using the modified Cockcroft-Gault formula
- Cardiac-Recent myocardial infarction within 6 months, Congestive Heart Failure with EF <50%, active pericarditis or myocarditis
- Pulmonary-Need for supplemental oxygen to maintain oxygen saturation >92%
- Adrenal-Adrenal insufficiency requiring physiologically-dosed steroids

7) Women who are pregnant or lactating

8) Previous or known hypersensitivity to biological agents or constituents of flotetuzumab or its source material

9) Concurrent use of any other investigational drugs

10) Uncontrolled infection with human immunodeficiency virus (HIV) or chronic infection with hepatitis B virus or hepatitis C virus (HCV)

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

12) Previous treatment with radiotherapy or an immunotherapeutic agent in the 14 days prior to study drug administration (Cycle 1 Day 1) or 5 half-lifes, whichever is longer

13) Requirement, at the time of study entry, for concurrent steroids > 10 mg/day of oral prednisone or equivalent, except steroid inhaler, nasal spray, or ophthalmic solution

14) Use of granulocyte colony stimulating or granulocyte-macrophage colony stimulating factor in the 2 weeks prior to study drug administration

15) Prior adverse event with CD123 therapy necessitating therapy discontinuation

3.3. Inclusion of Women and Minorities

The proposed study is open to both men and women and to all racial/ethnic subgroups. There is no explicit mention of different treatment effects in male and female patients or in different racial/ethnic subgroups in the literature. Therefore, this study will not have separate accrual targets for these groups.

3.4. Informed Consent

All patients eligible for the study must be evaluated by one of the study investigators. Informed consent must be obtained and the consent form signed. An eligibility checklist will be completed following fulfillment of the on-study requirements for laboratory work and eligibility criteria. For enrollment of non-English-speaking candidates, the policies and procedures mandated by the JHM IRB will be followed as listed on the following website: https://irb.jhmi.edu/Guidelines/nonenglishconsent.html

4. Study Design and Schedule

4.1. Pre-Treatment Plan

A complete history and physical examination and list of medications will be documented for each patient within two weeks of enrollment. A bone marrow aspirate or biopsy confirming a diagnosis of relapsed AML will be documented in the electronic medical record. A bone marrow aspirate/biopsy documenting a CR (<5% blasts) must be performed prior to transplantation. Evaluations within 14 days of beginning therapy will include:

1. A history including a list of active medications and physical exam including body height and weight
2. Hematologic studies: complete blood count with differential cell counts.
3. Chemistry panel (including electrolytes, creatinine, uric acid, albumin, total protein, calcium and phosphate)
4. Thyroid Stimulating Hormone
5. Lactate Dehydrogenase
6. Hepatic panel: serum AST, ALT, alkaline phosphatase, total bilirubin, PT/PTT
7. Urinalysis
8. An ECG
9. Pregnancy test (where appropriate)
10. ECOG performance status
11. A bone marrow biopsy and/or aspirate including chimerism testing

4.2. Treatment Schedule

All patients will undergo a bone marrow biopsy prior to enrollment to assess for relapse and must have gross morphologic evidence of relapsed leukemia (≥5% blasts) for enrollment in this group. Patients may enroll at any time after day +30 post-transplant. Patients enrolled on DL1 will receive flotetuzumab by continuous infusion using multi-step lead-in dosing as outlined in

Table 1, and then 500 ng/kg/day on days 7-28. After one cycle, all patients will undergo a bone marrow biopsy to assess response including assessment of MRD. Patients who fail to achieve a CR, CRi, CRh, or MLFS may continue with subsequent induction cycles as a continuous infusion up to a total of five cycles. If there is evidence of response (CR, CRi, CRh, or MLFS) and the toxicities of treatment are acceptable, patients will be eligible for two consolidation cycles as outlined in Table 1. Additional bone marrow biopsies for response assessment will be performed after the second cycle. Results will be censored should patients undergo a second alloHSCT. If there is a need to de-escalate dosing based on toxicity, then patients will be enrolled on DL-1 using multi-step lead-in dosing as outlined in Table 1, and then 300 ng/kg/day on days 5-28 of the first cycle and days 1-28 of subsequent cycles.

Table 1			**Dosing Schedule**	
Cycle	**Week**	**Days**	**ng/kg/day**	
Induction: Cycle 1				
			DL1	DL-1
1	1	Day 1	30	30
		Day 2	60	60
		Day 3	100	100
		Day 4	200	200
		Day 5	300	300
		Day 6	400	300
		Day 7	500	300
	2	Days 8-14	500	300
	3	Days 15-21	500	300
	4	Days 22-28	500	300
Consolidation/Reinduction: Cycle 2 and beyond				
2	1	Days 1 – 28	500	300

4.3 Flotetuzumab Dosing

Flotetuzumab dosing in this trial will be based on maximum tolerated dose schedule as determined in the phase I trial and subsequently modified with multi-step lead-in dosing during the first week of treatment.[60,61] During week 1 of treatment, patients will receive flotetuzumab via continuous infusion using multi-step lead-in dosing as described in table 1. If they tolerate this lead-in dose, then they will be escalated to a dose of 500 ng/kg/day on days 7-28 for DL1 or

300 ng/kg/day on days 5-28 for DL-1. Dosing in subsequent cycles will be as outlined above without multi-step lead-in dosing.

4.4 Timing of Initiation and Criteria for Subsequent Treatment

After HSCT the majority of acute GVHD occurs in the first 60 days. To ensure patients are stable prior to initiating flotetuzumab therapy, the study will start no sooner than day 30 post-transplant and must be off of immunosuppression for minimum of 30 days prior to initiating treatment. Approximately half of relapses occur within the first year after SCT, with 80% occurring within the first two years.[63]

Patients eligible for subsequent cycles of therapy as consolidation or continued induction, must meet the following criteria:

- Peripheral blast count ≤20,000/mm^3
- Meeting criteria for end organ function as defined in exclusion criteria #6
- Have had resolution to ≤ Grade 1 of any non-hematologic adverse event deemed to be possibly, probably, or definitely related to flotetuzumab

4.5. Premedications and Prophylaxis

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

Prior to the first dose (Week 1):

All patients are to be treated as described in table 2. No steroids should be administered for infusion-related reaction prophylaxis except as indicated. Patients should receive adequate hydration during study therapy, and the addition of IV fluids to maintain intravascular volume (e.g., 50-100 mL/hr of normal saline) is recommended for at least 48 hours after the start of therapy.

Prior to Week 2 dosing:

The premedication schedule should be followed as described below in table 2. In addition, these premedications should be considered in patients if they are resuming flotetuzumab treatment after a dose interruption lasting > 1 day in Cycle 1.

Steroids should not be administered for infusion-related reaction prophylaxis except as indicated below, but may be used for the treatment of emerging symptoms as clinically appropriate. Patients should receive adequate hydration during study therapy, and the addition of IV fluids to maintain intravascular volume (e.g., 50-100 mL/hr of normal saline) is recommended for at least 48 hours after the start of therapy in Week 2.

Prior to subsequent doses (after Week 2):

The premedication schedule should be followed as described below in table 2.

For infusion-related reactions during the course of treatment, supportive care measures should be implemented as outlined in **Section 4.7.1**.

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

Table 2 Premedications/Prophylaxis for CRS/IRR

Medication	7-day Continuous Infusion Schedule (Induction: Cycle 1*)			
	Week 1	Week 2	Week 3	Week 4
Acetaminophen (1000 mg PO) Or Paracetamol (1000 mg PO) Or Ibuprofen (400 mg PO)	Day 1: 30 minutes prior to dosing; Then q 8 hrs for 48 hours Day 7: 30 minutes prior to syringe change; Then q 8 hrs for 48 hrs	None	None	None

Diphenhydramine (25-50 mg IV or PO) or equivalent	Day 1: 30 minutes prior to dosing; Then q 8 hrs for 48 hrs Day 7: 30 minutes prior to syringe change; Then q 8 hrs for 48 hours	None	None	None
Famotidine (20 mg IV) or equivalent	Day 1: 30 minutes prior to dosing; Then q 12 hrs for 48 hrs Day 7: 30 minutes prior to syringe change; Then q 12 hrs for 48 hours	None	None	None
Dexamethasone (or equivalent)	Day 1: 10-20 mg IV up to 30 minutes prior to dosing; Then 4 mg IV at 12 hours after dosing Day 7: 10 mg IV up to 30 minutes prior to syringe change; Then 4 mg 12 hours later	None	None	None
7-day Continuous Infusion Schedule (Reinduction/Consolidation: Cycle 2 and Beyond)*				
Acetaminophen (1000 mg PO) Or Paracetamol (1000 mg PO) Or Ibuprofen	Day 1: 30 minutes prior to dosing; Then q 8 hrs for 48 hours	None	None	None

Diphenhydramine (25-50 mg IV or PO) or equivalent	Day 1: 30 minutes prior to dosing; Then q 8 hrs for 48 hrs	None	None	None
Famotidine (20 mg IV) or equivalent	Day 1: 30 minutes prior to dosing; Then q 12 hrs for 48 hrs	None	None	None
Dexamethasone (or equivalent)	Day 1: 10-20 mg IV up to 30 minutes prior to dosing; Then 4 mg IV at 12 hours after dosing	None	None	None

* If dosing is interrupted for >24 hours during continuous infusion, the premedication schedule for Week 1 of the Reinduction/Consolidation: Cycle 2 and Beyond schedule should be followed.

4.6. Study Parameters

The studies planned during patient evaluation and therapy are listed in Table 3. This represents the basic follow-up plan and will change based on patient condition. All attempts will be made to collect all correlative studies, however, due to laboratory constraints (weekends, holidays, laboratory emergencies or staffing difficulties) and patient constraints (related to intercurrent disease, transportation, or inadequate sample), some samples will not be collected or will not be suitable for evaluation. A patient will be considered suitable for evaluation independent of the availability of correlative study material. A variation of +/- 3 days for all study testing and visits is permitted.

Table 3: Study Calendar

Pre-Treatment		Cycle 1			Subsequent Cycles			End of Treatment	Post-Treatment Follow-up
Parameter	**Pre-treatment** Within 14 days prior to Day 1	**Day 1**	**Day 8, Day 15, Day 22**	**Day 28**	**Day 1**	**Day 8, Day 15, Day 22**	**Day 28**		
Screening/Administrative									
Informed Consent	X								
Pre-Treatment		**Cycle 1**			**Subsequent Cycles**			**End of Treatment**	**Post-Treatment Follow-up**
Parameter	**Pre-treatment** Within 14 days prior to Day 1	**Day 1**	**Day 8, Day 15, Day 22**	**Day 28**	**Day 1**	**Day 8, Day 15, Day 22**	**Day 28**		
Demography/Medical History	X								
Eligibility Criteria	X								
Study Drug Administration									
Flotetuzumab		X	X		X	X			
Safety Assessments									
Physical Examination	X	X			X			X	
ECOG Performance Status	X	X			X				
Vital Signs	X	X			X				
Weight[1]	X	X			X				
Height	X								
ECG	X								
Concomitant Medications	Continuous from the time of signing of ICF until at least 30 days after the last dose of flotetuzumab								
Adverse Events	Continuous from the time of signing of ICF until at least 30 days after the last dose of flotetuzumab								
Laboratory Assessments									
CBC with Differential	X	X	X	X	X	X	X	X	
PT, PTT	X	X			X				

CMP	X	X	X	X	X	X	X	X	
Magnesium	X	X			X				
Phosphate	X	X			X				
LDH	X								
HBV surface antigen and hepatitis C (antibodies)	X								
HIV Antibody	X								
Serum HCG or urine pregnancy test for applicable women only	X	X			X				
Pre-Treatment		**Cycle 1**			**Subsequent Cycles**			**End of Treatment**	**Post-Treatment Follow-up**
Parameter	**Pre-treatment** Within 14 days prior to Day 1	**Day 1**	**Day 8, Day 15, Day 22**	**Day 28**	**Day 1**	**Day 8, Day 15, Day 22**	**Day 28**		
Peripheral Blood and Bone Marrow studies for MRD	X			X			X		
Bone Marrow studies for chimerism	X			X			X		
Peripheral Blood and Bone Marrow studies for immunologic correlates	X		X (Day 15 ONLY/PB ONLY)	X			X (Cycle 2 ONLY)		X (at 6 and 12 months post-transplant and relapse)[4]
Peripheral Blood serum samples for Cytokine Measurement	X[5]	X[5]	X[5]						
Disease Evaluations									
Bone Marrow aspirate/biopsy	X			X			X	X	

MRD Assessment via Peripheral Blood and Bone Marrow[2]	X			X			X	X	
Survival, subsequent therapy, other malignancies[3]									X

[1] Must be assessed prior to dosing on Day 1 of each specified cycle, in case of weight loss of >10% within a cycle, the dose of study drug must be recalculated.

[2] See section 5.5. MRD assessment will be performed on bone marrow using MFC as previously described by Gerber et al., where any detectable disease will be considered positive.[35,54] Samples will be sent for standard-of-care MFC from the bone marrow aspirate, and if a bone marrow aspirate cannot be obtained, tben samples will be sent from the peripheral blood for standard-of-care MFC.

[3] This data will be obtained through standard-of-care provider visits that occur following alloHSCT.

[4] The correlative samples will be collected at the time of the patient's standard of care bone marrow biopsies that are performed at 6 and 12 months after transplant. There is considerable variability in the time at which these are performed.

[5] Samples will be collected pre-dose and once per infusion bag change.

4.7. Dosing Delays and Modification for Toxicity

4.7.1. Management of Cytokine Release Syndrome (CRS)/Infusion-related reactions (IRR)

In the first weeks of treatment, patients are at risk for cytokine release syndrome (CRS) and infusion-related reactions (IRR). While these reactions occur in the majority of patients treated with flotetuzumab (81%), most events are either grade 1 or 2. The severity of these events seems to correlate with the baseline absolute CD3+ T cell count and increasing cytokine levels, especially interleukin-6 (IL-6). The risk of CRS was decreased with two-step lead-in dosing.[64] Due to the relationship between IL-6 levels and the severity of CRS, an early intervention strategy with the IL-6 receptor antagonist tocilizumab was used to mitigate toxicity in the phase I trial. CRS/IRR should be graded according to the ASTCT CRS Consensus Grading System[65]

Table 4 ASTCT CRS Consensus Grading[65]

Table 2
ASTCT CRS Consensus Grading

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever*	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C
		With		
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
		And/or†		
Hypoxia	None	Requiring low-flow nasal cannula‡ or blow-by	Requiring high-flow nasal cannula‡, facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (eg, CPAP, BiPAP, intubation and mechanical ventilation)

Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.
* Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretic or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.
† CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS.
‡ Low-flow nasal cannula is defined as oxygen delivered at ≤6 L/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at >6 L/minute.

Table 5 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*

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 version 5.

IRR/CRS should be managed as described below. Every attempt should be made to continue the infusion during management of the reaction.

Table 6 CRS Severity and Management

CRS Severity	**Management**

Non-life-threatening • Fever • Tachycardia • Nausea • Fatigue • Headache • Malaise • Myalgias	○ Slow the infusion rate by 10-20%. ○ Monitor the patient for worsening of condition. ○ Administer IV fluids, diphenhydramine hydrochloride (or institutional equivalent) 50 mg IV, acetaminophen 1000 mg PO or ibuprofen 400 mg PO for fever, and oxygen and bronchodilators for mild bronchospasm, as appropriate; ○ Administer tocilizumab (4-8 mg/kg IV) if the above symptoms do not resolve within 2 hours with supportive therapy, or is associated with any of the following signs and symptoms: ▪ Temperature > 101.5oF (38.6oC) ▪ Signs of pending respiratory compromise as indicated by RR > 20/min ▪ HR > 20 bpm above pre-treatment baseline ▪ Note: isolated changes in hemodynamic parameters alone that do not require intervention without pulmonary compromise or fever should not prompt immediate use of tocilizumab. ○ Corticosteroids should not be used. ○ Continue infusion at reduced rate and slowly increase infusion rate to the original rate in 2 steps after stabilization or resolution of symptoms every 4-6 hours, as tolerated. A more gradual increase in the rate of infusion may be undertaken after consultation with the Principal Investigator.
Requiring mild intervention defined by • Decreases in BP requiring fluid boluses or low-dose vasopressors, requiring supplemental oxygen (≤6 L/min by supplemental oxygen) to maintain oxygen saturation >92%, or • Grade 2 organ toxicity	○ Slow the infusion rate by 25-50%. ○ Administer IV fluids, diphenhydramine hydrochloride 50 mg IV or institutional equivalent, acetaminophen 1000 mg PO or ibuprofen 400 mg PO for fever, and oxygen and bronchodilators for bronchospasm, as appropriate and if not administered previously; ○ Administer vasopressors (at doses < 20 ng/min of norepinephrine) as needed for circulatory support. ○ Tocilizumab (8 mg/kg IV) should be used for the above signs if they do not resolve with other measures within 2 hours, or that requires the use of supplemental oxygen > 4 L by nasal cannula or low dose vasopressors.

	○ Corticosteroids may be used for patients that do not respond to other measures, including tocilizumab. ○ Continue infusion at reduced rate and slowly increase infusion rate to the original rate gradually by half-rate increments (i.e. if rate is reduced from 5 mL/hr to 2.5 mL/hr increase to ~3.75 mL/hr, then 4.25 mL/hr, then 5 mL/hr) after stabilization or resolution of symptoms every 4-6 hours, as tolerated. A more gradual increase in the rate of infusion may be undertaken after consultation with the principal investigator. ○ Report the event as a serious adverse event (SAE), if appropriate. ○ Monitor for worsening condition;
Life threatening defined by • Need for high-dose vasopressors, >6 L/min of oxygen by nasal cannula to maintain oxygen saturations >92%, • Grade 3 organ toxicity (except transaminitis), or • Grade 4 transaminitis infusion reactions and infusion-related events	○ Stop the infusion. ○ TO AVOID EXACERBATION OF INFUSION REACTION OR CRS: DO NOT FLUSH THE TUBING – ASPIRATE RESIDUAL DRUG FROM THE PORT LUMEN. ○ Administer IV fluids, diphenhydramine hydrochloride (or institutional equivalent) 50 mg IV, acetaminophen 1000 mg PO or ibuprofen 400 mg PO for fever, and oxygen and bronchodilators for mild bronchospasm, as appropriate. ○ Provide appropriate circulatory support including vasopressors as medically indicated; ○ Administer tocilizumab if not administered previously. If administered previously, an additional dose may be used for prolonged or recurrent episodes. ○ Symptoms that are refractory to tocilizumab should be treated with any of the following: ▪ Corticosteroids; doses of dexamethasone (or equivalent) of greater than 30 mg may be required ▪ Etanercept (or equivalent anti-TNFa) 50 mg IV, and/or daclizumab (anti-IL2 receptor) 1 mg/kg IV (where approved); ○ Resume the infusion at previously tolerated dose once the infusion reaction has resolved or decreased to Grade 1. Increase dose rate to the original rate, as prescribed by protocol, e.g., 500 ng/kg/day, by increasing the dose in near double

	increments as tolerated after stabilization or resolution of symptoms every 4-6 hours. A more gradual increase in the rate of infusion may be undertaken after consultation with the Principal Investigator. ○ Report the event as a serious adverse event (SAE), if appropriate. ○ Discontinue the infusion if not resolved to Grade 1 within 72 hours.
Resulting in death	○ Notify the regulatory authorities. ○ Report the event as an SAE.

4.7.2. Neurotoxicity Monitoring

Neurotoxicity, including changes in mental status, has been reported with other T-cell directed therapies including chimeric-antigen receptor (CAR)-T cells and CD3xCD19 based bispecific antibodies such as blinatumomab and rarely with flotetuzumab. 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.

Patients need to be counseled during the consent process that they should NOT drive or operate heavy machinery while they are receiving flotetuzumab treatment and for 30 days after their last dose of flotetuzumab.

4.7.3. Tumor Lysis Syndrome

Prophylaxis for TLS should be administered for patients with active 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.

4.7.4. Other Clinically Relevant Adverse Events

If a grade 3 non-hematological adverse event occurs that is deemed by the investigator to be possibly, probably or definitely related to the study drug, then flotetuzumab will be withheld until the toxicity resolves to no more than grade 1 (mild) after which flotetuzumab may be restarted at the previously tolerated dose. If the toxicity persists for more than 14 days, then flotetuzumab will be permanently discontinued. If a grade 4 non-hematological adverse event occurs that is deemed by the investigator to be possibly, probably or definitely related to the study drug, then flotetuzumab should be permanently discontinued.

4.7.5. GVHD

If a patient develops definitive evidence of acute GVHD[66], flotetuzumab will be permanently discontinued. Study therapy will be permanently discontinued should a patient develop definitive evidence of chronic GVHD.[67] See appendix for specific GVHD assessment criteria.

4.7.6. Immune-related Adverse Experiences

Immune checkpoint blockade has been associated with several syndromes associated with the breaking of immunological tolerance (19). Although not observed in non-clinical studies to date, similar events might occur in patients treated with flotetuzumab. These syndromes include pneumonitis, colitis, autoimmune hepatitis, arthritis, glomerulonephritis, myocarditis and cardiomyopathy, hypophysitis, thyroiditis, or other autoimmune endocrinopathies. The occurrence of any of these syndromes is an AESI. Their occurrence dictates interruption, and potentially discontinuation, of study drug administration pending further evaluation. In the event of suspected immune-related adverse experiences, the investigator should promptly consult with the Sponsor and early consideration should be given to the prompt implementation of systemic immune suppression utilizing agents that may include, but are not limited to steroids, anti-TNFα antibodies, and/or IL-6 receptor inhibitors.

4.7.7. Capillary Leak Syndrome

Risk of 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 CRS 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 the 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 IV 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.

4.7.8. Epstein-Barr Virus Reactivation

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.

4.8. Permitted Medications and Supportive Therapies

All concomitant medications for medical conditions other than AML are permitted, as clinically indicated. All supportive therapies, other than anticancer treatment needed for the management of subjects enrolled in this study, are permitted including transfusional support as per institutional standards.

The following medications and supportive therapies are examples of supportive therapies that may be used during the study:

- Antibacterial, antifungal, or antiviral agents as prophylaxis and to treat active infections
- Antiemetics, antidiarrheals, anticholinergics, antispasmodics, antipyretics, antihistamines, analgesics, 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 concomitant medication form.

4.9. Prohibited Therapies

The following medications are prohibited during this study:

- Concurrent systemic corticosteroids at doses >10 mg of prednisone daily (or equivalent) except for mandatory premedication
- Any investigational agent
- Any vaccination except annual inactivated influenza and SARS-CoV-2 vaccination

4.10. Concurrent Antineoplastic Therapies

Cytoreduction with hydroxyurea will be allowed for the purposes of achieving an initial peripheral blast count ≤20,000/mm^3 but must stop at least 24 hours prior to the protocol therapy. 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. The use of the following antineoplastic therapies will be specifically disallowed across all groups:

- Other cytotoxic chemotherapy
- Donor Lymphocyte Infusion
- Other monoclonal antibodies, antibody-drug conjugates, or bispecific T cell engager antibody constructs

The institution of any of the above should occur in the context of disease relapse or treatment failure leading to the discontinuation of the study treatment.

4.11. Duration of Therapy

Treatment is planned for up to five induction cycles and two cycles of consolidation. Treatment will continue until one of the following occurs:

1. Disease relapse after achieving a response

2. Serious illness that prevents further administration of treatment (i.e. progressive infection not responding to appropriate antibacterial and/or anti-fungal therapies)
3. Unacceptable adverse event(s)
4. Elective withdrawal of consent by the patient
5. Determination by the physician that it is no longer in the patient's best interest to continue the trial
6. Inability to tolerate the study drug
7. Initiation of breast feeding by the patient
8. Patient pregnancy
9. Delay of the study drug for more than 14 consecutive days due to unresolved toxicities
10. Completion of two consolidation cycles OR five induction cycles
11. No evidence of anti-leukemic activity (PR or better) after 2 successive cycles
12. Evidence of progressive disease

4.12. Duration of Follow-up

Patients will be followed for relapse and death for two years from the first day of the first cycle of treatment. Patients are routinely followed and monitored by JH physicians after alloHSCT or chemotherapy, and patients who complete the study and continue to receive their care at JH will have survival captured annually.

5. Disease Evaluations

5.1. Disease Status Definitions

Assessment of disease status will be performed based on the following definitions that have been modified from Dohner et al:[68]

Complete Remission (CR)

1 Absence of extramedullary disease
2 Bone marrow blasts <5% blasts and absence of blasts with Auer Rods
3 Absolute neutrophil count (ANC) >1,000/μL
4 Platelets >100,000/μL
5 Red cell transfusion independence

CR with incomplete blood count recovery (CRi)

- Meets all criteria for CR except platelets and/or ANC

CR with partial hematologic recovery (CRh)

- Meets all criteria for CR except platelets (≤100,000/μL BUT >50,000/μL) AND/OR ANC (≤1,000/μL BUT >500/μL)

Partial Remission (PR)

- ANC >1,000/μL AND platelets >100,000/μL with a decrease of bone marrow blasts to 5-25% with at least a 50% reduction in bone marrow blasts from pretreatment levels

Morphologic leukemia-free state (MLFS)

- Bone marrow blasts <5%; absence of Auer rods; absence of extramedullary disease; no hematologic recovery required

Refractory Disease

- Failure to achieve CR after 1-2 cycles of treatment

Progressive Disease (PD)

- Increase of at least 25% in the absolute number of circulating or bone marrow blasts or development of extramedullary disease

Relapsed Disease

- Reappearance of blasts in the blood or bone marrow (≥5%) or in any extramedullary site after a CR

Minimal Residual Disease (MRD)

- MRD in AML refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. Patients who achieved a CR by morphologic assessment alone can potentially harbor a large number of leukemic cells in the bone marrow. This will be assessed using conventional MFC, wherein any measurable level of leukemic cells will be considered positive.[30] The MFC assay for LSCs developed by Gerber et al, NGS assay developed by

Onecha et al, and the 5-gene RQ-PCR assay developed by Goswami et al will also be used as exploratory correlative studies.[54]

5.2. Disease Status Monitoring

Disease status will be confirmed by a bone marrow biopsy within 14 days of study enrollment. Additional bone marrow biopsies will be performed at the end of all cycles of treatment, and at 6 and 12 months post-transplant per the standard-of-care for all patients. MRD status will be assessed concurrently with all bone marrow biopsies via peripheral blood and bone marrow samples. If a bone marrow aspirate cannot be obtained, then MFC should be sent by peripheral blood for MRD monitoring.

6. Pharmaceutical Information

6.1. Flotetuzumab

1. Other Names: MGD006, S80880

2. Classification: Dual-affinity Re-targeting (DART) molecule

3. Mechanism of Action: The dual-affinity re-targeting (DART) molecule flotetuzumab has been shown to increase the interaction between CD123-expressing leukemia cells and T cells by 17-fold *in vitro.*[56] This interaction has been shown to selectively promote CD4 and CD8 T-cell proliferation in a dose dependent manner, while also activating both CD4 and CD8 T-cells as evidenced by increased CD25 expression. Furthermore, this interaction leads to cellular cytotoxicity in primary AML blasts *in vitro* in the absence and presence of stroma. Subsequently, flotetuzumab was shown to be effective in a murine AML model with a 1415-fold increase in photon flux from leukemic cells in untreated mice as opposed to those treated with flotetuzumab, while a control DART had no effect on tumor growth.[56]

4. Pharmacokinetics: The distribution and clearance of flotetuzumab has been investigated extensively in cynomolgus macaques. The cynomolgus macaque is a reasonable model for humans, as the tissue distribution of CD3 and CD123 is comparable between the two species. The distribution half-life of flotetuzumab is short (~4 minutes) with a volume of distribution of ~1-2 L/kg), which suggests rapid and extensive binding to target cells in tissues. Flotetuzumab was rapidly cleared at the end of infusion with a mean residence time of 7-9 hours, as expected for a molecule of its size (59 kD) that is likely renally cleared.[57]

5. Pharmacodynamics: As CD123 is also highly expressed on plasmacytoid dendritic cells, monocytes, and basophils in addition to leukemic stem cells, it is possible to measure levels of these cells types as a pharmacodynamics marker.[56] In the cynomolgus macaque, a dose of 100 ng/kg per day led to rapid and extensive depletion of CD123-positive cells with subsequent recovery following treatment discontinuation.[57]

6. Drug Supply: Flotetuzumab drug product (DP) is provided as a sterile aqueous solution with a protein concentration of 0.1 mg/mL in a buffer composed of 10 mM sodium phosphate at pH 6.0, 150 mM sodium chloride and 0.1 mg/mL polysorbate 80. The concentration and function of each component in the DP are summarized below.

Name of Ingredient	Concentration (mg/mL)	Nominal Amount per Vial (mg) [a]	Function
MGD006 drug substance (DS)	0.1	0.5	Active ingredient
Sodium phosphate monobasic monohydrate	1.13	5.65	Buffer component
Sodium phosphate dibasic	0.26	1.3	Buffer component
Sodium Chloride	8.78	43.9	Tonicifier
Polysorbate 80	0.1	0.5	Surfactant

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

7. MGV002 IV Solution Stabilizer: When administered by a single ambulatory pump or infusion pump configuration, flotetuzumab must be diluted into 0.9% Sodium Chloride Injection, USP (normal saline), containing custom MGV002 Flotetuzumab IV Solution Stabilizer.

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

8. Drug Dose Solution Preparation and Administration: Flotetuzumab DP and MGV002 Flotetuzumab IV Solution Stabilizer will be supplied by MacroGenics for use in this study as appropriate to accommodate the method of study drug administration.

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 (mg)]

<u>Errors in dilution could result in fatal Cytokine Release Syndrome (CRS).</u>

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.

The method of drug administration is using a single ambulatory pump or infusion pump with flotetuzumab dose solution prepared with 0.9% Sodium Chloride Injection, USP (normal saline) and MGV002 IV Solution Stabilizer, which allows for intravenous infusion up to 7 days before an intravenous bag change is required based on treatment plan.

9. IV Bag for single ambulatory pump infusion: The flotetuzumab dose solution is prepared for administration using either 150 mL or 250 mL empty intravenous bag composed of either polyolefin or PVC.

The method of drug administration is using a single ambulatory pump or infusion pump with flotetuzumab dose solution prepared with MGV002 IV Solution Stabilizer and normal saline which allows for intravenous infusion up to 7 days before an intravenous bag change is required based on treatment plan.

Before administration, the parenteral investigational medicinal products should be visually inspected. Drug should not be administered if discoloration or foreign particulates are observed in the solution.

Prior to adding 0.9% Sodium Chloride Injection, USP (normal saline), MGV002 IV Solution Stabilizer, and flotetuzumab IMP to an empty IV bag as described below for dose preparation, the volume of normal saline needs to be calculated based on the predefined total target volume, the specified MGV002 IV Solution Stabilizer volume, and the flotetuzumab IMP volume calculated from the *Dose Calculation Worksheet*.

- The dose solution preparation begins with the addition of calculated volume of normal saline to an empty IV bag, followed by the addition of a predefined volume of MGV002 IV Solution Stabilizer calculated using the *Dose Calculation Worksheet*. **Normal Saline and MGV002 IV Solution Stabilizer must be added to the IV bag prior to adding flotetuzumab to prevent adsorption of flotetuzumab to the IV bag and administration set**.
- Add calculated amount of flotetuzumab IMP into the IV bag containing both normal saline and MGV002 IV Solution Stabilizer. The amount of flotetuzumab IMP calculated for dilution is based upon the patient's weight and the assigned dosing cohort using the Dose Calculation Worksheet and the description in the pharmacy manual.
- When diluted during dose preparation, final flotetuzumab concentrations must be within concentration range of **50 ng/mL and 4,167 ng/mL** (concentration will vary per patient's weight) to enable 24 hours or up to 168 hours of IV infusion of doses in the range of 30 ng/kg/day up to 500 ng/kg/day.

Upon completing the dose preparation steps, gently invert the prepared IV bag to mix the dose solution. **THE BAG MUST NOT BE SHAKEN**. Each intravenous bag contains dose-formulated flotetuzumab that is intended to be used for no more than 7-days of intravenous infusion. Flotetuzumab is stable for 192 hours after dilution in MGV002-stabilized normal saline. The dose-formulated flotetuzumab is stable for up to 24 hours of storage after preparation prior to 7-days of administration. During the time of the infusion, the infusion bag and extension set can be exposed to room temperature. However, if administration does not immediately begin, the bag should be stored refrigerated at 2-8ºC (36−46ºF) until administration can begin, with the time of administration adjusted to account for the maximum of 192 hours of dose-formulated flotetuzumab stability. DO NOT FREEZE the prepared dose solution.

6.2 Toxicity

6.2.1. Definition of Dose-Limiting Toxicity

DLT will be defined employing the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 5.0 (CTCAE v5.0), except IRR/CRS which will be defined by the modified criteria proposed by ASCT and described in section 4.7.1 and in Table 4.

DLT is defined as:

- Grade 4 neutropenia lasting ≥ 42 days from start of cycle in absence of evidence of active AML (< 5% blasts).
- The development of acute or chronic graft-versus-host disease during treatment
- Grade 3−5 nonhematologic toxicity that is possibly, probably or definitely related to the study drug
  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;
  - Infection, bleeding, or other expected direct complication of cytopenias due to active underlying disease;
  - Grade 3 or 4 tumor lysis syndrome (TLS) or disseminated intravascular coagulopathy (DIC) if it is successfully managed clinically and resolves within 7 days without end-organ damage;
  - Grade 3 or 4 isolated electrolyte 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 CRS/IRR that is successfully managed and resolves within 72 hours, as this is an expected event that does not require permanent treatment discontinuation
- Any non-leukemia-related death occurring within 90 days of treatment initiation

Treatment discontinuation for adverse events prior to the completion of one cycle will be considered a DLT unless the adverse event is disease progression, which necessitates alternative therapy, or the adverse event is not possibly, probably, or definitely due to the study treatment. Patients who discontinue treatment prior to the completion of one cycle for progressive disease or due to an event that is not possibly, probably, or definitely due to the study treatment a will be replaced.

6.2.2. Infusion-related Reaction

Infusion-related reaction including cytokine release syndrome (CRS), which may be life-threatening or fatal, occurred in 81% of patients receiving flotetuzumab. The majority of these reactions were mild to moderate in severity, whereas 8% of patients treated at the RP2D experienced at least one severe (Grade 3) event. None of these events have been life threatening or fatal. Infusion reactions may be clinically indistinguishable from manifestations of

CRS. This typically occurs during the initial 48 hours of the first infusion of flotetuzumab. Symptoms of infusion-related reactions including CRS may include chills, fever, headache, fatigue, flushing, nausea, bronchospasm, and hypotension. Patients should be closely monitored for signs or symptoms of these events. For all patients receiving inpatient flotetuzumab infusion, vital signs should be monitored according to the inpatient standard-of-care. Management of these events may require either temporary interruption or discontinuation of flotetuzumab as described in Section 4.5.1.

6.2.3. Other Toxicities

In addition to CRS/infusion reactions, a number of adverse events that were felt to have possible, probable, or definite relationship to flotetuzumab were observed in ≥10% of patients enrolled in the phase I study. The most common such events included pyrexia in 27.3% of patients (Gr ≥3 in 3.5%); nausea in 22.7%; decreased lymphocyte count, decreased platelet count, diarrhea, and vomiting in 13.6%; and chills, fatigue, decreased white blood cell count, and hypocalcemia in 12.1%; and increased alanine aminotransferase, hypomagnesemia, and anemia in 10.6%. The following grade 3/4/5 treatment-related adverse events have been reported:

- Grade 3: infusion-related reaction (12/66, 18.2%); anemia (7/66, 10.6%); febrile neutropenia (5/66, 7.6%); and pyrexia, white blood cell count decreased, C-reactive protein increased, hypocalcemia, hypophosphatemia, pulmonary edema, hypotension, hypertension, and delirium (2/66, 3.0% each); and lymphocyte count decreased, alanine aminotransferase increased, weight increased, urine output decreased, diarrhea, syncope, hypofibrinogenemia, thrombocytopenia, arthralgia, musculoskeletal pain, joint range of motion decreased, dyspnea, laryngeal inflammation, mental status changes, hyperbilirubinemia, and infection (1/66, 1.5% each).
- Grade 4: lymphocyte count decreased and platelet count decreased (8/66, 12.1% each); white blood cell count decreased (5/66, 7.6%); neutrophil count decreased (4/66, 6.1%); and alanine aminotransferase increased, aspartate aminotransferase increased, and respiratory failure (1/66, 1.5% each).
- Grade 5: none.

7. Correlative Studies

The correlative laboratory studies will be attempted on all patients. However, they are not a requirement for participation, and are not formal study endpoints. The overarching goals of the correlative studies are to:

1. Explore the impact of MRD as measured by conventional multi-color flow cytometry (MFC), RNA/DNA-based approaches, and our group's MFC LSC assay[54,55], and their impact on RFS in AML patients who receive flotetuzumab following transplant.
2. Characterize the effect of flotetuzumab on allogeneic T-cells, particularly on their re-distribution, *in vivo* expansion, activation, and effector differentiation after treatment using MFC.
3. Characterize the effect of flotetuzumab on T cell receptor diversity.
4. Characterize the effect of flotetuzumab on T cell gene expression using MFC and RNA sequencing.
5. Characterize gene expression in the tumor microenvironment (TME) using the NanoString PanCancer IO360 assay, and assess the impact of TME gene expression patterns on response to therapy.[69]
6. Characterize T-cell infiltration of the bone marrow using multiplex immunohistochemistry (IHC).[70]

7.1. MRD Monitoring

The high relapse rate seen in adult AML following alloHSCT indicates the lack of eradication of MRD despite morphologic remission. In AML, the presence of MRD by conventional MFC is highly correlated with the expression of leukemia-specific fusion transcripts, and patients with persistent MRD prior to and following transplant have worse overall survival.[29] Correlative specimens will be banked to allow exploration of novel MRD assays in addition to conventional MFC. One such novel assay is a 5-gene RQ-PCR assay developed by Goswami et al. that can be run on peripheral blood samples.[71] The RQ-PCR assay has previously been used to assess MRD in AML patients who underwent a MAC alloHSCT, and demonstrated 89% sensitivity and 100% specificity in predicting post-transplant relapse within one year of transplant based on the presence of MRD pre-transplant.[35] The RQ-PCR assay can be performed on peripheral blood samples, and it would be more convenient to use in future trials, assuming its performance is comparable to a conventional MFC assay for MRD. An additional advantage of this approach is that it does not require a baseline sample of the patient's disease, which may not be available in

this patient population. Another approach will use next generation sequencing (NGS) with a panel of 32 genes that are commonly mutated in AML as well as qPCR for NPM1, which cover approximately 82% of all AML at a sensitivity of 10^{-4} for single nucleotide variants and 10^{-5} for insertions/deletions.[72] This DNA-based next generation sequencing (NGS) has shown a 71% concordance rate with conventional MFC, and pre-transplant MRD by NGS and MFC show a similar association with post-transplant relapse and survival.[73] Notably, the simultaneous presence of MRD by NGS and MFC seems to confer the greatest risk of relapse, so it will be particularly helpful to assess both simultaneously. One limitation of this approach will be the need for diagnostic samples, which may be not be available for all enrolled patient. In addition to conventional MFC, we will also use our group's MFC assay to detect LSCs from bone marrow samples.[35,54] Our group's MFC LSC assay has been shown to be more sensitive for MRD than conventional MFC assays; this probably results from the fact that LSCs in a large fraction of patients do not express the same antigen aberrancy as more differentiated progeny.[54,55,74]

7.2. Flow Cytometry Studies

We will perform multi-color flow cytometry on PB and BM specimens before and after flotetuzumab using three pre-determined panels of mAbs including but not limited to those specific for CD3, CD4, CD8, PD-1, CD45RA, CCR7, CD25, CD27, CD28, CD69, Ki-67, T-bet, CD127, FoxP3, HLD-DR, CTLA-4, TNFRII, TIM3, LAG-3, CD160, 2B4, BTLA, KLRG-1, CD16, and CD56. This multi-color approach allows the examination of different $CD4^+$ and $CD8^+$ T cell subpopulations (expressing CD45RA, CCR7, CD27, and CD28), phenotypic separation of human CD4+FOXP3+ T cells into three distinct subpopulations, and assessment of their proliferative status (% Ki-67 cells).[75,76] The expression of CD69, HLA-DR and TNFRII will be measured to characterize T cell activation status and the expression of co-inhibitory molecules (PD-1, TIM3, LAG-3, CD160, 2B4, BTLA, and KLRG-1) will be studied. Natural killer cells (NK), NK-T cells will be enumerated using CD16 and CD56 (NK/NK-T cells).

7.3. Immune Profiling of the T cell repertoire

The studies proposed will evaluate the T cell receptor (TCR) diversity in T cells isolated from PB and BM from patients before and after treatment with flotetuzumab. We will determine TCR diversity and clonal composition using a molecular and computational approach based on high-throughput DNA sequencing of rearranged TCRβ CDR3 regions from T cell genomic DNA. This approach allows direct measurement of the TCRβ CDR3 region sequence diversity in any

arbitrarily complex population of T cells and also permits quantitative description of the clonal composition of the population.[77] The Luznik laboratory in collaboration with the E. Warren laboratory from the Fred Hutchinson Cancer Research Center (FHCRC) has performed extensive monitoring and tracking of the TCR repertoires in patients undergoing alloHSCT (manuscript in preparation). We are utilizing an established multiplex PCR strategy to amplify the CDR3 region of the TCR, spanning the variable region formed by the junction of the V, D, and J segments and their associated non-template insertions followed by Illumina-based sequencing methodology, a well-characterized methodology developed by Adaptive Technology (http://www.adaptivebiotech.com/technology/). Sequencing is followed by comprehensive bioinformatics analyses focused on determining the diversity of the T cell and B cell repertoires as well as the entropy and clonality of each repertoire consistent with previous studies. Through these ongoing studies we have developed substantial experience not only in using and analyzing DNA retrieved from unsorted PBMCs but also from sorted T cell subpopulations (naïve vs. memory vs. regulatory), paired PB and BM samples as well as DNA retrieved from FFPE archived tissues.

We hypothesize that a primary action of flotetuzumab will be to re-direct allogeneic T cells toward the CD123+ cells resulting in increased TCR diversity and the emergence of unique TCR clonotypes that may be relevant in allogeneic post-transplant anti-tumor immunity. The long-term goal of this correlative work is to uncover biomarkers that predict which patients will respond to flotetuzumab. This correlative work will also provide deeper insight into allogeneic anti-tumor immunity, effects of anti-CD123/CD3 mAbs on GVHD, and mechanisms of failure to eradicate anti-CD123+ malignancies after flotetuzumab treatment. Exploratory studies to characterize T cell transcriptional signature before and after treatment using Human Prime View Gene Expression Array (http://www.affymetrix.com) will be also considered to gain further insight into T cell function in patients with AML before and after treatment. While analysis will focus on $CD8^+$ T cells, as part of T cell isolation we are routinely sorting $CD8^+$, $CD4^+$, B cells, NK cells and tumor cells; these other populations will be stored for future analysis if of interest.

7.4. Cytokine Profiling

As a part of PB and BM collection and mononuclear cell isolation we will collect plasma that will be used for the measurement of defined cytokines (IL-2, IL-6, IL-10, IFN-γ, TNF-α) to assess flotetuzumab and checkpoint inhibitors-induced global T-cell activation. We will examine the levels of cytokines before and after treatment with flotetuzumab.

7.5. Operating Procedures for Specimen Collection

Peripheral blood and bone marrow specimen collection will be performed as delineated in the study calendar (Section 4.3) and shown in Figure 2. Correlative studies will only be collected at the times when peripheral blood and bone marrow collections are performed for clinical care. All enrolled patients will have bone marrow and peripheral blood samples collected prior to treatment with flotetuzumab (day -14-0), following treatment with flotetuzumab (day 28), at days 180 and 365 post-transplant, and at the time of relapse as illustrated in **Figure 2**. An additional peripheral blood sample will be collected during the first cycle of treatment with flotetuzumab on day 14.

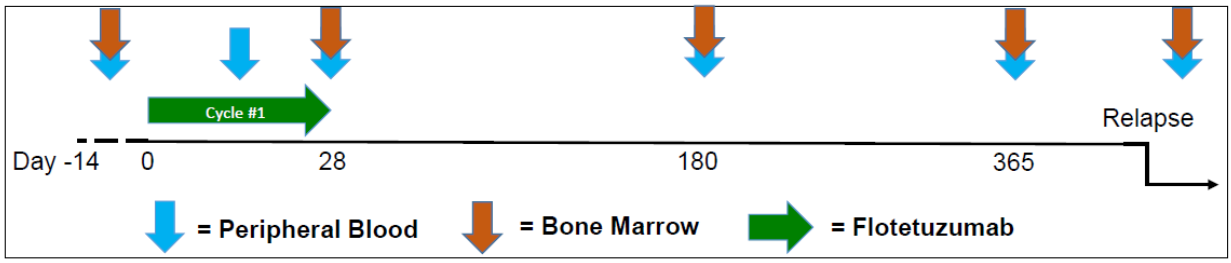

Figure 2: Sample Collection for post-transplant flotetuzumab

Bone marrow: 5 x 8mL will be collected in green top ACD tubes (~40 mL). Re-adjustment of the direction of bone marrow aspirate needle should take a place after each 10 cc is collected to prevent hemodilution. At the time of collection, green top tubes must be thoroughly mixed to prevent clotting.

Peripheral blood: 5x8mL ACD green top tubes of blood (~40mLs) will be collected at each time point.

Specimens should be labeled with the patient's study number (given at the time of registration), study protocol number, sample collection date and time, and sample source (PB or BM). Thus, the samples will be de-identified, but the principal investigator and relevant study staff will have access to the study numbers to allow clinical correlation. Sample collection date and time, and sample source (PB or BM) will be recorded. All data should be kept in the laboratory log. At each sampling time, BM and PB mononuclear cells (PBMC) will be processed via Ficoll density gradient centrifugation. The washed cells will be counted, triaged for DNA isolation, and viably

cryopreserved using a controlled-rate freezer with transfer to the vapor phase of liquid nitrogen for long-term storage.

The specimens should be delivered to the Jones Laboratory at the Sidney Kimmel Comprehensive Cancer Center immediately after collection. Members of the Jones Laboratory will have access to the de-identified samples for the purposes of the studies outlined herein. Analysis of the results including correlation with clinical information will be performed at the Sidney Kimmel Comprehensive Cancer Center. Upon completion of the research protocol (i.e. two years from the end of treatment of the final patient), all specimens will be destroyed or transferred to the Johns Hopkins Biospecimen and Genomic Data Bank in the Department of Pathology based on the patients' agreement.

8. Statistical Methods

This is a pilot study of flotetuzumab in patients with AML as post-alloHSCT therapy among patients in remission and those with relapsed disease.

8.1. Endpoints

8.1.1. Primary Endpoints

1. Adverse events and toxicities will be tabulated and reported by type and grade and the proportions reported with exact 95% binomial confidence intervals.
2. The maximum tolerated dose (MTD) of flotetuzumab in patients with relapsed/refractory AML following alloHSCT will be determined.

8.1.2. Secondary Endpoints

8.1.2.1. Response-related Endpoints

1) Report the complete response (CR + CRi) rate in relapsed AML to flotetuzumab following alloHSCT.

8.1.2.2. Toxicity-related Endpoints

2) Report the incidence of acute GVHD
3) Report the incidence of chronic GVHD
4) Report cumulative incidence of non-leukemia mortality

8.2. Study Design

This study proposal will be a phase 1 trial of flotetuzumab in patients with relapsed/refractory AML following alloHSCT. The first part of the study will be a dose escalation following a 3+3 design for post-transplant flotetuzumab. The study will start with the recommended dose of flotetuzumab in relapsed/refractory ALL (DL1). The first three patients will be enrolled and monitored for the first cycle of treatment for DLTs through the full DLT period (90 days) before enrolling additional patients. If less than 2 patients experience a DLT, an additional 3 patients will be enrolled. If 0 or 1 out of 6 patients have a DLT, then the dose will be deemed safe and considered the MTD of flotetuzumab in the post-transplant setting. If 2 or more of the first 3 or 6 patients experience a DLT, then the trial will proceed to enroll patients at DL-1 starting with 3 patients. The first three patients on DL-1 will be enrolled and monitored in the first cycle of treatment for DLTs through the full DLT period (90 days) before enrolling additional patients. If less than 2 patients experience a DLT at DL-1, an additional 3 patients will be enrolled at DL-1. If 0 or 1 out of 6 patients have a DLT at DL-1, then the dose will deemed safe and the MTD. If 2 or more of the first 3 or 6 patients experience a DLT, then the trial will be stopped. An expansion cohort of 4 subjects will be also pursued at MTD for flotetuzumab to further characterize safety, tolerability and any preliminary signs of biological or clinical activity in this cohort. DLT monitoring will continue in this expansion cohort, and the trial will be stopped if two additional subjects experience a DLT. Any patient who discontinues treatment prior to the completion of one cycle of flotetuzumab for disease progression or for an adverse event that is not possibly, probably, or definitely due to the study treatment will be replaced.

8.3. Analysis of secondary endpoints

8.3.1. Response

Due to practical considerations, our sample size will be limited to a maximum of 16 patients. CR rate will be reported as a proportion for the endpoint, along with the exact 90% confidence intervals for binomial proportion.

Prior reports indicate that the CR rate in this population of patients is around 30%.[49] In Table 7, we report the precision and compute the exact 90% confidence intervals for the CR rate assuming a range of reasonably anticipated values for the flotetuzumab treatment.

Table 7. CR rates and 90% exact binomial confidence intervals for maximum enrollment at a single dose level (N=10)

CR rate	20	30	40	50	60
Exact 90% CI	0-41	6-54	15-65	24-76	35-85

8.3.2. Toxicity-related Endpoints

The cumulative incidence of acute (grade I-IV, grade III-IV) and chronic GVHD (overall, and by extent) will be estimated through competing-risk analysis, wherein death is a competing risk for GVHD.

9. Data Safety and Monitoring Plan

The study PI will be responsible for the conduction of the study, including the monitoring of the study's safety and oversight of the data collection. The PI will follow the Data Safety and Monitoring plan outlined in the Sidney Kimmel Comprehensive Cancer Center's DSMP Policy.

9.1. Data Reporting

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring.

The PI is responsible for monitoring the study. Data must be reviewed to assure the validity of the data, as well as the safety of the subjects. The PI will also monitor the progress of the trial, review safety reports, and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study. The PI will be responsible for maintaining the clinical protocol, reporting adverse events, assuring that consent is obtained and documented, reporting of unexpected outcomes, and reporting the status of the trial in the continuing renewal report submitted to the IRB and to the monitoring review group. The report should be submitted in a timely manner according to the schedule defined by the Johns Hopkins Medicine Institutional Review Board.

9.2. Management of Safety Data

This Study has been designated as an interventional study. As such, all adverse events/product quality complaints for study drug (flotetuzumab) regardless of causality and special situations will be reported from the time a subject has signed an Informed Consent Form (ICF) until 30 days after the last dose of study drug excluding those from subjects not exposed to study drug.

Serious adverse events will be reported for 30 days after the last dose of study drug.

9.3. Adverse Event Definition

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non- investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonisation [ICH])

This includes any occurrence that is new in onset or increased in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities (except Grade 1 and 2 hematologic and metabolic abnormalities) that are deemed clinically significant by the principal investigator.

This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 for adverse event reporting, except for IRR/CRS, which has been defined in Table 4. In cases where CTCAE version 5.0 cannot be applied to the toxic event, the investigator will quantify the toxicity based on the intensity as defined:

1. Mild: The subject is aware of the signs or symptoms but they are easily tolerated; usually does not require additional therapy or discontinuation of study drugs.
2. Moderate: The signs and symptoms are sufficient to restrict but do not prevent usual activity; possibly requires additional therapy but usually does not require discontinuation of the study drug.
3. Severe: The subject is unable to perform usual activities and usually requires discontinuation of the study drug.

Patients are to be followed for adverse events for 30 days after the last dose of the study drug and any adverse event occurring in a patient up to 30 days after stopping the study drug or until the start of a subsequent systemic anticancer therapy, if earlier, must be reported. The surveillance period after study drug discontinuation may be extended if there is a strong

suspicion that the drug has not yet been eliminated or if the nature of a particular event may suggest long-term effects from the investigational drug, as assessed by the investigator.

9.4. Serious Adverse Event (SAE) Definition

A serious adverse event based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via study drug
- Is medically important determined by the investigator(s)*

*The investigator should exercise medical and scientific judgment in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

NOTE: DEATH FOR ANY REASON SHOULD BE REPORTED AS A SERIOUS ADVERSE EVENT.

9.4.1. Hospitalization or prolongation of existing hospitalization following the completion of flotetuzumab

For reports of hospitalization, it is the sign, symptom or diagnosis which led to the hospitalization that is the serious event for which details must be provided. In the event of prolongation of an already existing hospitalization, the event that triggered the increased hospital stay will be considered as SAE.

Any event requiring hospitalization or prolongation of hospitalization that occurs during the study must be reported as a serious adverse event, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study. [Note: Hospitalizations that were planned before the start of data collection and where the underlying condition for which the hospitalization was planned has not worsened will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.]
- The investigator will be required to hospitalize the subject for the duration of the treatment period.

9.4.2. Life-Threatening Conditions

An AE or SAE is considered "life-threatening" if, in the view of the Investigator, its occurrence places the subject at immediate risk of death. It does not include an AE or SAE that, had it occurred in a more severe form, might have caused death.

Disease progression should not be recorded as an adverse event or serious adverse event term; instead, signs and symptoms of clinical sequelae resulting from disease progression/lack of efficacy will be reported if they fulfill the serious adverse event definition.

9.5. Unexpected (unlisted) Adverse Event/Reference Safety Information

An adverse event is considered unexpected if the nature or severity is not consistent with the applicable product reference safety information. For a medicinal product(s) with a marketing authorization, the expectedness of an adverse event will be determined by whether or not it is listed in the applicable product information.

For flotetuzumab, the expectedness of an adverse event will be determined by whether or not it is listed in the Investigator's Brochure. Any unexpected clinical event, regardless of grade requires expedited reporting to the regulatory authorities.

9.6. Adverse Drug Reaction and Toxicity Monitoring

The study team (research nurse, study coordinator or attending) will assign toxicity scores using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, weekly through the first cycle of therapy and at least every cycle thereafter. A copy of the CTCAE version 5.0 is available at (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_refer

ence_5x7.pdf). If unexpected and serious toxicity occurs that would result in patients being subjected to unacceptable risk, the trial will be placed on hold while the toxicity is investigated.

9.7. Toxicity Reporting

The Principal Investigator is responsible for ongoing safety evaluation of the study. The Principal Investigator is required to notify his/her Institutional Review Board (IRB) of a serious adverse event according to institutional policy. The requirements for IRB Protocol Problem Reporting at Johns Hopkins are can be found at this website: http://www.hopkinsmedicine.org/institutional_review_board/guidelines_policies/guidelines/

FDA reporting requirements

7 Calendar-Day IND Safety Report

Fatal or life threatening SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 7 calendar days after awareness of the event. Serious adverse events (SAEs) that are unexpected, and at least possibly associated to the study drugs, as assessed by the Investigator, should be reported promptly to the Food and Drug Administration (FDA).

15 Calendar-Day Written IND Safety Report

The Investigator is required to notify the FDA, and all participating investigators in a written IND Safety Report, of any serious, unexpected adverse event considered by the Investigator to be possibly related to the use of study drugs within 15 calendar-days of first learning of the event. If applicable, the Investigator must also notify the FDA, and all participating investigators, of any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity within 15 calendar-days of first learning of the event.

All other SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 15 calendar days after awareness of the event. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

All IND safety reports must be submitted to the FDA on Form 3500A and be accompanied by Form 1571. The report must be submitted to an appropriate Review division that has the responsibility to review the IND application under which the safety report is submitted. All IND

safety reports are recommended to be submitted electronically. Other means of rapid communication to the respective review divisions Regulatory Project Manager (e.g., telephone, facsimile transmission, email) may also be used.

Reporting Protocol Deviations to the JHM IRB

There are several types of deviations from protocol procedures recognized by the JHM IRB, and each type has a different IRB reporting requirement:

A. Protocol deviations that constitute unanticipated problems involving risks require prompt reporting to the JHM IRB: A protocol deviation that constitutes an "unanticipated problem involving risks to subjects or to others" (Policy No. 103.6(b) for the definition of an unanticipated problem) must be reported promptly to the IRB, as follows:

1. Emergency deviations: When a deviation occurs in an emergency situation, such as when a departure from the protocol is required to protect the life or physical well-being of a participant. The Sponsor (JHU Principal Investigator) and the reviewing IRB must be notified as soon as possible, but not later than 5 days after the emergency situation occurred (21 CFR 812.150(a)(4)).
2. Major, non-emergent deviations without prior approval: A planned deviation that is non-emergent and represents a major change in the protocol as approved by the IRB. The Principal Investigator and the IRB must approve the request before the proposed change is implemented. If a major, non-emergent deviation occurs without prior IRB approval the event is considered non-compliance. Noncompliance must be reported to the IRB promptly.

B. Protocol deviations that are only minor or administrative: At JHM, minor or administrative protocol deviations are defined as those which do not "affect the scientific soundness of the research plan or the rights, safety, or welfare of human subjects." If a protocol deviation occurs which meets this definition, the deviation should be reported to the JHM IRB at the time the continuing review application is submitted. Examples of minor or administrative deviations could include: follow up visits that occurred outside the protocol required time frame because of the participant's schedule, or blood samples obtained at times close to but not precisely at the time points specified in the protocol.

9.7.1. Special Reporting Situations

Safety events of interest for a Macrogenics medicinal product that require expediting reporting and/or safety evaluation include, but are not limited to:

- Drug exposure during pregnancy (maternal and paternal)
- Overdose of a Macrogenics medicinal product
- Exposure to a Macrogenics medicinal product from breastfeeding
- Suspected abuse/misuse of a Macrogenics medicinal product
- Inadvertent or accidental exposure to a Macrogenics medicinal product
- Any failure of expected pharmacological action (i.e., lack of effect) of a Macrogenics medicinal product
- Medication error involving a Macrogenics medicinal product (with or without patient exposure to the Macrogenics medicinal product, e.g., name confusion)
- Suspected transmission of any infectious agent via administration of a medicinal product
- Unexpected therapeutic or clinical benefit from use of a Macrogenics medicinal product

These safety events may not meet the definition of an adverse event; however, from a Macrogenics Scientific Affairs, LLC perspective, they are treated in the same manner as adverse events. Special situations should be recorded on the Adverse Event page of the CRF.

Any special situation that meets the criteria of a serious adverse event should be recorded on a Serious Adverse Event Report Form and be reported to Macrogenics Scientific Affairs, LLC **within 24 hours of the submission to regulatory authorities.**

9.7.2. SAEs

All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct

- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

The principal investigator will transmit all SAEs and special situations following exposure to a Macrogenics product under study in a form provided by Macrogenics Scientific Affairs, LLC in accordance with Section 9.11.4 Transmission Methods, in English **within 24-hours of the submission to regulatory authorities.**

All follow-up information for serious adverse events that are not resolved at the end of the study or by the time of patient withdrawal must be reported directly by the principal investigator, **within 24 hours of the submission to regulatory authorities**, to Macrogenics Scientific Affairs, LLC using the Macrogenics Scientific Affairs, LLC Serious Adverse Event Report.

All available clinical information relevant to the evaluation of a related SAE, serious ADR or special situation is required.

- The principal investigator is responsible for ensuring that these cases are complete and if not are promptly followed-up. A safety report is not considered complete until all clinical details needed to interpret the case are received. Reporting of follow-up information should follow the same timeline as initial reports.

- Copies of any and all relevant correspondences with regulatory authorities and ethics committees regarding any and all serious adverse events, irrespective of association with the Macrogenics Product under study, are to be provided to Macrogenics Scientific Affairs, LLC using a transmission method in Section 9.11.4.

9.7.3 Non-Serious AEs

All non-serious adverse events should be reported to Macrogenics Scientific Affairs, LLC according to the timeframe agreed between all involved parties.

9.8. Pregnancies

All initial reports of pregnancy must be reported to Macrogenics Scientific Affairs, LLC by the principal investigator **within 24 hours of the submission to regulatory authorities** using the Serious Adverse Event Form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, fetal death, stillbirth, congenital anomaly, ectopic pregnancy) are considered serious adverse events and must be reported using the Serious Adverse Event Form.

Any subject who becomes pregnant during the study must be promptly withdrawn from the study and discontinue further study treatment.

Because the effect of the Macrogenics medicinal product on sperm is unknown, pregnancies in partners of male subjects exposed to a Macrogenics medicinal product will be reported by the principal investigator **within 24 hours of the submission to regulatory authorities** using the Serious Adverse Event Form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

9.9. Drug Relationship

The investigator will classify the study product relationship of an adverse event to the investigational product according to the following definition:

1. None: The time course between the administration of the study product and the occurrence or worsening of the adverse event rules out a causal relationship and or another cause is confirmed and no indication of involvement of the study product in the occurrence/worsening of the adverse event exists.
2. Unlikely: the time course between the administration of the study product and the occurrence or worsening of the AE makes a causal relationship unlikely; the known product effects of the study product or of the substance class provide no indication of involvement in the AE and another cause adequately explains the AE; regarding the AE, a plausible causal chain may be deduced from the known side effects of the study product or the substance class but another cause is much more probably; or another cause is confirmed and involvement of the study product in the AE is unlikely.
3. Possible: Regarding the AE, a plausible causal chain may be deduced from the pharmacological properties of the study product or the substance class, but another cause just as likely to be involved is also known; although the pharmacological

properties of the study product or the substance class provide no indication of involvement in the AE, no other cause gives adequate explanation.

4. Probable: the pharmacological properties of the study product or of the substance class and/or the course of the AE suggest involvement of the study product in the AE, although another cause cannot be ruled out.

5. Definite: the pharmacological properties of the study product or of the substance class and the course of the AE indicate involvement of the study product in the AE and no indication of other causes exists.

6. Unclassifiable: only used for SAE: the available information is not sufficient for causality assessment.

9.10. Outcome

The investigator will record the outcome of the AE choosing one of the following categories:

1. Recovered/resolved
2. Recovering/resolving
3. Not recovered/not resolved
4. Recovered/resolved with residual effects as specified
5. Fatal
6. Unknown

9.11. Data Handling and Record Keeping

9.11.1. Maintenance of Safety Information

All safety data should be maintained in a clinical database in a retrievable format. The principal investigator shall provide all adverse events, both serious and non-serious, in report format. However, in certain circumstances more frequent provision of safety data may be necessary, e.g. to fulfill a regulatory request, and as such the data shall be made available within a reasonable timeframe at Macrogenics Scientific Affairs, LLC request.

9.11.2. Procedures for Reporting Safety Data and Product Quality Complaints (PQCs) for Macrogenics Medicinal Products to Macrogenics Scientific Affairs, LLC

All adverse events and special situations, whether serious or non-serious, related or not related, following exposure to a Flotetuzumab are to be documented by the investigator and recorded in the CRF and in the subject's source records. Investigators must record in the CRF their opinion concerning the relationship of the adverse event to Flotetuzumab.

All (serious and non-serious) adverse events reported for Flotetuzumab should be followed-up in accordance with clinical practice.

9.11.3. Reporting Procedures for Reporting Safety Data and Product Quality Complaints (PQCs) for Non-Macrogenics Medicinal Products

For SAEs, special reporting situations and PQCs following exposure to a non-Macrogenics medicinal product under study, the principal investigator should notify the appropriate regulatory/competent authority or the manufacturer of that medicinal product (in the absence of appropriate local legislation) as soon as possible.

9.11.4. Transmission Methods

The following methods are acceptable for transmission of safety information to Macrogenics Scientific Affairs, LLC:

- Electronically via Macrogenics SECURE Email service (preferred), For business continuity purposes, if SECURE Email is non-functional:
    - Facsimile (fax), receipt of which is evidenced in a successful fax transmission report, to
- Telephone (if fax is non-functional).

Please use the contact information and process information provided by Macrogenics Scientific Affairs, LLC. The report will be submitted to MacroGenics Product Safety via:

Fax: 301.354.3800 OR

Email: saereports@MacroGenics.com

10. Ethics

10.1. Institutional Review Board

The study protocol and any amendment that is not solely of an administrative nature must be approved by an Institutional Review Board (IRB).

10.2. Ethical Conduct of the Study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki.

10.3. Evaluation of Benefits and Risks/Discomforts

10.3.1. Potential Benefits

Patients will receive evaluation and treatment of their malignancy as a result of participating in this trial. The trial will provide information on the safety of administering flotetuzumab to patients following alloHSCT but may or may not help a specific patient personally. This treatment may enhance relapse free survival compared to alloHSCT alone. Alternative approaches to entering this trial, including standard supportive care only, will also be discussed before the verbal and written consent is obtained regarding the risks, benefits, and the treatment requirements for this trial.

10.3.2. Measure for Minimizing Risk

Administering flotetuzumab to patients may involve risks that are currently unforeseeable. Side effects can be unpredictable in nature and severity, although all care will be taken to minimize them. If patients suffer any physical injury as a result of participating in this study, immediate medical treatment is available at the treatment center. Frequent blood work will be taken to monitor side effects. Although no compensation is available, any injury will be evaluated and treated in keeping with benefits of care to which patients are entitled under applicable regulations. Patients may experience significant treatment-related morbidity, and/or progression of their disease.

10.3.3. Risks/Benefits Analysis

Data gathered from both clinical and laboratory evaluations in this trial will be analyzed frequently to ensure safety of the patients. Any new or significant finding(s) found during the course of the research will be shared and explained to each participant since that may affect a patient's willingness to participate further. Patient's anonymity will be protected to the maximum extent in all publications and presentations that result from research.

10.3.4. Patient Information and Consent

The investigator or consent designee will explain the nature of the study, its purpose and associated procedures, the expected duration, and the potential benefits and risks of participation to each patient prior to his/her entry into the study (i.e. before examinations and procedures associated with selection for the study are performed). Each patient will have ample opportunity to ask questions and will be informed about the right to withdraw from the study at

any time without any disadvantage and without having to provide reasons for this decision. Following this informative discussion, a patient will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the patient voluntarily agrees to sign the informed consent statement and has done so, may he/she enter the study. The patient will receive a copy of the signed and dated informed consent form. The signed informed consent statement is to remain in the investigator's files. The informed consent form and any other written information provided to the patients will be revised whenever important new information becomes available that may be relevant to the patient's consent, or there is an amendment to the protocol which necessitates a change to the content of the written informed consent form. The investigator will inform the patient of changes as per IRB recommendations in a timely manner and will ask the patient confirm continuation of his/her participation in the study by his/her signature on the revised informed consent form. Any revised written informed consent form must receive the IRB's approval/favorable opinion in advance of use.

10.4. Financial Disclosure

Each investigator (including the principal investigator and any sub-investigators) who is directly involved in the treatment or evaluation of research subjects must disclose certain financial arrangements. A financial disclosure statement must be provided for each investigator (including each sub-investigator in IND studies identified on FDA Form 1572) at a study site.

The following arrangements with, and interests of, investigators (including the spouse and dependent children) should be disclosed to the FDA:

1. Compensation made to the investigator in which the value of compensation could be affected by study outcome (e.g. higher compensation for favorable outcome than for an unfavorable outcome, or a royalty interest related to product sales).
2. A proprietary interest by the investigator in the tested product, including, but not limited to, a patent, trademark, copyright or licensing agreement
3. Any equity interest in the study supporter, Macrogenics Scientific Affairs, LLC (i.e. any ownership interest, stock options, or other financial interest whose value cannot be readily determined through reference to public prices, or any equity interest in a publicly held company that exceeds $50,000 in value held during the time the investigator is carrying out the study and for 1 year following completion of the study)
4. Significant payments of other sorts, i.e., payments that have a cumulative monetary value of $25,000 or more, made by the study supporter (Macrogenics Medical

Affairs, LLC) of a covered study to the investigator or the investigator's institution to support activities of the investigator exclusive of the costs of conducting the clinical study or other clinical studies (e.g., a grant to fund ongoing research, compensation in the form of equipment or retainers for ongoing consultation or honoraria) during the time the investigator is carrying out the study and for 1 year following completion of the study

5. In this context "investigator" is defined as all individuals listed on FDA form 1572 – or for non-IND studies performed outside the U.S. listed in the signature list.
6. This shall also apply to the spouse and each dependent child of the investigator.

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12. Appendix

12.1. GVHD Staging and Grading

12.1.1. Acute GVHD[66]

GVHD Target Organ Staging

Stage	Skin (Active Erythema Only)	Liver (Bilirubin)	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	<2 mg/dL	No or intermittent nausea, vomiting, or anorexia	Adult: <500 mL/day or <3 episodes/day Child: <10 mL/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2-3 mg/dL	Persistent nausea, vomiting or anorexia	Adult: 500-999 mL/day or 3-4 episodes/day Child: 10-19.9 mL/kg/day or 4-6 episodes/day
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL		Adult: 1000-1500 mL/day or 5-7 episodes/day Child: 20-30 mL/kg/day or 7-10 episodes/day
3	Maculopapular rash >50% BSA	6.1-15 mg/dL		Adult: >1500 mL/day or >7 episodes/day Child: >30 mL/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) *plus* bullous formation and desquamation >5% BSA	>15 mg/dL		Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume).

Overall clinical grade (based on most severe target organ involvement):
Grade 0: No stage 1-4 of any organ.
Grade I: Stage 1-2 skin without liver, upper GI, or lower GI involvement.
Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI.
Grade III: Stage 2-3 liver and/or stage 2-3 lower GI, with stage 0-3 skin and/or stage 0-1 upper GI.
Grade IV: Stage 4 skin, liver, or lower GI involvement, with stage 0-1 upper GI.

12.1.2. Chronic GVHD[67]

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capabl of self-care, >50% of waking hours ou of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN† **SCORE % BSA** ☐ *GVHD features to be scored by BSA:* **Check all that apply:** ☐ Maculopapular rash/erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1-18% BSA	☐ 19-50% BSA	☐ >50% BSA
SKIN FEATURES SCORE:	☐ No sclerotic features		☐ Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration

Other skin GVHD features (NOT scored by BSA)
Check all that apply:
☐ Hyperpigmentation
☐ Hypopigmentation
☐ Poikiloderma
☐ Severe or generalized pruritus
☐ Hair involvement
☐ Nail involvement
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____

MOUTH *Lichen planus-like features present:* ☐ **Yes** ☐ **No**	☐ No symptoms	☐ Mild symptoms **with** disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake

☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* ☐ **Yes** ☐ **No** ☐ **Not examined**	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
GI Tract ***Check all that apply:*** ☐ Esophageal web/ proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss ≥5%* ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss* (<5%)	☐ Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____				
LIVER	☐ Normal total bilirubin and ALT or AP < 3 x ULN	☐ Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	☐ Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	☐ Elevated total bilirubin > 3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LUNGS**				
Symptom score:	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung score: **%** FEV1 ☐	☐ FEV1≥80%	☐ FEV1 60-79%	☐ FEV1 40-59%	☐ FEV1 ≤39%
Pulmonary function tests ☐ Not performed				
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____				

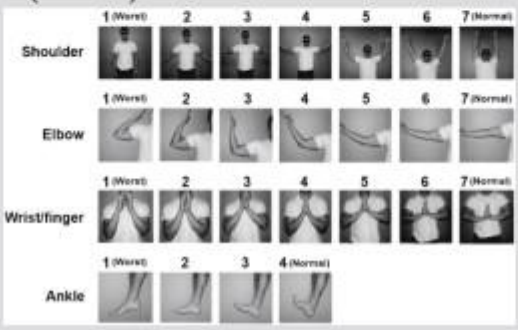
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*[†]) ☐ Not examined *Currently sexually active* ☐ Yes ☐ No	☐ No signs	☐ Mild signs[‡] and females with or without discomfort on exam	☐ Moderate signs[‡] and may have symptoms with discomfort on exam	☐ Severe signs[‡] with or without symptoms
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0-3) based on functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

☐ Ascites (serositis)___	☐ Myasthenia Gravis___	
☐ Pericardial Effusion___	☐ Peripheral Neuropathy___	☐ Eosinophilia > 500/µl___
☐ Pleural Effusion(s)___	☐ Polymyositis___	☐ Platelets <100,000/µl ___
☐ Nephrotic syndrome___	☐ Weight loss>5%* without GI symptoms ___	☐ Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ❑ **No GVHD** ❑ **Mild** ❑ **Moderate** ❑ **Severe**

Photographic Range of Motion (P-ROM)



Mild chronic GVHD

1 or 2 Organs involved with no more than score 1 *plus*

Lung score 0

Moderate chronic GVHD

3 or More organs involved with no more than score 1

OR

At least 1 organ (not lung) with a score of 2

OR

Lung score 1

Severe chronic GVHD

At least 1 organ with a score of 3

OR

Lung score of 2 or 3

Key points:

In skin: higher of the 2 scores to be used for calculating global severity.

In lung: FEV1 is used instead of clinical score for calculating global severity.

If the entire abnormality in an organ is noted to be unequivocally explained by a non-GVHD documented cause, that organ is not included for calculation of the global severity.

If the abnormality in an organ is attributed to multifactorial causes (GVHD plus other causes) the scored organ will be used for calculation of the global severity regardless of the contributing causes (no downgrading of organ severity score).