CHILDREN'S ONCOLOGY GROUP

The world's childhood cancer experts

NCI Pediatric Early Phase Clinical Trials Network

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PEPN1812

A PHASE 1 TRIAL OF THE CD123 X CD3 DART® MOLECULE FLOTETUZUMAB (NSC#808294, [redacted]) IN CHILDREN, ADOLESCENTS, AND YOUNG ADULTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

Lead Organization: COG Pediatric Early Phase Clinical Trials Network (PEP-CTN)

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For PEP-CTN Operations and Data/Statistics Contacts see: http://members.childrensoncologygroup.org

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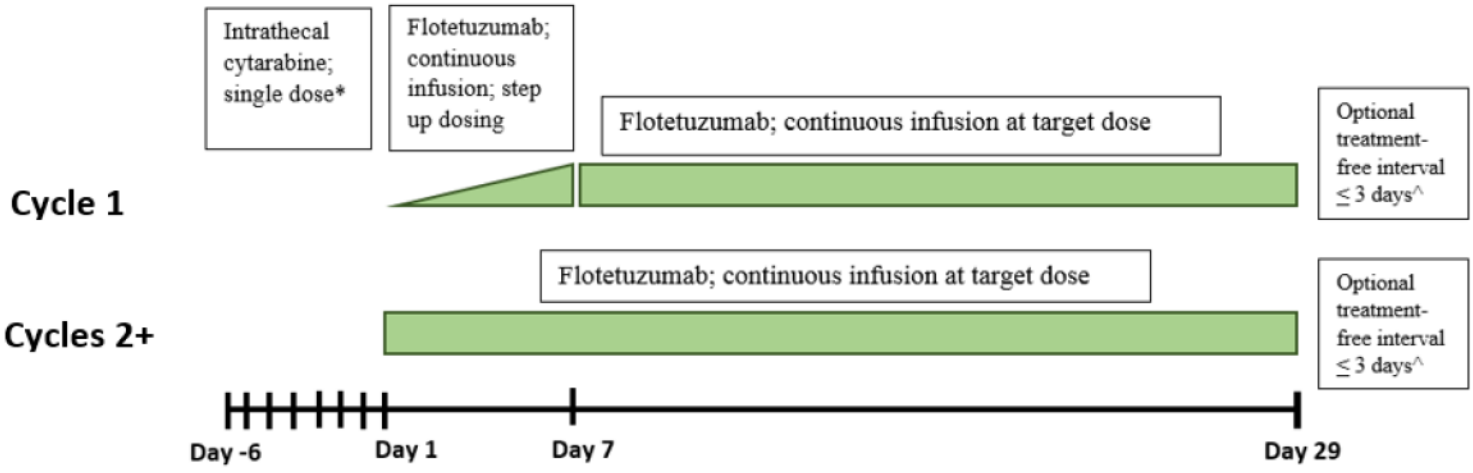
The Certificate of Confidentiality will not protect against mandatory disclosure by the researchers of information on suspected child abuse, reportable communicable diseases, and/or possible threat of harm to self or others.

ABSTRACT

PEPN1812 is a Phase 1 study designed to evaluate the toxicity profile of flotetuzumab in pediatric patients with relapsed/refractory acute myeloid leukemia (AML). Flotetuzumab is a bispecific antibody designed to target CD3 and CD123. Patients will receive a continuous infusion of flotetuzumab due to the favorable toxicity and efficacy profile of this administration schedule in adults with AML. Patients may receive up to a total of 6 cycles of flotetuzumab unless they are unable to achieve a partial response (PR) after Cycle 2 or are found to have progressive disease at any time point. The objectives of this study are to determine the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) of flotetuzumab, as well as to describe the toxicities, pharmacokinetics, and pharmacodynamics properties of flotetuzumab in the pediatric population. PEPN1812 includes correlative laboratory studies to identify biomarkers to predict toxicities and response to flotetuzumab.

EXPERIMENTAL DESIGN SCHEMA



*Patients should receive a dose of intrathecal (IT) cytarabine prior to the start of Cycle 1 between Days -6 and 0. Patients may receive an optional dose of IT cytarabine during subsequent cycles on Day 1 or with bone marrow evaluations at the discretion of the treating physician.

^The duration of the optional treatment-free interval (up to a maximum of 3 days) is at the treating physician's discretion. If no

treatment-free interval is considered necessary, patients may proceed immediately to the next cycle of therapy as long as they do not have progressive disease. In cases where the patient immediately proceeds to the next cycle of therapy, Day 29 of the previous cycle will be Day 1 of the subsequent cycle.

A cycle of therapy is 29 days. The starting dose will be 500 nanograms/kg/day (DL1) with dose levels for subsequent groups of patients as follows:

Dose Level	Dose (nanograms/kg/day)
-1	300
1*	**500**
2	700

* Starting Dose Level

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To assess the safety and tolerability of flotetuzumab administered by continuous IV infusion to pediatric patients < 21 years of age with relapsed or refractory AML.

1.1.2 To estimate the maximum tolerated dose (MTD) and/or recommended Phase 2 dose (RP2D) of flotetuzumab administered by continuous IV infusion to pediatric patients < 21 years of age with relapsed or refractory AML.

1.2 Secondary Aims

1.2.1 To characterize the pharmacokinetics of flotetuzumab in pediatric patients with relapsed of refractory AML.

1.2.2 To define preliminarily the anti-tumor activity of flotetuzumab within the confines of a Phase 1 study and correlate potential activity with baseline disease burden at study entry.

1.2.3 To monitor anti-drug antibody (ADA) production and characterize the immunogenicity of flotetuzumab.

1.3 Exploratory Aims

1.3.1 To evaluate changes in T-lymphocyte population numbers before and after flotetuzumab treatment.

1.3.2 To evaluate the tumor microenvironment and cytokine production by immune effector cells before and after flotetuzumab treatment.

1.3.3 To quantify CD123 surface expression on AML cells at baseline and evaluate expression as a potential biomarker of flotetuzumab response.

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

Despite maximal intensification of multi-agent cytotoxic chemotherapy, long-term cure rates for children with AML remain approximately 60%.[1] The development of new AML immunotherapies is thus of great interest, as these therapies may improve outcomes or allow reduction in cytotoxic chemotherapy and decrease long-term toxicities. DART molecules are bispecific antibody-based agents that bind two distinct antigens simultaneously. DARTs are similar to single-chain bispecific antibodies, such as blinatumomab, but instead have two independent polypeptides each composed of the V_H of one antibody in tandem with the V_L of the other antibody attached by a disulfide bridge.[2] This unique architecture improves effector-to-target (E:T) cell-to-cell contact and induces greater *in vitro* target cell killing and T cell-specific activation.[3] Flotetuzumab is a CD123 x CD3 DART designed to target CD123+ tumor cells for recognition and elimination by CD3-expressing T lymphocytes. CD123 (IL3R alpha chain) is expressed at high levels on the majority of AML cells, as well at lower levels on normal plasmacytoid dendritic cells (pDC), basophils, monocytes, and eosinophils.[4] CD123 overexpression in AML has been associated with higher rates of chemoresistance and with high-risk genetic alterations, including *FLT3* internal tandem duplication (*FLT3*-ITD).[5-7] CD123 is also expressed at high levels on leukemia stem cells (LSCs), a small and dormant cellular population that likely contributes to chemoresistance and leukemia progression.[8,9] CD123 overexpression combined with the differential CD123 expression on LSCs and probable lack of expression by normal hematopoietic stem cells suggests that targeting CD123 may provide both preferential debulking and more durable remission.[10,11] To that end, robust anti-leukemia efficacy has been observed in preclinical models of adult and childhood AML treated with a variety of CD123-targeted agents, such as fusion proteins, monoclonal antibodies, antibody-drug conjugates, and chimeric antigen receptor (CAR) T cell immunotherapies.[11-18] Several of these agents have advanced to Phase 1 clinical trial evaluation in adults with relapsed or refractory AML (clinicaltrials.gov NCT02159495, NCT03190278, NCT02848248, NCT02715011), and clinical responses in a small number of patients treated with CD123 immunotherapies were recently reported.[19-24] Significant myeloablation and cardiac toxicity as on target/off tumor effects have not been reported to date, suggesting potential clinical tolerability of CD123 targeted therapies.

2.2 Preclinical Studies

2.2.1 Anti-Leukemia Activity of Flotetuzumab

Preclinical studies have demonstrated potent activity of flotetuzumab against human AML. *In vitro* incubation of CD123+ AML cells with flotetuzumab induced pronounced T cell activation via increased CD25 expression, T cell proliferation, T cell central memory compartment expansion, cytokine production (IFN-γ and IL-6), and AML cell killing versus minimal activity against CD123- control cells.[14] This study reported that AML cell killing was likely mediated by CD8+ T cells via granzyme B and perforin cytotoxic pathways. Anti-leukemia activity appeared to correlate directly with the level of CD123 cell surface expression.[12]

Dose-dependent *in vivo* inhibition of tumor growth was also demonstrated in AML cell line xenograft models.[12,14]. Flotetuzumab was delivered continuously for up to a week via peritoneally-implanted osmotic pumps in immunodeficient mice (NSG/$\beta 2m^{-/-}$) injected intradermally with the CD123+ KG-1a AML cell

line. The mice were previously reconstituted intravenously with normal human peripheral blood mononuclear cells (PBMCs) to provide a source of human CD3+ effector cells. Significant tumor regression was observed at all tested doses of flotetuzumab, whereas no activity was observed in animals treated with a CD3x4420 control DART (Figure 1).[14]

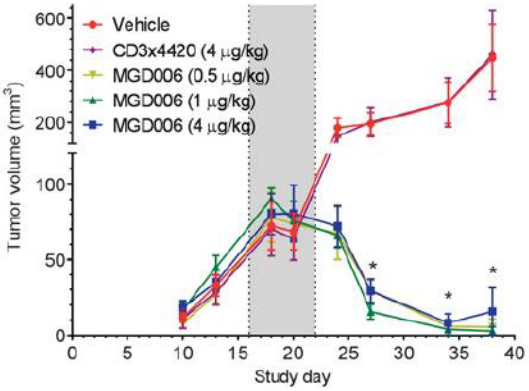


Figure 1: Tumor volumes in mice treated with intraperitoneal flotetuzumab (MGD006) by continuous infusion. Gray area indicates continuous delivery of flotetuzumab or controls. * indicates $p \leq 0.05$ by two-way ANOVA.[14]

In addition, cynomolgus monkeys (n=48) have been treated with flotetuzumab at various dose levels and frequencies to assess potential tolerability in non-human primates and effects of flotetuzumab upon normal tissues. Normal CD123+ cell depletion in the peripheral blood was observed as early as 4 hours from the start of infusion and at doses as low as 3 nanograms/kg/day. In animals that received interrupted dosing (4-days-on/3-days-off schedule), CD123+ cell depletion persisted during the 3-days-off period. CD123+ leukocyte numbers in the blood generally returned to baseline over the subsequent 3 – 4 weeks following flotetuzumab discontinuation (Figure 2). Similarly, within the bone marrow, CD123+ lineage-negative cells were decreased at the end of treatment in MGD006-treated animals compared to the vehicle-treated group and returned to baseline during the recovery period.[14]

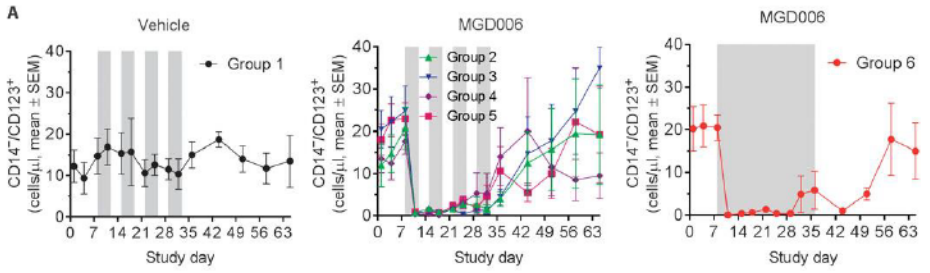


Figure 2: Preclinical study of flotetuzumab administration in cynomolgus monkeys. Data depict mean number of CD14-/CD123+ leukocytes in peripheral blood by study day and group. Group 1 received vehicle, Groups 2-5 received 4-day weekly flotetuzumab infusions reaching target doses of 100, 300, 600, and 1000 nanograms/kg/day, and Group 6 received a continuous infusion of flotetuzumab reaching a target dose of 1000 nanograms/kg/day. Gray area indicates delivery of flotetuzumab or vehicle.[14]

2.2.2 Animal Toxicology

On target/off tumor toxicities have been reported with various leukemia immunotherapies. CD123 is expressed on some normal committed hematopoietic precursors, such as common myeloid progenitors (CMP).[4] Aplasia of normal myeloid precursor cells is a potential on target/off tumor toxicity of CD123-based immunotherapies, as was observed in one study of AML xenograft models treated with CD123 CAR T cells.[17] In the non-human primate study discussed above, cynomolgus monkeys were treated with flotetuzumab to assess safety. White blood cell counts and platelets showed transient fluctuations, but largely remained within normal limits throughout the study at various dose schedules and levels. Reversible reductions in hematocrit were also observed at the highest doses. Bone marrow morphology and cellularity remained unchanged, and while bone marrow CD123+ precursor cells were reduced, hematopoietic stem cells were not. After treatment cessation, the majority of hematopoietic parameters returned to baseline, consistent with repopulation from spared hematopoietic stem cells which express low or no CD123.[14]

CD123 is also expressed at low levels on endothelial cells.[25] Capillary leak and exacerbation of hypotension related to cytokine release syndrome (CRS) are potential on target/of tumor toxicities. Another preclinical study specifically explored this potential toxicity and reported minimal effects of CD123 CAR T cells against primary human endothelial cells.[26]

Other unique toxicities associated with bispecific therapies include infusion-related reactions (IRR) and CRS. The predicted mechanism of action of flotetuzumab is the creation of an immunological synapse between the leukemic blast target cell and the T cell, leading to T cell activation and killing of the leukemic cell. Activation of T cells is associated with the elaboration of various cytokines. *In vitro* preclinical testing of flotetuzumab incubated with human PBMCs demonstrated most vigorous cytokine production of IFN-γ, but increased TNF-α, IL-2, IL-6, IL-4, and IL-10 production was also detected. These cytokines have all been implicated in the pathophysiology of cytokine release syndrome (CRS),[27,28] a common and expected side effect of antibody and cellular immunotherapies. In preclinical non-human primate toxicology studies, cynomolgus macaques tolerated a continuous infusion of flotetuzumab with 0.1 ug/kg/day escalated weekly to up to 1 ug/kg/day. CRS was predominantly a first-dose effect that was mitigated by using a within-subject dose escalation strategy. Depletion of circulating normal CD123+ hematopoietic cells was observed as early as 72 hours after treatment initiation and persisted throughout the infusion period.[14]

2.2.3 Preclinical Pharmacokinetic Studies

Formal pharmacokinetic analyses have been conducted in NOD/SCID mice and cynomolgus monkeys; in general, flotetuzumab concentrations appear to be dose-dependent. In a non-human primate study, 32 treatment-naïve cynomolgus monkeys were treated on a weekly basis for 5 weeks with continuous vehicle or flotetuzumab at escalating doses (100, 300, 600, 1000 nanograms/kg/day). Following each dose level, flotetuzumab levels appeared to be linear and stationary. Flotetuzumab was rapidly eliminated from plasma

with an estimated elimination half-life of 3.5 hours, which is comparable to that of blinatumomab in humans. Peripheral T cell counts rapidly decreased during drug infusion and then increased slightly above initial values (i.e., rebound) once flotetuzumab was eliminated from the system. Peripheral CD123+ cells were depleted after the first drug administration with the lowest dose in all animals, but with variable duration of depletion. Anti-drug antibody development led to the suppression of T cell trafficking, but did not prevent flotetuzumab-mediated CD123+ cell depletion. A pharmacokinetic model was created to describe the time-course of free drug plasma concentrations and was able to reproduce drug concentration-time profiles reliably in a group of representative animals after a 7-day infusion.[29]

2.3 Clinical Studies in Adults

2.3.1 Phase 1 Clinical Trial

Flotetuzumab monotherapy is currently under clinical investigation in adults with relapsed or refractory AML (rrAML) after treatment failure via the phase 1 trial CP-MGD006-01 (NCT02152956; MacroGenics). This trial was planned to evaluate up to 10 dose levels and/or schedules of flotetuzumab monotherapy. In this study, a maximally tolerated dose (MTD) and recommended Phase 2 dose (RP2D) was defined as 500 nanograms/kg/day administered continuously for a 28 day cycle.[30] Dose-limiting toxicity of grade 3 IRR/CRS occurred in 3 of 9 adults (n=1 each for CRS, syncope, and arthralgia/myalgia) treated at 700 nanograms/kg/day. Data from the CP-MGD006-01 trial demonstrate tolerability of flotetuzumab with a step-up dose regimen, which utilizes lower-dose flotetuzumab at initiation followed by a two-stage intra-patient escalation during the first week and continuous dosing at the MTD for the following 21 days. This approach has minimized infusion-related reactions (IRR)/CRS while preserving anti-leukemia activity. The study initially explored an intermittent dosing schedule (4 days on/3 days off) but, due to safety and efficacy concerns, the trial has subsequently been amended to treat all patients with continuous 28-day infusions. At the cut-off date on November 1, 2018, 31 patients have been treated at the RP2D. Anti-leukemic activity was reported in 18/27 (67%) response-evaluable patients, with an overall response rate (CR/CRi/MLF [morphologic leukemia free state]/PR) of 26% (6/27) and a CR/CRi rate of 19% (5/27). Interestingly, all 5 of the patients who had a CR/CRi were considered primary chemotherapy-refractory patients (defined as refractory to $\geq$ 2 induction attempts, or first CR with initial CR < 6 months) compared to none of the patients with relapsed AML.[31]

The most common treatment-related adverse event (AE) observed in treated patients has been IRR/CRS. Most patients experienced mild to moderate CRS (grade 1 25.8%, grade 2 58.1%) of short duration (median of 1 day) and were conservatively managed to full resolution. Grade $\geq$3 events occurred in 4/31 (12.9%) of patients with a median duration of 2.5 days. Twenty-one patients (67%) received at least one dose of the anti-IL6 receptor antibody tocilizumab.[31,32] Given the high anticipated incidence of IRR/CRS, step-up dosing and CRS prophylaxis with dexamethasone have been instituted via protocol amendments, which have decreased the incidence of IRR/CRS. Other AEs include nausea (32.3%), peripheral edema (29%), diarrhea (25.8%),

pyrexia (25.8%), C-reactive protein increase (19.4%), dyspnea (16.1%), headache (16.1%), hypotension (16.1%), and myalgia (16.1%). Hematologic AEs include lymphocyte count decrease (22.6%), anemia (19.4%), platelet count decrease (19.4%), white blood cell count decrease (16.1%), and neutrophil count decrease (12.9%).

2.3.2 Phase 2 Studies

There are currently no Phase 2 studies.

2.3.3 Pharmacology/Pharmacokinetics/Correlative and Biological Studies

Based on data from the ongoing adult Phase 1 clinical trial, flotetuzumab has been found to have linear pharmacokinetics with a high volume of distribution and clearance.[33]

In regards to predicting CRS, of the 30 patients treated at the RP2D, CRS severity showed a relationship with the baseline frequency of circulating CD4+ cells while C8+ cell frequency, disease burden, CD123 expression on AML blasts, monocyte levels or effector-to-target ration in the peripheral blood did not show a relationship with CRS severity. Additionally, CRS severity was not correlated with anti-leukemic activity.[32]

Gene expression analysis was performed on baseline bone marrow samples from patients being treated on the adult Phase 1 clinical trial. Based on this analysis, samples were able to be separated into either immune-infiltrated or immune-depleted immunologic clusters. Compared to relapsed patients, those patients with primary refractory AML were more likely to have immune-infiltrated phenotypes. Immune-infiltrated samples could be further subdivided into immune-enriched and immune-exhausted phenotypes. Chemorefractory patients were more likely to be immune-enriched and hypomethylating refractory patients were more likely to be immune-exhausted with higher levels of checkpoint expression and T regulatory cells. Further, responders to flotetuzumab showed a significantly higher IFN-γ signaling score at baseline compared to non-responders, consistent with the greater frequency of responders in primary refractory patients compared to relapse patients.[34]

2.4 Pediatric Studies

2.4.1 Prior Experience in Children

There are currently no pediatric studies published or in development.

2.5 Overview of Proposed Pediatric Study

This is a Phase 1 study using a rolling six design[35] with one potential dose escalation cohort. The primary aim of the study is to identify safe and tolerable dosing of the CD123 x CD3 DART flotetuzumab in children < 21 years of age with AML in second or greater relapse, refractory AML after two or more chemotherapy cycles, first relapse after primary chemotherapy-refractory disease, or first relapse after hematopoietic stem cell transplantation (HSCT).

Flotetuzumab will be administered continuously for a total of 28 days (**NOTE:** infusion ends on Day 29). Step-up dosing will be used in the first 7 days of Cycle 1 to minimize

IRR/CRS, followed by 21 days at the adult recommended phase 2 dose (RP2D) (500 nanograms/kg/day; dose level 1, [DL1]).[31] If DL1 is safe and tolerable based on standard immunotherapy DLT assessments [27], an additional cohort of patients will dose-escalate flotetuzumab to 700 nanograms/kg/day (DL2). If fewer than 2 DLTs occur at DL2, this will be considered the RP2D. If there are $\geq$ 2 DLTs at DL2, DL1 will be considered the MTD of flotetuzumab monotherapy. If flotetuzumab monotherapy at DL1 is not tolerable, dose de-escalation will occur to 300 nanograms/kg/day (DL-1). If DL-1 is deemed tolerable, this dosing will be the MTD. Further dose de-escalation beyond DL-1 will not be permitted and will limit further exploration of flotetuzumab in this patient population.

Bone marrow evaluation will be performed on Day 29 (+/- 1 day) as an exploratory measurement of response. Following the end of flotetuzumab infusion on Day 29, patients will be allowed a treatment-free interval of up to 3 days to allow treatment respite. The duration of this treatment-free interval will be up to the treating physician's discretion. However, patients may also skip the treatment-free interval and proceed immediately to the next cycle of therapy while awaiting results from response evaluation.

If a patient experiences progressive disease at any time point, they must discontinue protocol therapy. Patients with at least a partial response (PR) following Cycle 2 may continue on subsequent cycles of flotetuzumab for a total of up to 6 cycles. Patients should discontinue protocol therapy if they have not achieved at least a PR following Cycle 2. No planned step-up dosing will occur during subsequent cycles unless there is an infusion interruption of > 3 days between cycles of flotetuzumab. For patients with no evidence of disease after Cycle 1, subsequent cycles should be interrupted until ANC $\geq$ 500 µL and platelets $\geq$ 20,000/mm^3. Patients with persistent disease are permitted to continue flotetuzumab regardless of ANC and platelet counts.

Pharmacokinetic sampling and monitoring for ADAs will be performed in all patients (see Appendix VI-A, Appendix VI-B, Appendix VI-C, and Appendix VII). In consenting patients, correlative studies to characterize the tumor microenvironment will be obtained, which will include peripheral blood studies and bone marrow (see Appendix X and Appendix XII). Correlative plasma studies as a measurement of CRS and biomarkers of response will also be obtained in consenting patients (see Appendix XI).

3.0 SCREENING AND STUDY ENROLLMENT PROCEDURES

Patient enrollment for this study will be facilitated using the Slot-Reservation System in conjunction with the Oncology Patient Enrollment Network (OPEN), a web-based registration system available on a 24/7 basis. It is integrated with the NCI Cancer Trials Support Unit (CTSU) Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient position in the RAVE database.

Access requirements for OPEN:
Investigators and site staff will need to be registered with CTEP and have a valid and active Cancer Therapy Evaluation Program-Identity and Access Management (CTEP-IAM) account (check at < https://ctepcore.nci.nih.gov/iam/index.jsp >). This is the same account (user id and password) used for credentialing in the CTSU members' web site. To perform registrations in OPEN, the site user must have been assigned the 'Registrar' role on the relevant Group or CTSU roster. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

3.1 Current Study Status

Investigators should refer to the COG website to determine if the study is currently open for accrual. If the study is listed as active, investigators should then access the Studies Requiring Reservations page to ensure that a reservation for the study is available. To access the Studies Requiring Reservations page:

1. Log in to https://open.ctsu.org/open/
2. Click the **Slot Reservation** Tab. *The Site Patient page opens.*
3. Click the **Report** Tab. *The Slot Reservation Report opens. Available Slots are detailed per study strata.*

3.2 IRB Approval

NCI Pediatric CIRB approval or local IRB approval of this study must be obtained by a site prior to enrolling patients. Sites must submit CIRB/IRB approvals to the NCI's Cancer Trials Support Unit (CTSU) Regulatory Office and allow 3 business days for processing. The CTSU IRB Certification Form may be submitted in lieu of the signed IRB approval letter. All CTSU forms can be located on the CTSU web page (www.ctsu.org). Any other regulatory documents needed for access to the study enrollment screens will be listed for the study on the CTSU Member's Website under the Regulatory Tab.

Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing or amendment review. This information will be provided to the CTSU Regulatory Office from the CIRB at the time the site's Signatory Institution accepts the CIRB approval. The Signatory site may be contacted by the CTSU Regulatory Office or asked to complete information verifying the participating institutions on the study.

Submitting Regulatory Documents:

Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab → Regulatory Submission
When applicable, original documents should be mailed to:

CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support. For general (non-regulatory) questions, call the CTSU General Helpdesk at 1-888-823-5923 or contact CTSU by email at ctsucontact@westat.com.

Study centers can check the status of their registration packets by accessing the Site Registration Status page on the CTSU Member's Website under the Regulatory Tab. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

3.3 Patient Registration

Prior to enrollment on study, patients must be assigned a COG patient ID number. This number is obtained via the COG Registry in the OPEN system once authorization for the release of protected health information (PHI) has been obtained.

3.4 Reservation and Contact Requirements

Before enrolling a patient on study, a reservation must be made through the OPEN website and the Study Chair or Vice Chair should be notified. (The patient will need a COG patient ID number in order to obtain a reservation). Patients must be enrolled within 7 calendar days of making a reservation.

Reservations may be obtained 24-hours a day through the OPEN website.

3.5 Informed Consent/Assent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the patient or the patient's parents or guardian if the patient is a child, and a signed informed consent and assent will be obtained according to institutional guidelines.

3.6 Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial must only be done after obtaining written informed consent. This can be accomplished through one of the following mechanisms: a) the COG screening protocol, b) an IRB-approved institutional screening protocol or c) the study-specific protocol. Documentation of the informed consent for screening will be maintained in the patient's research chart. Studies or procedures that were performed for clinical indications (not exclusively to determine eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

3.7 Eligibility Checklist

Before the patient can be enrolled, the responsible institutional investigator must sign and date the completed eligibility checklist. A signed copy of the checklist will be uploaded into RAVE immediately following enrollment.

3.8 Institutional Pathology Report

Immediately following enrollment, the institutional pathology report for the diagnosis

under which the patient is being enrolled must be uploaded into RAVE. The report must include the associated study number and COG patient registration and accession numbers. Personal identifiers, including the patient's name and initials must be removed from the institutional pathology report prior to submission.

3.9 Study Enrollment

Patients may be enrolled on the study once all eligibility requirements for the study have been met. Patients who give informed consent for the protocol in order to undergo screening for eligibility are not considered enrolled and should not be enrolled until the screening is completed and they are determined to meet all eligibility criteria. Study enrollment is accomplished by going to the CTSU OPEN (Oncology Patient Enrollment Network) https://open.ctsu.org/open/. For questions, please contact the Study Assigned Research Coordinator, or the CTSU OPEN helpdesk at https://www.ctsu.org/CTSUContact.aspx. Patients must be enrolled before treatment begins. The date protocol therapy is projected to start must be no later than five (5) calendar days after the date of study enrollment. **Patients must not receive any protocol therapy prior to enrollment.** The only exception to this is for intrathecal cytarabine, which can be given within 1 week prior to administration of flotetuzumab. If IT cytarabine is given prior to enrollment, a separate institutional consent must be obtained.

3.10 Dose Assignment

The dose level will be assigned via OPEN at the time of study enrollment.

4.0 PATIENT ELIGIBILITY

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated. Laboratory values used to assess eligibility must be no older than seven (7) days at the start of therapy. Laboratory tests need **not** be repeated if therapy starts **within** seven (7) days of obtaining labs to assess eligibility. If a post-enrollment lab value is outside the limits of eligibility, or laboratory values are older than 7 days, then the following laboratory evaluations must be re-checked within 48 hours prior to initiating therapy: CBC with differential, bilirubin, ALT (SGPT) and serum creatinine. If the recheck is outside the limits of eligibility, the patient may not receive protocol therapy and will be considered off protocol therapy. Imaging studies, bone marrow biopsy and/or aspirate must be obtained within 14 days prior to start of protocol therapy (repeat the tumor imaging if necessary).

Clarification in timing when counting days: As an example, please note that if the patient's last day of prior therapy is September 1st, and the protocol requires waiting at least 7 days for that type of prior therapy, then that patient cannot be enrolled until September 8th.

Important note: The eligibility criteria listed below are interpreted literally and cannot be waived (per COG policy posted 5/11/01). All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical or research record which will serve as the source document for verification at the time of audit.

4.1 Inclusion Criteria

4.1.1 Age:

Patients must be < 21 years of age at the time of study enrollment.

4.1.2 Weight: Patients must weigh ≥ 17 kg*.

*Weight limit is due to constraints related to the concentration of the current drug formulation. If a new formulation of flotetuzumab becomes available to allow dosing of smaller patients, the protocol will be amended.

4.1.3 Diagnosis: Patients with recurrent or refractory AML are eligible. Patients must have histologic verification of malignancy at relapse.

4.1.4 Disease Status:

i. Patients with leukemia must have ≥ M2 marrow by morphology and/or flow cytometry and one of the following:
   a. Second or greater relapse
   b. Refractory after 2 or more chemotherapy cycles
   c. First relapse after primary chemotherapy-refractory disease
   d. First relapse after HSCT

ii. CNS disease:
   a. Patients must have the status of CNS1 (see Section 12.3) and no clinical signs or neurologic symptoms suggestive of CNS leukemia, such as cranial palsy.
   b. Patients with CNS3 or CNS2 status may receive antecedent intrathecal chemotherapy to achieve CNS1 status prior to study entry (see Section 12.3).
   c. Patients with a history of CNS chloromatous disease are required to have no radiographic evidence of disease prior to enrollment.

4.1.5 Therapeutic Options: Patient's current disease state must be one for which there is no known curative therapy or therapy proven to prolong survival with an acceptable quality of life.

4.1.6 Performance Level: Patients must have a performance status corresponding to ECOG scores of 0, 1, or 2. Use Karnofsky for patients > 16 years of age and Lansky for patients ≤ 16 years of age (See Appendix I and https://members.childrensoncologygroup.org/prot/reference_materials.asp under Standard Sections for Protocols). Use appropriate score for study population. **NOTE:** Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

4.1.7 Prior Therapy:

4.1.7.1 Patients must have fully recovered from the acute toxic effects of all prior anti-cancer therapy and must meet the following minimum duration from prior anti-cancer directed therapy prior to enrollment. If after the required timeframe, the numerical eligibility criteria are met, e.g., blood count criteria, the patient is considered to have recovered adequately.

   a. Cytotoxic chemotherapy or other anti-cancer agents known to be myelosuppressive. See DVL homepage for commercial and Phase 1 investigational agent classifications. For agents not listed, the duration

of this interval must be discussed with the study chair and the study-assigned Research Coordinator prior to enrollment.

- ≥ 14 days must have elapsed after the completion of other cytotoxic therapy with the exception of hydroxyurea. Additionally, patients must have fully recovered from all acute toxic effects of prior therapy.

  NOTE: Cytoreduction with hydroxyurea is recommended to be discontinued ≥ 24 hours prior to the start of protocol therapy.

  No waiting period is required for patients having received intrathecal cytarabine, methotrexate, and/or hydrocortisone.

b. Anti-cancer agents not known to be myelosuppressive (e.g. not associated with reduced platelet or ANC counts): ≥ 7 days after the last dose of agent. See DVL homepage for commercial and Phase 1 investigational agent classifications. For agents not listed, the duration of this interval must be discussed with the study chair and the study-assigned Research Coordinator prior to enrollment.

c. Antibodies: ≥ 21 days must have elapsed from infusion of last dose of antibody, and toxicity related to prior antibody therapy must be recovered to Grade ≤ 1.

d. Corticosteroids: See Section 4.2.2.1. If used to modify **immune adverse events** related to prior therapy, ≥ 14 days must have elapsed since last dose of corticosteroid.

e. Hematopoietic growth factors: ≥ 14 days after the last dose of a long-acting growth factor (e.g. pegfilgrastim) or 7 days for short-acting growth factor. For agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time during which adverse events are known to occur. The duration of this interval must be discussed with the study chair and the study-assigned Research Coordinator.

f. Interleukins, Interferons and Cytokines (other than Hematopoetic Growth Factors): ≥ 21 days after the completion of interleukins, interferon or cytokines (other than Hematopoetic Growth Factors)

g. Stem cell Infusions (with or without TBI):
- Allogeneic (non-autologous) bone marrow or stem cell transplant, or stem cell boost: ≥ 84 days after infusion and no evidence of GVHD.
- Donor leukocyte infusion: ≥ 42 days.
- Autologous stem cell infusion including boost infusion: ≥ 42 days.

h. Cellular Therapy: ≥ 42 days after the completion of any type of cellular therapy (e.g. modified T cells, NK cells, dendritic cells, etc.)

i. XRT/External Beam Irradiation including Protons: ≥ 14 days after local XRT; ≥ 84 days after TBI, craniospinal XRT or if radiation to ≥ 50% of the pelvis; ≥ 42 days if other substantial BM radiation.

j. Radiopharmaceutical therapy (e.g., radiolabeled antibody, 131I-MIBG): ≥ 42 days after systemically administered radiopharmaceutical therapy.

k. Patients must not have received prior exposure to flotetuzumab.

4.1.8 Organ Function Requirements

4.1.8.1 Adequate Bone Marrow Function Defined as:

- Platelet count ≥ 20,000/mm^3 (may receive platelet transfusions).
- Hemoglobin ≥ 8.0 g/dL at baseline (may receive RBC transfusions)

These patients must not be known to be refractory to red cell or platelet transfusion.

4.1.8.2 Adequate Renal Function Defined as:

- Creatinine clearance or radioisotope GFR ≥ 70ml/min/1.73 m^2 or
- A serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	Female
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

4.1.8.3 Adequate Liver Function Defined as:

- Bilirubin (sum of conjugated + unconjugated) ≤ 1.5 x upper limit of normal (ULN) for age regardless of baseline.
- SGPT (ALT) ≤ 3x ULN. For the purpose of this study, the ULN for SGPT is 45 U/L regardless of baseline.
- SGOT (AST) ≤ 3x ULN. For the purpose of this study, the ULN for SGPT is 50 U/L regardless of baseline.
- Serum albumin ≥ 2 g/dL.

4.1.8.4 Adequate Cardiac Function Defined As:

- Shortening fraction of ≥ 27% by echocardiogram, or
- Ejection fraction of ≥ 50% by gated radionuclide study.

4.1.8.5 Adequate Neurologic Function Defined as:

- Patients with seizure disorder may be enrolled if on anticonvulsants and well controlled.
- Nervous system disorders (CTCAE v5) resulting from prior therapy must be ≤ Grade 2 with the exception of decreased tendon reflex (DTR). Any Grade of DTR is eligible.

4.1.9 Informed Consent: All patients and/or their parents or legally authorized representatives must sign a written informed consent. Assent, when appropriate, will be obtained according to institutional guidelines.

4.1.10 Access: Permanent central access should be established with a central line. A central line that contains 2 lumens is preferred.

4.2 Exclusion Criteria

4.2.1 Pregnancy or Breast-Feeding

Pregnant or breast-feeding women will not be entered on this study because there is yet no available information regarding human fetal or teratogenic toxicities. Pregnancy tests must be obtained in girls who are post-menarchal. Males or females of reproductive potential may not participate unless they have agreed to use an effective contraceptive method for the duration of study therapy and for 12 weeks after flotetuzumab discontinuation.

4.2.2 Concomitant Medications

4.2.2.1 Corticosteroids: Patients must be off steroids (unless physiologic replacement dosing) for at least 7 days prior to enrollment. If used to modify **immune adverse events** related to prior therapy, ≥ 14 days must have elapsed since last dose of corticosteroid (See Section 4.1.7.1.d).

4.2.2.2 Investigational Drugs: Patients who are currently receiving another investigational drug are not eligible.

4.2.2.3 Anti-cancer Agents: Patients who are currently receiving other anti-cancer agents are not eligible [except hydroxyurea, which may be continued until 24 hours prior to start of protocol therapy].

4.2.2.4 Anti-GVHD agents post-transplant:
Patients who are receiving cyclosporine, tacrolimus or other agents to treat graft-versus-host disease post bone marrow transplant are not eligible for this trial.

4.2.3 Study Specific:

4.2.3.1 Patient has French-American-British classification (FAB) type M3 leukemia (acute promyelocytic leukemia) or identification of t(15;17).

4.2.3.2 Patient has isolated CNS involvement or isolated extramedullary relapse.

4.2.3.3 Patient with known HIV infection are eligible if he or she has a negative HIV serology and an undetectable viral load.

4.2.3.4 Patient known to have one of the following concomitant genetic syndromes: Bloom syndrome, ataxia-telangiectasia, Fanconi anemia, Kostmann syndrome, Shwachman syndrome or any other known bone marrow failure syndrome. Patients with Down syndrome ARE eligible for the study.

4.2.3.5 Patients must not weigh < 17 kg.

4.2.3.6 Patients must not have received prior therapy with a CD123 directed antibody or CD123 directed CAR T cells.

4.2.4 Infection: Patients who have an uncontrolled infection are not eligible.

4.2.5 Patients who have received a prior solid organ transplantation are not eligible.

4.2.6 Patients who in the opinion of the investigator may not be able to comply with the safety monitoring requirements of the study are not eligible.

4.2.7 Known hypersensitivity to murine, yeast, or recombinant proteins; polysorbate 80; recombinant human serum albumin; benzyl alcohol; or any excipient contained in the flotetuzumab drug formulation. Additionally, those patients who have had a hypersensitivity reaction to etoposide that is considered likely related to polysorbate 80 are not eligible.

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

5.0 TREATMENT PROGRAM

5.1 Overview of Treatment Plan

A cycle of therapy is 29 days. Following the end of flotetuzumab infusion on Day 29, patients will be allowed a treatment free interval of up to 3 days to allow respite. The duration of this treatment free interval will be up to the treating physician's discretion. Keeping interruptions to $\leq$ 3 days will reduce the chance of disease rebound and lower the risk for recurrent CRS based upon clinical experience in flotetuzumab-treated adult patients. Alternatively, the treating physician may opt to skip the treatment-free interval and allow the patient to proceed immediately to the next cycle without respite, as long as the patient is experiencing clinical benefit. Clinical benefit may be defined as a reduction in leukemia related symptoms, and/or a reduction in transfusion frequency and/or a reduction or clearance of peripheral blasts. Patients with at least a PR following Cycle 2 may continue on subsequent cycles of flotetuzumab for a total of up to 6 cycles, lasting approximately 6 months. Patients should discontinue protocol therapy if they have not achieved at least a PR following Cycle 2.

During the dose escalation portion of the study, flotetuzumab will be administered by continuous intravenous infusion for a total of 28 days (**NOTE:** infusion ends on Day 29).

All doses of flotetuzumab in this study are described as nanograms/kg/day. However, the drug labels are expressed in milligrams/mL. Step-up dosing will be used in the first 7 days of Cycle 1 to minimize IRR/CRS, followed by 21 days at the adult recommended phase 2 dose (RP2D) (500 nanograms/kg/day, Dose Level 1 [DL1]). If DL1 is deemed safe and tolerable based on standard immunotherapy DLT assessments (Section 5.5), an additional cohort of patients will dose-escalate to flotetuzumab at 700 nanograms/kg/day (DL2). Step-up dosing will occur during the first 8 days of Cycle 1 for this cohort. If fewer than 2 DLTs occur at DL2, this will be considered the RP2D. If there are ≥ 2 DLTs at DL2, DL1 will be considered the MTD. If DL1 is not tolerable, dose de-escalation will occur to 300 nanograms/kg/day (DL-1) with initial step-up dosing during the first 8 days of Cycle 1 for this cohort. If DL-1 is deemed tolerable, this dosing will be the MTD. Further dose de-escalation will not be permitted and will limit further exploration of flotetuzumab in this patient population. No planned step-up dosing will occur during subsequent cycles unless there is an infusion interruption of more than 3 days between cycles of flotetuzumab. If an interruption lasts > 3 days, subsequent cycle dosing should be discussed with the study chair.

Bone marrow evaluation will be performed on Day 29 (+/- 1 day) to evaluate response.

Drug doses should be adjusted based on the patient's weight measured within 7 days prior to the beginning of each cycle. Flotetuzumab continuous infusion must be administered through an IV line with a low protein binding polyethersulfone (PES) 0.2 micron in-line filter. Due to the risk of CRS, sites should have sufficient supply of tocilizumab (enough to treat a 70 kg patient x three 8 mg/kg doses) at all times for each patient on therapy. Patients will remain hospitalized for the duration of Cycle 1 for safety monitoring. Patients may receive subsequent cycles as an outpatient if the drug can be accurately and safely delivered (see Appendix XIV), and at the discretion of the treating physician and after discussion with the study chair.

Patients should receive a single dose of IT cytarabine prior to the start of Cycle 1 between Days -6 to 0. Patients may receive IT cytarabine on Day 1 of subsequent cycles or with bone marrow evaluations at the discretion of the treating physician.

IT Cytarabine Age-based dosing:

Age (yrs)	**Dose**
< 1	20 mg
1-1.99	30 mg
2-2.99	50 mg
≥ 3	70 mg

Infusion-Related Reaction/Cytokine Release Syndrome (IRR/CRS) Prophylaxis for Cycle 1:

Acetaminophen: 10 – 15 mg/kg/dose (max: 650 mg/dose) IV or PO

Week 1 Step-up Dosing: once daily, 30 minutes prior to starting each new dose, then q4-6h prn
Final Dose Escalation: 30 minutes prior to dose escalation to goal dose, then scheduled q8h x 48 hours, then q4-6h prn

Diphenhydramine: 0.5 – 1 mg/kg/dose (max: 50 mg) IV or PO
Week 1 Step-up Dosing: once daily, 30 minutes prior to starting each new dose, then q6-8h prn
Final Dose Escalation: 30 minutes prior to dose escalation, then scheduled q8h x 48 hours, then q6-8h prn

Histamine 2 Receptor Antagonists:
Ranitidine: 1 mg/kg/dose IV (max single dose: 50 mg) or 2 mg/kg/dose PO (max single dose: 150 mg); or equivalently dosed H2 receptor antagonist (e.g., famotidine 0.25 mg/kg/dose IV (max single dose: 20 mg) or 0.5 mg/kg/dose PO (max single dose: 40 mg))
Week 1 Step-up Dosing: 30 minutes prior to starting each new dose, then ranitidine q8h (IV) or q12h (PO) prn OR famotidine q12h IV/PO prn
Final Dose Escalation: 30 minutes prior to dose escalation, then scheduled ranitidine q8h (IV) or q12h (PO) OR famotidine q12h IV/PO x 48 hours, then prn (at same frequency)

Dexamethasone
Week 1, Day 1: 5 mg/m^2/dose (max single dose: 5 mg) IV or PO once, 30 minutes prior to starting flotetuzumab, then 5 mg/m^2/dose (max single dose: 5 mg) IV or PO given 12 hours later x 1 (total daily dose: 10 mg/m^2)

DL1, Cycle 1

	Day 0	1	2	3	4	5	6	7	8-28	29
IT cytarabine*	X									End of flotetuzumab infusion
Flotetuzumab 30 nanograms/kg/day		X								
Flotetuzumab 60 nanograms/kg/day			X							
Flotetuzumab 100 nanograms/kg/day				X						
Flotetuzumab 200 nanograms/kg/day					X					
Flotetuzumab 300 nanograms/kg/day						X				
Flotetuzumab 400 nanograms/kg/day							X			
Flotetuzumab 500 nanograms/kg/day								X	X	

*can be given between Days -6 and 0

DL-1, Cycle 1

	Day 0	1	2	3	4	5	6	7	8	9-28	29
IT cytarabine*	X										End of flotetuzumab infusion
Flotetuzumab 30 nanograms/kg/day		X									
Flotetuzumab 60			X	X							

nanograms/kg/day											
Flotetuzumab 100 nanograms/kg/day					X	X					
Flotetuzumab 200 nanograms/kg/day							X	X			
Flotetuzumab 300 nanograms/kg/day									X	X	

* can be given between Days -6 and 0

DL2, Cycle 1

	Day 0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9-28**	**29**
IT cytarabine*	X										End of flotetuzumab infusion
Flotetuzumab 30 nanograms/kg/day		X									
Flotetuzumab 100 nanograms/kg/day			X								
Flotetuzumab 200 nanograms/kg/day				X							
Flotetuzumab 300 nanograms/kg/day					X						
Flotetuzumab 400 nanograms/kg/day						X					
Flotetuzumab 500 nanograms/kg/day							X				
Flotetuzumab 600 nanograms/kg/day								X			
Flotetuzumab 700 nanograms/kg/day									X	X	

*can be given between Days -6 and 0

All Cohorts, Cycles 2+

	Day 1	**Day 2-28**	**29**
IT cytarabine*	X		End of flotetuzumab infusion
Flotetuzumab at Cycle 1 target dose	X	X	

*Patients may receive an optional dose of IT cytarabine during subsequent cycles on Day 1 or with bone marrow evaluations at the discretion of the treating physician.

If a treatment-free interval > 3 days occurs between any 2 cycles, the subsequent cycle will require step-up dosing. If a treatment-free interval > 3 days occurs between any 2 cycles, the Study Chair must be notified and the subsequent cycle dosing schedule must be discussed.

IT Cytarabine Age-based dosing:

Age (yrs)	**Dose**
< 1	20 mg
1-1.99	30 mg

2-2.99	50 mg
≥ 3	70 mg

Infusion Related Reaction/Cytokine Release Syndrome Prophylaxis prior to each subsequent cycle and for prolonged interruptions:

Prophylaxis is required prior to subsequent cycles if the treatment-free interval is greater than 24 hours. Prophylaxis is also recommended following any interruption in cycles that last >24 hours. For interruptions in cycles that last <24 hours, prophylaxis can be given at the discretion of the treating physician.

Acetaminophen: 10 – 15 mg/kg/dose (max: 650 mg/dose) IV or PO
Day 1: 30 minutes prior to starting flotetuzumab infusion, then q4-6h prn

Diphenhydramine: 0.5 – 1 mg/kg/dose (max: 50 mg) IV or PO
Day 1: 30 minutes prior to flotetuzumab infusion, then q6-8h prn

Ranitidine: 1 mg/kg/dose IV (max single dose: 50 mg) or 2 mg/kg/dose PO (max single dose: 150 mg); or equivalently dosed H2 receptor antagonist (e.g., famotidine)
Day 1: 30 minutes prior to starting flotetuzumab infusion, then q8h (IV) or q12h (PO) prn

Dexamethasone
Day 1: 5 mg/m^2/dose (max single dose: 5 mg) IV or PO once 30 minutes prior to starting flotetuzumab infusion, then 5 mg/m^2/dose (max single dose: 5 mg) IV or PO given 12 hours later (total daily dose: 10 mg/m^2)

5.2 Criteria for Starting Subsequent Cycles

A cycle may be repeated every 29 days, with an optional ≤ 3 day respite period between each cycle. The optional ≤ 3 day respite period will be up to the treating physician's discretion. If a patient has progressive disease at any time point or is unable to achieve a PR following Cycle 2, subsequent protocol therapy must be discontinued. Due to a risk for disease rebound and recurrent CRS, patients are allowed to proceed to subsequent cycles while awaiting disease evaluation at the discretion of the treating physician. For patients with no evidence of disease after Cycle 1, subsequent cycles should be interrupted until ANC ≥ 500 µL and platelets ≥ 20,000/mm^3. Patients with persistent disease are permitted to continue flotetuzumab regardless of ANC and platelet counts. Laboratory parameters as defined in the eligibility section, Section 4.0, must be met, and the patient must be eligible to continue agent administration per the requirements in Section 6.0.

5.3 Dose Escalation Schema

5.3.1 Inter-Patient Escalation

The starting dose will be 500 nanograms/kg/day (DL1) with dose levels for subsequent groups of patients as follows:

Dose Level	Dose (nanograms/kg/day)
-1	300
1*	**500**
2	700

* Starting Dose Level

There will be no planned escalations beyond Dose Level 2 (700 nanograms/kg/day), as previous pediatric Phase 1 studies have rarely defined a MTD greater than 160% of the adult MTD. If estimated clearance is high in this pediatric study population, we will consider amending the protocol to allow further dose escalation to 900 nanograms/kg/day.

If the MTD has been exceeded at the first dose level, then the subsequent cohort of patients will be treated at a dose of 300 nanograms/kg/day (Dose Level -1). If Dose Level -1 is not well tolerated, further de-escalation will not occur. The study will be closed to accrual.

5.3.2 Intra-Patient Escalation

Intra-patient dose escalation will occur during the first 7 days of Cycle 1 on Dose Level 1 and the first 8 days of Cycle 1 on Dose Levels -1 and 2 as described in Section 5.1. Intra-patient dose escalation occurring outside of this time period is not allowed.

5.4 Grading of Adverse Events

Adverse events (toxicities) will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Any suspected or confirmed dose-limiting toxicity should be reported immediately (within 24 hours) to the Study Chair.

5.5 Definition of Dose-Limiting Toxicity (DLT)

DLT will be defined as any of the following events that are possibly, probably or definitely attributable to protocol therapy. The DLT observation period for the purposes of dose-escalation will be the first cycle of therapy.

Dose limiting hematological and non-hematological toxicities are defined differently.

5.5.1 Non-hematological dose-limiting toxicity

5.5.1.1 Any Grade 3 or greater non-hematological toxicity at least possibly attributable to protocol therapy with the specific exclusion of:

- Alopecia
- Grade 3 fatigue, anorexia, nausea, vomiting, and diarrhea not requiring hospitalization, tube-feeding, or use of TPN.
- Grade 3 liver enzyme elevation, including ALT/AST, occurring after flotetuzumab administration that returns to Grade $\leq$ 1 or baseline within 7 days from the start of therapy. Note: For the purposes of this study the ULN for ALT is defined as 45 U/L.
- Grade 3 or 4 fever, febrile neutropenia, or infection
- Grade 3 or 4 isolated electrolyte abnormalities that resolve with or without intervention to $\leq$ Grade 2 within 72 hours. Electrolyte supplementation is encouraged.
- Grade 3 or 4 tumor lysis syndrome that resolves within 7 days without end-organ damage.
- Grade 3 rash that resolves (with or without supportive care) to $\leq$

Grade 2 within 7 days

- Grade 3 mucositis that resolves (with or without supportive care) to ≤ Grade 2 within 7 days
- Early intervention with TPN or enteral tube feeding for anorexia, nausea not attributable to protocol therapy, or concern for poor nutritional status clearly and incontrovertibly not attributable to protocol therapy will not be considered a DLT
- Grade 3 or greater pain due to leukemia, mucositis, typhlitis, infection, or obvious injury
- Grade 3 IRR (including CRS) resolving to ≤ Grade 2 within 72 hours. CRS will be defined by ASBMT consensus grading[36]
- Cases of Hy's law (> 3x ULN of ALT/AST along with > 2x ULN in total bilirubin without evidence of cholestasis) will be considered a DLT if no other reason can be found to explain the observed injury (e.g. leukemia, infection, another drug, etc.).

5.5.1.2 Non-hematological toxicity that causes a delay of ≥ 14 days between treatment cycles.

5.5.1.3 Note: Allergic reactions that necessitate discontinuation of study drug will not be considered a dose-limiting toxicity.

5.5.2 Hematological dose limiting toxicity

Hematological dose limiting toxicity will be defined as failure to recover a peripheral ANC > 500/mm^3 and platelets > 20,000/mm^3 by 42 days after the first treatment day. Patients with detectable leukemia (inclusive of MRD) will be unevaluable for a hematologic DLT.

5.5.2.1 Note: Grade 3 or 4 febrile neutropenia will not be considered a dose-limiting toxicity.

6.0 DOSE MODIFICATIONS FOR ADVERSE EVENTS

The Study Chair must be notified of any dosage modification or use of myeloid growth factor.

6.1 Dose Modifications for Hematological Toxicity

6.1.1 Patients who have dose-limiting thrombocytopenia or neutropenia should receive subsequent cycles at the next lower dose level.

6.1.2 Patients who experience dose-limiting thrombocytopenia or neutropenia after one dose reduction must be removed from protocol therapy. The use of hematopoietic growth factors is discouraged. However, granulocyte colony stimulating factor may be used outside of Cycle 1 for culture proven bacteremia or invasive fungal infection. The Study Chair should be notified before growth factors are initiated.

6.1.3 Patients who have a dose-limiting hematological toxicity that does not resolve to eligibility parameters within 56 days of any cycle in the absence of

detectable leukemia must be removed from protocol therapy.

6.2 Dose Modifications for Non-Hematological Toxicity

6.2.1 Patients who have any dose-limiting non-hematological toxicity (as defined in Section 5.5.1) may continue on protocol therapy upon meeting eligibility lab requirements or baseline but should receive subsequent doses at the next lower dose level.

6.2.2 If a non-hematological dose-limiting toxicity recurs after one dose reduction, the patient must be removed from protocol therapy.

6.2.3 Patients who have a dose-limiting non-hematological toxicity that does not resolve to baseline or eligibility within 21 days after the planned start of the next treatment cycle must be removed from protocol therapy.

6.3 Dose Modifications for Infusion-Related Reactions and Cytokine Release Syndrome

The mechanism of action of flotetuzumab is the creation of an immunological synapse between the leukemic blast bearing CD123 and T cells, leading to T cell activation and killing of the leukemic cell. Activation of T cells is associated with the elaboration of various cytokines including IFN-γ, TNF-α, IL-2, IL-6, IL-4 and IL-10. This T cell activation and subsequent cytokine production can lead to infusion related reactions (IRRs) or cytokine release syndrome (CRS).

Patients should be monitored closely for the development of IRRs during flotetuzumab infusion. Medications and supportive measures for the treatment of such reactions should be available for immediate use for an infusion reaction during study drug administration and may include, but are not limited to: subcutaneous epinephrine, antihistamines, corticosteroids, IV fluids, vasopressors, oxygen, bronchodilators, and antipyretics. Resuscitation equipment and other supplies for the emergency management of an allergic/toxic reaction must be available. The patient should be treated according to the best available local practices and procedures. All supportive measures consistent with optimal patient care will be provided throughout the study according to institutional standards.

If possible, a serum sample should be obtained at the time of diagnosis of CRS for evaluation of circulating cytokines. Follow-up samples should also be obtained at the time of resolution of symptoms. Application of appropriate diagnostic or treatment procedures should not be delayed or hindered to obtain such samples.

Other types of adverse reactions may be observed during infusion that are not interpreted as related to cytokine release, but may be related to flotetuzumab. Such reactions should be diagnosed, graded and managed according to best practices. Early and frequent consultation with the study medical monitor is encouraged.

6.3.1 CRS Management

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

CRS is a commonly observed event during treatment with flotetuzumab based on experience in the adult population, with the majority of patients experiencing at least 1 event of Grade 1 or 2. Based on current understanding gained from patients treated with blinatumomab and CAR T cells, CRS is likely to be a common toxicity that can be managed through supportive care and anti-cytokine interventions to allow for full activity of T cells during therapy. A consensus grading system for CRS has been proposed by the American Society for Blood and Marrow Transplantation which will be utilized for this study (Table 1).

Table 1: ASBMT 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 supplemental oxygen	Requiring high-flow nasal cannula[#@], facemask, non-rebreather mask, or Venturi mask supplemental oxygen	Requiring positive pressure (*e.g.*, 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.

Source: Lee et al., 2018[36]

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

6.3.1.1 Grade 1 Infusion Reactions, Including CRS and IRR:

- Slow the infusion rate by 20%;
- Monitor the patient for worsening of condition;
- Administer IV fluids, diphenhydramine IV, acetaminophen PO/IV for fever, and oxygen and bronchodilators for mild bronchospasm, as appropriate;
- Administer tocilizumab (4-8 mg/kg [max 800 mg/dose] IV) for Grade 1 IRR/CRS that does 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 or pulse oximetry < 93% in the absence of supplemental oxygen
    - Decreases in diastolic or systolic BP to < 15 mmHg below pretreatment baseline
    - HR > 20 bpm above pre-treatment baseline
    - Note: isolated changes in hemodynamic parameters alone without pulmonary compromise or fever should not prompt immediate use of tocilizumab.
- Corticosteroids should not be used for Grade 1 IRR/CRS.
- Continue rate at 20% reduction and increase infusion rate to the original rate by increasing the infusion rate after stabilization or resolution of symptoms, as tolerated. If multiple dose reductions are required or if a more gradual increase in the rate of infusion is desired, dose increasing must be discussed with the study chair.

6.3.1.2 Grade 2 Infusion Reactions and Infusion-Related Events:

- Slow the infusion rate by 50%;
- Administer IV fluids, diphenhydramine IV, acetaminophen PO/IV for fever, and oxygen and bronchodilators for bronchospasm, as appropriate and if not administered previously;
- Tocilizumab should be used for Grade 2 IRR/CRS that does not resolve with other measures within 2 hours, or that requires the use of supplemental oxygen > 4L by nasal cannula or low dose vasopressors.
    - Tocilizumab Dosing:
        - < 30 kg: 12 mg/kg
        - ≥ 30 kg: 8 mg/kg [max 800 mg]

- In patients with CRS who respond to tocilizumab, fever and hypotension often resolve within 6 hours, and pressors and other supportive care measures can be weaned quickly thereafter. In some cases, however, symptoms may not completely resolve, and continued aggressive support may be necessary for several days. If the patient's condition does not improve or stabilize within 8 – 12 hours of the tocilizumab dose, administration of a second dose of tocilizumab and/or a second immunosuppressive agent, such as additional dexamethasone, should be considered. Tocilizumab is generally not used in the management of CNS symptoms without significant hemodynamic instability or other life-threatening symptomatology.
- Corticosteroids may be used for Grade 2 IRR/CRS that does not respond to other measures, including tocilizumab. Dexamethasone is the corticosteroid of choice administered at a total daily dose of at least 0.2 – 0.4 mg/kg/day (maximum 24 mg per day, in addition to dexamethasone administered during daily dose escalation) administered preferably intravenously divided 3 – 4 times daily for at least 1 day but no more than 4 days. The dose should be stopped or tapered as clinically indicated.
- Continue rate at 50% reduction and increase infusion rate to the original rate by doubling the infusion rate after stabilization or resolution of symptoms, as tolerated. If multiple dose reductions are required or if a more gradual increase in the rate of infusion is desired, dose increasing must be discussed with the study chair.
- Monitor for worsening condition.

6.3.1.3 Grade 3 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 IV, acetaminophen PO/IV 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.

- Grade 3 CRS that is refractory to tocilizumab should be treated with corticosteroids. Dexamethasone is the corticosteroid of choice administered at a total daily dose of at least 0.2 – 0.4 mg/kg/day (maximum 24 mg per day) administered preferably intravenously divided 3 – 4 times daily for at least 1 day but no more than 4 days. The dose should be stopped or tapered as clinically indicated.
- If infusion reaction improves to ≤ grade 2 within 72 hours, infusion may be resumed at no greater than 50% of the original rate once symptoms decrease to Grade 1. The rate may then be escalated to the original rate after 30 minutes, as tolerated. A more gradual increase in the rate of infusion may be undertaken after consultation with the study chair;
- If infusion reaction DOES NOT improve to ≤ grade 2 within 72 hours or if there are multiple grade 3 infusion reactions, flotetuzumab treatment must be permanently discontinued;
    - Report as an immediately reportable event (IRE) immediately;
    - Report the event as a serious adverse event (SAE) if appropriate.

6.3.1.4 Grade 4 Infusion Reactions and Infusion-Related Events

- Stop the infusion and disconnect the infusion tubing from the patient;
- TO AVOID EXACERBATION OF INFUSION REACTION OR CRS: DO NOT FLUSH THE TUBING – ASPIRATE RESIDUAL DRUG FROM THE PORT LUMEN;
- Administer IV fluids, diphenhydramine IV, acetaminophen PO/IV for fever, and oxygen and bronchodilators for mild bronchospasm, as appropriate.
- Provide appropriate ventilator and circulatory support as medically indicated;
- Agents including those listed below have been described in the management of patients with severe complications of cytokine storm and/or severe immune-related adverse events, and may be required:
    - Higher doses of corticosteroids and/or
    - Tocilizumab (anti-IL-6 receptor):
        - <30 kg: 12 mg/kg IV
        - ≥30 kg: 8 mg/kg [max 800 mg] IV, and/or
    - Etanercept (anti-TNFα) 0.8 mg/kg (max: 50 mg/dose) subQ
- Notify the Sponsor's Medical Monitor immediately;
- Report the event as an SAE. Patients who have a Grade 4 CRS should not receive further flotetuzumab.

6.3.1.5 Grade 5 Infusion Reactions and Infusion-Related Events:

- Notify the Sponsor's Medical Monitor or designee immediately;
- Report the event as an SAE.

6.4 Dose Modifications for Capillary Leak Syndrome

Risk of capillary leak syndrome (CLS) has been associated with various immunotherapies, including antibody-drug conjugates, T cell-engaging bispecific antibodies, and chimeric antigen receptor T cells. While CLS has not been observed with frequency in patients treated with flotetuzumab, rapid weight gain, decreased albumin, and systemic edema have been reported in some patients. All episodes have occurred during the multi-step lead-in dosing period and have occurred concomitantly with symptoms and signs of cytokine release syndrome (CRS).

Patients should be monitored closely for the development of CLS during flotetuzumab infusion with specific attention during the lead-in dosing period and/or during CRS occurrence. Early signs and symptoms of CLS include weight gain, new onset or worsening edema, hypotension, and decreased serum albumin. Daily weights and strict 'ins and outs' (I/Os) are strongly recommended during the dose escalation period (Cycle 1/Days 1-7 and beyond as clinically indicated). Medications and supportive measures for the treatment of such reactions should be available for immediate use, including diuretics, corticosteroids, IV fluids, vasopressors, and supplemental oxygen.

CLS Management
The following are suggested treatment guidelines for CLS related to flotetuzumab, which may be modified as needed by treating physicians/investigators according to the best practices of medicine. Early intervention is recommended at the first clinical signs of CLS in the absence of clear alternative etiologies.

6.4.1 Weight gain

For any weight gain ≥5% from the previous treatment day, consider albumin 0.5 – 1 g/kg/dose (maximum dose 25 g) until body weight increase has resolved. If patient is normotensive or hypertensive, consider furosemide 1 mg/kg IV. For weight gain unresponsive to fluid management, consider administration of dexamethasone 5-10 mg/m^2/dose (max single dose10 mg) IV/PO or equivalent corticosteroid (e.g. methylprednisolone 1 mg/kg/dose IV). Interruption of flotetuzumab infusion may also be considered.

6.4.2 Hypotension

Hypotension can be a sign of CLS and/or CRS. If a patient is noted to be hypotensive, his or her fluid status should be managed as clinically indicated with intravenous fluids and vasopressors. Albumin or other colloids should be considered for patients whose perfusion is not restored by crystalloid alone. For hypotension unresponsive to fluid management, dexamethasone 5-10 mg/m^2/dose (max single dose 10 mg) IV/PO or equivalent corticosteroid (e.g, methylprednisolone 1 mg/kg/dose IV) should be given. Interruption of flotetuzumab dosing should also be considered, particularly in patients with severe hypotension requiring vasopressor medication support. Please also see CRS management guidelines in Section 6.3.1.

6.4.3 Hypoalbuminemia

For any decrease in albumin ≥ 0.5 g/dL below baseline level at the start of the cycle, consider administration of albumin 0.5 – 1 g/kg/dose (maximum dose 25 g); may be repeated up to 3 times daily based on the patient's clinical status.

7.0 SUPPORTIVE CARE AND OTHER CONCOMITANT THERAPY

7.1 Concurrent Anticancer Therapy

Concurrent cancer therapy, including chemotherapy, radiation therapy, immunotherapy, or biologic therapy may NOT be administered to patients receiving study drug. If these treatments are administered the patient will be removed from protocol therapy.

7.2 Investigational Agents

No other investigational agents may be given while the patient is on study.

7.3 Supportive Care

Appropriate antibiotics, blood products, antiemetics, fluids, electrolytes, and general supportive care are to be used as necessary. Patients should receive adequate hydration during study therapy, and the addition of IV fluids to maintain intravascular volume is recommended for at least 48 hours after the start of therapy. Patients should receive appropriate *Pneumocystis jiroveci* pneumonia prophylaxis (*e.g.*, trimethoprim-sulfamethoxazole) during study participation. Empiric anti-fungal (*e.g.,* caspofungin, fluconazole) and antibiotic prophylaxis (*e.g.,* levofloxacin) when ANC < 200 is strongly encouraged.

See COG Supportive Care Guidelines at https://childrensoncologygroup.or/index.php/cog-supportive-care-guidelines. See Section 7.5 for drugs that should not be used concomitantly due to potential interactions with flotetuzumab. See below for recommendations on management of specific toxicities associated with flotetuzumab.

7.4 Growth Factors

Growth factors that support platelet or white cell number or function can only be administered in accordance with Section 6.1.2 or for culture proven bacteremia or invasive fungal infection. The Study Chair should be notified before growth factors are initiated.

7.5 Concomitant Medications

Because flotetuzumab employs a mechanism of action dependent upon the engagement of T lymphocytes, the use of corticosteroids other than those employed for premedication should be limited. Chronic doses of corticosteroids in excess of 0.25 mg/kg (max 10 mg) daily of prednisone or equivalent are prohibited other than for the management of drug-related adverse experiences. Corticosteroid pretreatment should follow the recommended dose and schedule (see Section 5.1).

Vaccinations (with the exception of the annual inactivated influenza vaccine) are prohibited during the study.

8.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

8.1 Required Clinical, Laboratory and Disease Evaluation

All clinical and laboratory studies to determine eligibility must be performed within 7 days

prior to enrollment unless otherwise indicated. Laboratory values used to assess eligibility (see Section 4.0) must be no older than seven (7) days at the start of therapy. Laboratory tests need **not** be repeated if therapy starts **within** seven (7) days of obtaining labs to assess eligibility. If a post-enrollment lab value is outside the limits of eligibility, or laboratory values are older than 7 days, then the following laboratory evaluations must be re-checked within 48 hours prior to initiating therapy: CBC with differential, bilirubin, ALT (SGPT) and serum creatinine. If the recheck is outside the limits of eligibility, the patient may not receive protocol therapy and will be considered off protocol therapy. Imaging studies, bone marrow aspirate, and biopsy must be obtained within 14 days prior to start of protocol therapy (repeat the imaging if necessary).

STUDIES TO BE OBTAINED	Pre-Study	During Cycle 1	Prior to Subsequent Cycles^
History	X	Weekly	X
Physical exam with vital signs	X	Weekly	X
Height, weight, BSA	X		X
Performance status	X		
CBC, differential, platelets	X	Twice Weekly (every 3 to 4 days)[3]	Weekly[4]
Pharmacokinetics		X[1]	X[1]
Anti-drug antibody (ADA)		X[10]	X[10]
Urinalysis	X		
Electrolytes including Ca^{++}	X	Twice weekly (every 3 to 4 days) during Week 1, then weekly	X
Creatinine, ALT, bilirubin, AST	X	Weekly	X
Albumin	X		X
Imaging (CT or MRI) of chloroma (if clinically indicated)	X	End of Cycle 1 (if present at baseline)	End of each cycle (if present on previous study)
Bone marrow aspiration	X[5, 6, 12, 13]	End of Cycle 1[5]	End of every cycle
Pregnancy test[2]	X		
ECHO or gated radionuclide study	X		X
EKG	X		X
Neurologic exam	X		X
Next-generation sequencing (NGS) MRD analysis	With bone marrow evaluation[7,13]	With bone marrow evaluation[7]	With bone marrow evaluation[7]
Peripheral T cell quantification		X[8]	X[8]
Cytokine analysis		X[9]	X[9]
Tumor Microenvironment Profiling	X[11, 13]		
Flow cytometry with minimal residual disease determination (MRD)[14]		With bone marrow evaluation	With bone marrow evaluation

^ Studies may be obtained within 72 hours prior to the start of the subsequent cycle.

1 PK samples will be collected from all patients during Cycle 1. The PK sampling schedule during Cycle 1 will differ depending on dose level assignment of the patient. See Section 8.2 for details. Blood samples will also be collected 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after the end of infusion

on the patient's last day of protocol therapy.

[2] Women of childbearing potential require a negative pregnancy test prior to starting treatment; sexually active patients must use an acceptable method of birth control. Abstinence is an acceptable method of birth control.

[3] If patients have Grade 4 neutropenia then CBCs should be checked at least every other day until recovery to Grade 3 or until meeting the criteria for dose limiting toxicity.

[4] If patients develop Grade 4 neutropenia then CBCs should be checked every 3 to 4 days until recovery to Grade 3.

[5] Bone marrow aspirate and clot section/biopsy.

[6] In consenting patients, CD123 site density analysis to be sent to Hematologics at baseline (see Section 8.4).

[7] In consenting patients only. See Section 8.5 for details.

[8] In consenting patients, blood samples will be collected on Cycle 1 Days 1, 8, 14, 22, and 29 and on Day 1 of subsequent cycles prior to infusion. See Section 8.6 for details.

[9] In consenting patients, blood samples will be collected on Cycle 1, Day 1 (pre-infusion) and on Cycle 1, Day 8. Additionally, if patients experience IRR/CRS, blood samples will be collected from consenting patients at the time of IRR/CRS and at the time of IRR/CRS resolution. See Section 8.7 for details.

[10] ADA samples will be collected from all patients on Cycle 1, Day 1 (pre-infusion), Cycle 3, Day 1 (pre-infusion), Cycle 5, Day 1 (pre-infusion). See Section 8.3 for details.

[11] In consenting patients, extra bone marrow aspirate will be obtained from the mandatory bone marrow biopsy prior to Cycle 1. See Section 8.8 for details.

[12] Cytogenetics and FISH will be performed on the collected bone marrow aspirate (it is recommended that cytogenetics/FISH be performed by a COG-approved laboratory).

[13] When possible, additional aliquots of bone marrow should be collected at the time of the screening bone marrow for optional central evaluations. The preferred specimen source is bone marrow, but if additional bone marrow cannot be obtained, peripheral blood may be sent in those patients who have an absolute blast count of at least 1,000/µl (2 mL of peripheral blood may be submitted for each 1 mL of required marrow). Alternatively, frozen aliquots in those patients previously enrolled on APEC can be utilized if permission is granted. See Section 8.4, Section 8.5, and Section 8.8 for details.

[14] Analyses to be performed at Hematologics. Ship samples to the address listed in Section 8.4.5.

8.2 Pharmacology (Required)

8.2.1 Description of Studies and Assay

Pharmacokinetics (PK) will be performed to determine the PK of flotetuzumab in children. Serum samples will be collected and flotetuzumab concentration will be determined by a validated electrochemiluminescent (ECL)-based sandwich assay. Samples will be analyzed by MacroGenics Pharmaceuticals. Bioanalytical measures will be combined with respective individual patient dosing history and demographic information. Pharmacometric analysis will be performed using an appropriate model-based approach. PK parameters will be correlated with toxicity parameters. PK exposures will be compared between patients who achieve CR/CRi and those who do not.

8.2.2 Sampling Schedule (See Appendix VI-A, Appendix VI-B, and Appendix VI-C)

The PK sampling schedule will vary according to the dose level assignment of the patient. For all patients being treated on DL1, blood samples will be collected at the following time points*:

- Cycle 1, Day 1: prior to start of infusion
- Cycle 1, Days 3 – 7: 30 minutes prior to syringe change and dose escalation

- Cycle 1, Day 8: 24 hours after Day 7 syringe change and final dose escalation
- Cycle 1, Day 12, Day 15, Day 19, Day 22, Day 27, and Day 29 (prior to infusion ending): any time

For all patients being treated on DL-1, blood samples will be collected at the following time points*:

- Cycle 1, Day 1: prior to start of infusion
- Cycle 1, Day 4, Day 6, and Day 8: 30 minutes prior to syringe change and dose escalation
- Cycle 1, Day 9: 24 hours after Day 8 syringe change and final dose escalation
- Cycle 1, Day 13, Day 17, Day 21, Day 25, and Day 29 (prior to infusion ending): any time

For all patients being treated on DL2, blood samples will be collected at the following time points*:

- Cycle 1, Day 1: prior to start of infusion
- Cycle 1, Days 3 – 8: 30 minutes prior to syringe change and dose escalation
- Cycle 1, Day 9: 24 hours after Day 8 syringe change and final dose escalation
- Cycle 1, Day 13, Day 17, Day 21, Day 25, and Day 29 (prior to infusion ending): any time

*All time points given are for patients that follow the dosing schedule exactly as outlined in Section 5.1. For patients that require dose interruptions during Cycle 1, PK samples should be drawn according to when the patient reaches certain dose levels (see Appendix VI-A, Appendix VI-B, and Appendix VI-C for details).

Blood samples will also be collected at the following time points on the patient's last day of protocol therapy:

- 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after the end of infusion

8.2.3 Sample Collection and Handling Instructions

Blood samples (2 – 3 ml) will be collected in SST tubes at a site distant from the infusion for pharmacokinetic evaluation. **NOTE**: Patients ≤ 23 kg should only have 2 ml of blood drawn per sample. Samples cannot be drawn from the 2nd lumen of a multi-lumen catheter through which drug is being administered. Record the exact time that the sample is drawn along with the exact time that infusion begins and ends.

8.2.4 Sample Processing

1. Collect blood into a 2 – 3 mL SST tube and invert the tube several times prior to allowing blood to clot.
2. Allow blood to clot for at least 60 minutes at room temperature.
3. Separate serum within 60 – 90 minutes of collection.
4. Centrifuge for 10 minutes at room temperature at 1100 – 1300 x g

5. Transfer serum equally into two 2 mL cryovials (primary and back up)
6. Immediately freeze at -60˚C to -80˚C.

8.2.5 Sample Labeling

Each tube must be labeled with the patient's study registration number and the study I.D. Data should be recorded on the Pharmacokinetic Study Form (Appendix VI-A, Appendix VI-B, and Appendix VI-C), which must accompany the sample(s).

Additionally, MacroGenics has standard naming conventions for samples. MGD006 is flotetuzumab. Sites should use the below labeling conventions for lab samples:

Pre MGD006 PK 1 (primary)
Pre MGD006 PK 2 (back up)
UNS PK (use this for unscheduled)

For all patients being treated on DL1, samples are to be labeled via the following conventions:

Sample Timepoint	Label Naming Convention
Cycle 1, Day 1: prior to start of infusion	Pre MGD006 infusion PK
Cycle 1, Days 3 – 7: 30 minutes prior to syringe change and dose escalation	30 min pre MGD006 infusion (include day) PK
Cycle 1, Day 8: 24 hours after Day 7 syringe change and final dose escalation	Day 8 PK
Cycle 1, Day 12, Day 15, Day 19, Day 22, Day 27, and Day 29 (prior to infusion ending): any time	Day 12 PK, Day 15 PK, etc.

For all patients being treated on DL-1, samples are to be labeled via the following conventions:

Sample Timepoint	Label Naming Convention
Cycle 1, Day 1: prior to start of infusion	Pre MGD006 infusion PK
Cycle 1, Day 4, Day 6, and Day 8: 30 minutes prior to syringe change and dose escalation	30 min pre MGD006 infusion (include day) PK
Cycle 1, Day 9: 24 hours after Day 8 syringe change and final dose escalation	Day 9 PK
Cycle 1, Day 13, Day 17, Day 21, Day 25, and Day 29 (prior to infusion ending): any time	Day 13 PK, Day 17 PK, etc.

For all patients being treated on DL2, samples are to be labeled via the following conventions:

Sample Timepoint	Label Naming Convention
Cycle 1, Day 1: prior to start of infusion	Pre MGD006 infusion PK
Cycle 1, Days 3 – 8: 30 minutes prior to syringe change and dose escalation	30 min pre MGD006 infusion (include day) PK
Cycle 1, Day 9: 24 hours after Day 8 syringe change and final dose	Day 9 PK

escalation	
Cycle 1, Day 13, Day 17, Day 21, Day 25, and Day 29 (prior to infusion ending): any time	Day 13 PK, Day 17 PK, etc.

Blood samples will also be collected at the following time points on the patient's last day of protocol therapy (EOT = end of treatment):

Sample Timepoint	Label Naming Convention
30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after the end of infusion	EOT PK 30 min post, EOT PK 1 hr post, etc.

8.2.6 Sample Shipping Instructions

Store samples at -70˚C until shipment. Ship on dry ice to MacroGenics as each patient completes the cycle or at site's discretion. If PK and immunogenicity samples are shipped in the same shipping container, place the PK and immunogenicity samples in separate cryoboxes within the shipping container. Prior to sample shipment, please email [redacted] to alert them of the sample shipment. Ship to the following address:

[redacted]

NOTE: Primary and back-up aliquots from the same time point should NOT be sent in the same shipment.

8.3 Immunogenicity Study (Required)

8.3.1 Description of Studies and Assay

Serum samples will be collected and anti-flotetuzumab antibodies will be detected using a validated bridging immunoassay by a MacroGenics, Inc. bioanalytical lab. Immunogenicity will be compared between patients who achieve CR/CRi and those who do not.

8.3.2 Sampling Schedule (See Appendix VII)

Blood samples will be collected at the following time points for all patients:

- Cycle 1, Day 1: pre-infusion
- Cycle 3, Day 1: pre-infusion
- Cycle 5, Day 1: pre-infusion

8.3.3 Sample Collection and Handling Instructions

Blood samples (2 – 3 ml) will be collected in SST tubes at a site distant from the infusion for immunogenicity evaluation. **NOTE:** Patients ≤ 23 kg should

only have 2 ml of blood drawn per sample.

8.3.4 Sample Processing

1. Collect blood into a 2 – 3 mL SST tube and invert the tube several times prior to allowing blood to clot
2. Allow blood to clot for at least 60 minutes at room temperature.
3. Separate serum within 60 – 90 minutes of collection.
4. Centrifuge for 10 minutes at room temperature at 1100 – 1300 x g.
5. Transfer serum equally into two 2 mL cryovials (primary and back up).
6. Immediately freeze at -60˚C to -80˚C.

8.3.5 Sample Labeling

Each tube must be labeled with the patient's study registration number, the study I.D., and the date and time the sample was drawn. Data should be recorded on the Pharmacokinetic Study Form (Appendix VII), which must accompany the sample(s).

Additionally, MacroGenics has standard naming conventions for samples. Sites should use the below labeling conventions for lab samples:

Pre MGD006 ADA
UNS ADA (use this for unscheduled)

For all patients, samples are to be labeled via the following conventions:

Sample Timepoint	Label Naming Convention
Cycle 1, Day 1: pre-infusion Pre MGD006 infusion	Pre MGD006 ADA
Cycle 3, Day 1: pre-infusion Pre MGD006 infusion C3	C3 Pre MGD006 ADA
Cycle 5, Day 1: pre-infusion Pre MGD006 infusion C5	C5 Pre MGD006 ADA

8.3.6 Sample Shipping Instructions

Store samples at -70˚C until shipment. Ship on dry ice to MacroGenics as each patient completes the cycle or at site's discretion. If PK and immunogenicity samples are shipped in the same shipping container, place the PK and immunogenicity samples in separate cryoboxes within the shipping container. Prior to sample shipment, please email [redacted] to alert them of the sample shipment. Ship to the following address:

Hua Li
MacroGenics, Inc.
15235 Shady Grove Road
Suite 304
Rockville, MD 20850 – 3234
Phone: (301) 354-2616

NOTE: Primary and back-up aliquots from the same time point should NOT be sent in the same shipment.

8.4 Baseline CD123 Site Density Quantification (Optional)

8.4.1 Description of Studies and Assay

Baseline bone marrow samples will be used to analyze CD123 site density on AML cells to aid in identifying biomarkers of therapy response and resistance. Correlation between changes in CD123 expression and patient's clinical response to flotetuzumab as well as cytogenetic/molecular features will be described. Samples will be analyzed at Hematologics, Inc.

8.4.2 Sampling Schedule (See Appendix VIII)

Analyses will be performed on the bone marrow samples retrieved prior to Cycle 1.

While the preferred specimen is bone marrow, if bone marrow samples cannot be obtained, peripheral blood may be sent in for patients who have an absolute blast count $\geq$ 1,000/µL.

8.4.3 Sample Collection and Handling Instructions

Bone marrow samples (5 mL) will be collected in a preservative-free sodium heparin vacutainer (green top).

In cases where the specimen is expected to take more than 24 hours to arrive, please add equal volume of RPMI 1640 medium to the marrow specimen.

If peripheral blood is obtained, 10 mL of blood will be collected in a preservative-free sodium heparin vacutainer (green top).

8.4.4 Sample Labeling

Each tube must be labeled with the patient's study registration number, the study I.D., the date and time the sample was drawn, and must be clearly labeled as bone marrow aspirate (BMA), if applicable. Data should be recorded on the Baseline CD123 Site Density Quantification Study Form (Appendix VIII), which must accompany the sample(s).

8.4.5 Sample Shipping Instructions

Samples must be shipped to Hematologics, Inc. Seattle, WA at the address listed below. All specimens must be shipped priority overnight.

Hematologics, Inc. 3161
Elliot Ave. Suite 200
Seattle, WA 98121
Phone: (800) 860-0934 or (206) 223-2700
Fax: (206) 223-5550
Weekends and After Hours: (206) 264-4459

8.5 NGS MRD Comparison with Flow Cytometry MRD (Optional)

8.5.1 Description of Studies and Assay

MRD levels at each bone marrow evaluation time point will be measured by standard flow cytometry and compared to MRD measured by NGS. The correlation between the measurements with each technique will be described to

aid in identifying biomarkers of therapy response and resistance.

8.5.2 Sampling Schedule (See Appendix IX)

Bone marrow samples will be collected from consenting patients at the following time point:

- With each bone marrow evaluation

While the preferred specimen is bone marrow, if bone marrow samples cannot be obtained prior to Cycle 1, peripheral blood may be sent in for patients who have an absolute blast count ≥ 1,000/μL. Bone marrow may be substituted for peripheral blood for the sample taken prior to Cycle 1 ONLY. All other samples must be bone marrow.

8.5.3 Sample Collection and Handling Instructions

Bone marrow samples (10 – 15 mL) will be collected in green top sodium heparin tubes at the time of bone marrow evaluation.

If peripheral blood is obtained for the prior to Cycle 1 time point, 20 – 30 mL of blood will be collected in large purple EDTA tubes. **NOTE**: Patients ≤ 23 kg should only have 20 mL of blood drawn per sample.

8.5.4 Sample Processing

Store samples at 4˚C until shipment.

8.5.5 Sample Labeling

Each tube must be labeled with the patient's study registration number, the study I.D., and the date and time the sample was drawn. Data should be recorded on the NGS MRD Comparison with Flow Cytometry MRD Study Form (Appendix IX), which must accompany the sample(s).

8.5.6 Sample Shipping Instructions

For questions regarding sample collection, processing, and shipping, please email [redacted]

Samples may be shipped at room temperature or with an ice pack. If samples are to be shipped with an ice pack, the collection tubes are to be wrapped in paper towels or other insulation to minimize tube freezing and direct contact with the ice pack.

Prior to sample shipment, please email [redacted] to alert them of the sample shipment.

Ship samples to the following address:

[redacted]

Following sample shipment, send another email to the addresses listed above

with the Fed-Ex tracking number and copy the PEPN1812 Research Coordinator on the email.

8.6 CD3 T-Lymphocyte Quantification (Optional)

8.6.1 Description of Studies and Assay

Peripheral blood samples will be collected from consenting patients to aid in identifying biomarkers of response through flow cytometric analysis of CD3+ T-cells. Changes in T cell number will be described and exploratory analysis will be conducted to assess their correlation with clinical features including occurrence of infections and response. Samples will be analyzed locally at the treating institution.

8.6.2 Sampling Schedule (See Appendix X)

Blood samples will be collected from consenting patients at the following time points:

- Cycle 1: Day 1 pre-infusion, and Days 8, 15, 22, and 29 (prior to the end of infusion)
- Subsequent cycles, Day 1: Pre-infusion

8.6.3 Sample Collection and Handling Instructions

Blood samples (2 – 3 ml) will be collected in EDTA tubes (or institutional standard) and analyzed locally via flow cytometry according to institutional standards. **NOTE:** Patients ≤ 23 kg should only have 2 ml of blood drawn per sample. Blood samples will be collected at a site distant from the infusion for T cell quantification. Samples cannot be drawn from the 2nd lumen of a multi-lumen catheter through which drug is being administered. Record the exact time that the sample is drawn along with the exact time that infusion begins on Day 1 of each cycle.

The date and time of sample collection will be recorded on the CD3 T-Lymphocyte Quantification Study Form (Appendix X) and uploaded into RAVE. Quantification results will be collected in RAVE per the guidance in the CRF packet.

8.7 Cytokine Production Measurement (Optional)

8.7.1 Description of Studies and Assay

Peripheral blood samples will be collected from consenting patients to improve mechanistic understanding of potential therapeutic responses and adverse effects with flotetuzumab therapy. Samples will be analyzed through a qualified ELISA assay by MacroGenics Pharmaceuticals.

8.7.2 Sampling Schedule (See Appendix XI)

Blood samples will be collected from consenting patients at the following time points:

- Cycle 1, Day 1: pre-infusion
- Cycle 1, Day 8

Additionally, if patients experience IRR/CRS, blood samples will be collected from consenting patients at the following time points:

- At the time of IRR/CRS
- At the time of IRR/CRS resolution

8.7.3 Sample Collection and Handling Instructions

Blood samples (2 – 3 ml) will be collected in SST tubes at a site distant from the infusion for cytokine production measurement evaluation. **NOTE:** Patients ≤ 23 kg should only have 2 ml of blood drawn per sample.

8.7.4 Sample Processing

1. Collect blood into a 2 – 3 mL SST tube.
2. Allow blood to clot for at least 60 minutes at room temperature.
3. Separate serum within 60 – 90 minutes of collection.
4. Centrifuge for 10 minutes at room temperature at 1100 – 1300 x g.
5. Transfer serum equally into two 2 mL orange capped cryovials (primary and back up).
6. Immediately freeze at -60˚C to -80˚C.

8.7.5 Sample Labeling

Each tube must be labeled with the patient's study registration number, the study I.D., and the date and time the sample was drawn. Data should be recorded on the Cytokine Analysis Study Form (Appendix XI), which must accompany the sample(s).

Additionally, MacroGenics has standard naming conventions for samples. Sites should use the below labeling conventions for lab samples:

Pre MGD006 CK
Day 8 CK
Start IRR/CRS CK
End IRR/CRS CK
UNS CK (use this for unscheduled)

8.7.6 Sample Shipping Instructions

Store samples at -70˚ C until shipment. Ship on dry ice to Washington University once all samples have been collected for each patient. Prior to sample shipment, please email [redacted] to alert her of the sample shipment. Ship to the following address:

[redacted]

NOTE: Primary and back-up aliquots from the same time point should NOT be sent in the same shipment.

8.8 Profiling of the Tumor Microenvironment (Optional)

8.8.1 Description of Studies and Assay

Bone marrow aspirate and biopsy will be collected from consenting patients to aid in identifying biomarkers of response through gene expression profiling, protein analysis, and patient-derived xenograft modeling. Studies will be performed via the Tasian Lab at the Children's Hospital of Philadelphia.

8.8.2 Sampling Schedule

Bone marrow aspirate and biopsy will be collected from consenting patients at the following time points:

- Prior to Cycle 1

While the preferred specimen is bone marrow, if bone marrow samples cannot be obtained, peripheral blood may be sent in for patients who have an absolute blast count $\geq$ 1,000/µL

In addition to the bone marrow aspirate or blood sample, 1 paraffin-embedded tissue block will be collected. If a block of tissue is unavailable, 10 unstained slides and 2 H&E stained slides may be collected instead.

8.8.3 Sample Collection and Handling Instructions

Bone marrow samples (10 – 15 mL) will be collected in green top sodium heparin tubes at the time of bone marrow evaluation.

If peripheral blood is obtained, 20 – 30 mL of blood will be collected in green top sodium heparin tubes. **NOTE:** Patients $\leq$ 23 kg should only have 20 mL of blood drawn per sample.

8.8.4 Sample Processing

Store samples at 4˚C until shipment.

8.8.5 Sample Labeling

Each tube must be labeled with the patient's study registration number, the study I.D., and the date and time the sample was drawn. Data should be recorded on the Tumor Microenvironment Study Form (Appendix XII), which must accompany the sample(s).

8.8.6 Sample Shipping Instructions

For questions regarding sample collection, processing, and shipping, please email [redacted]

Samples may be shipped at room temperature or with an ice pack. If samples are to be shipped with an ice pack, the collection tubes are to be wrapped in paper towels or other insulation to minimize tube freezing and direct contact with the ice pack.

Prior to sample shipment, please email [redacted]

Ship samples to the following address:

[redacted]

Following sample shipment, send another email to the addresses listed above with the Fed-Ex tracking number and copy the PEPN1812 Research Coordinator on the email.

9.0 AGENT INFORMATION

9.1 [redacted]

9.1.1 [redacted]

9.1.2 [redacted]

9.1.3 [redacted]

9.1.4 [redacted]

9.1.4.2 [redacted]

[redacted]

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

[redacted]

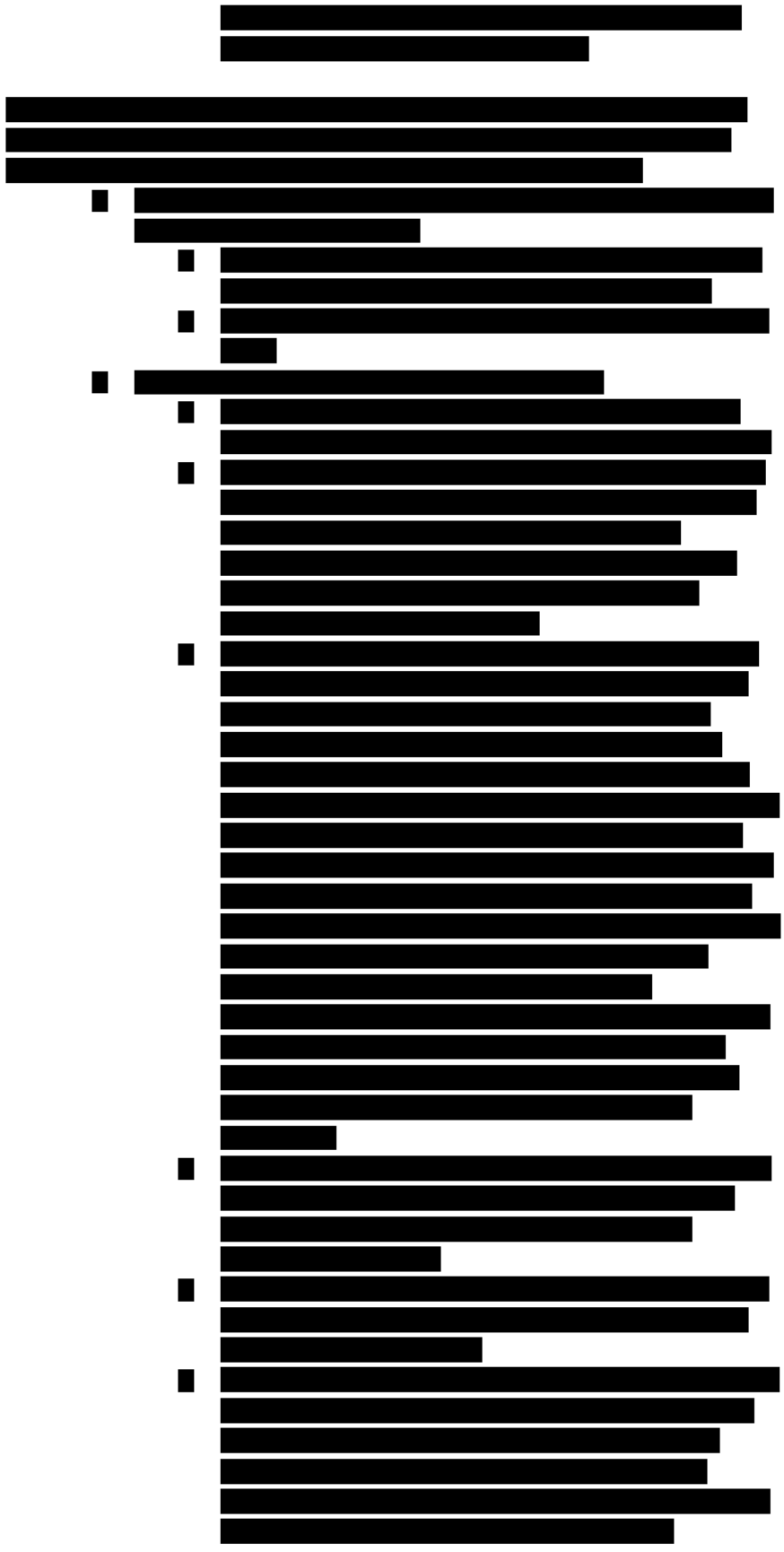
9.1.5 [redacted]

9.1.6 [redacted]

[redacted]

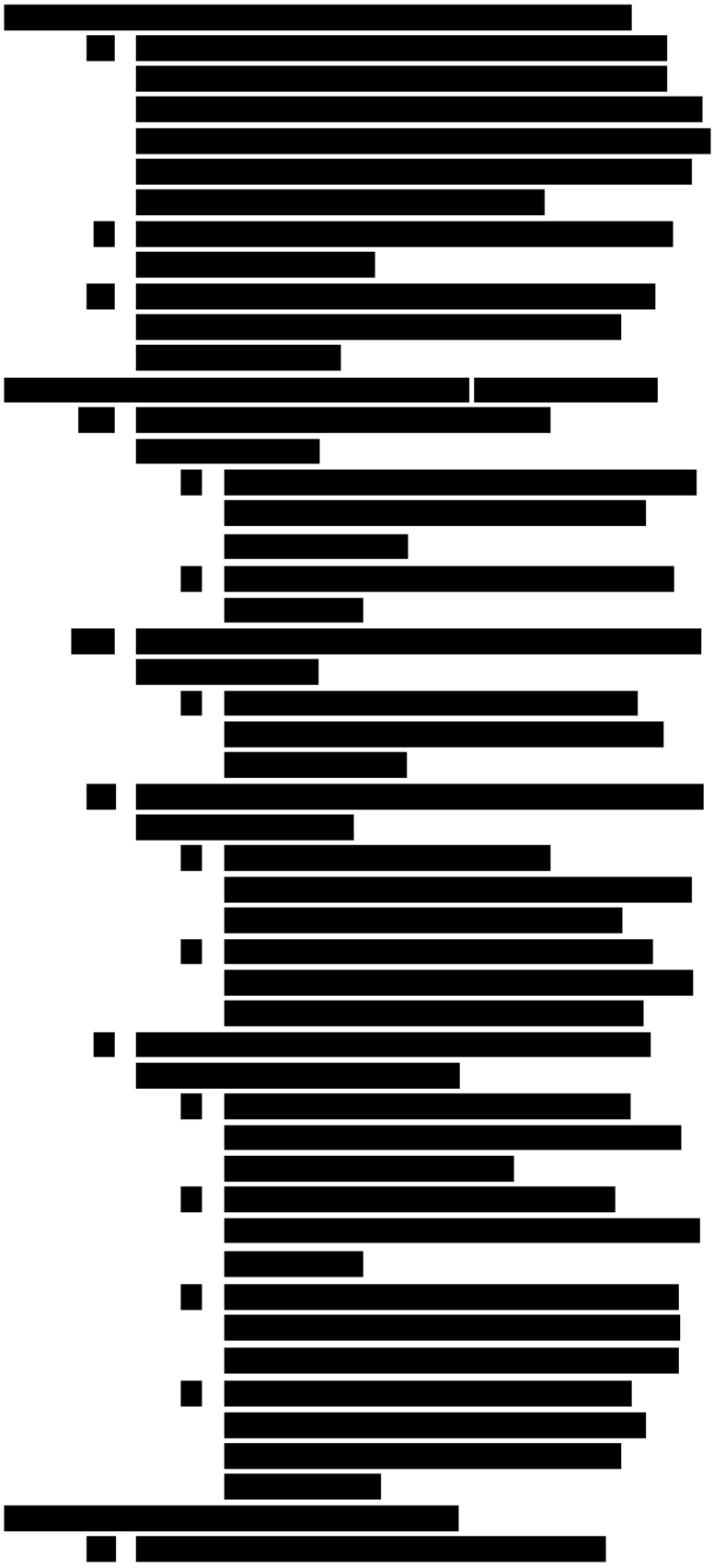
[redacted]

9.1.6.5 [redacted]



[redacted]

ii. [redacted]



[redacted]

9.1.7 [redacted]

[redacted]

[redacted]

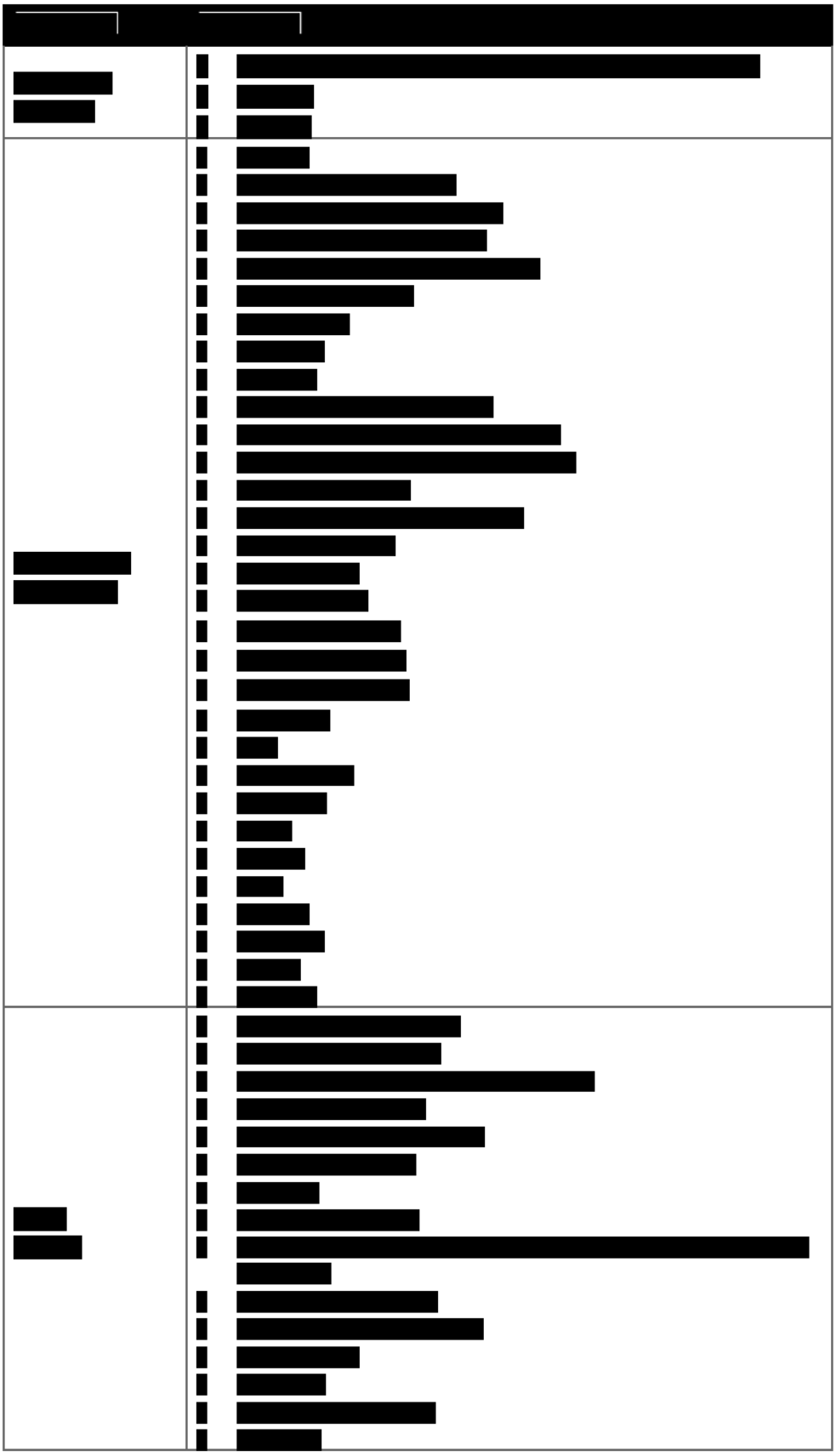
9.1.8 [redacted]

[redacted]

[redacted]

[redacted]

9.1.9 [redacted]



	• [redacted]
[redacted]	[redacted]

[redacted]

9.1.10 [redacted]

[redacted]

[redacted]

9.1.11 [redacted]

9.1.12 [redacted]

[redacted]

9.2 Cytarabine (07/13/15)

(Cytosine arabinoside, Ara-C, Cytosar®) NSC# 63878

9.2.1 Source and Pharmacology

Cytarabine appears to act through the inhibition of DNA polymerase. A limited, but significant, incorporation of cytarabine into both DNA and RNA has also been reported. It exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and under certain conditions blocking the progression of cells from the G1 phase to the S-phase. Cytarabine is metabolized by deoxycytidine kinase and other nucleotide kinases to the nucleotide triphosphate (Ara-CTP), an effective inhibitor of DNA polymerase. Ara-CTP is inactivated by a pyrimidine nucleoside deaminase, which converts it to the nontoxic uracil derivative (Ara-U). It appears that the balance of kinase and deaminase levels may be an important factor in determining sensitivity or resistance of the cell to cytarabine. It has an initial distributive phase $t_{½}$ of about 10 minutes, with a secondary elimination phase $t_{½}$ of about 1 to 3 hours. Peak levels after intramuscular or subcutaneous administration of cytarabine occur about 20 to 60 minutes after injection and are lower than IV administration. Intrathecally administered doses are metabolized and eliminated more slowly with a $t_{½}$ of about 2 hours.

9.2.2 Toxicity: (Intrathecal)

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, fever, headache	Arachnoiditis	Rash, somnolence, meningismus, convulsions, paresis
Prompt: Within 2-3 weeks, prior to the next course			Myelosuppression, ataxia
Delayed: Any time later during therapy, excluding the above condition			Necrotizing leukoencephalopathy, paraplegia, blindness (in combination with XRT & systemic therapy)

9.2.3 Formulation

Cytarabine for Injection is available as a preservative free solution 20 mg/mL (5 mL, 50 mL per vial) or 100 mg/mL (20 mL vial). Hydrochloric acid and/or sodium hydroxide may be added to adjust the pH. Store intact vials of solution at 15°-30°C (59°-86°F). Cytarabine solutions should be protected from light.

9.2.4 Guidelines for Administration

See Treatment and Dose Modification sections of the protocol.

Intrathecal:

For intrathecal administration, dilute with 5-10 mL (or volume per institutional practice) preservative free 0.9% sodium chloride injection, lactated Ringer's injection, Elliot's B solution. The volume of CSF removed should be equal to at least ½ the volume delivered.

Patient Age (years)	Recommended volume	10% CSF volume	CSF Volume *
1 – 1.99	5 – 10 mL	5 mL	50 ± 10 mL (babies)
2 – 2.99	5 – 10 mL	8 mL	80 ± 20 mL (younger children)
3 – 8.99	5 – 10 mL	10 mL	100 ± 20 mL (older children)
9 or greater	5 – 10 mL	13 mL	130 ± 30 mL (adults)

**Rieselbach, R.E. et.al. Subarachnoid distribution of drugs after lumbar injection; N Engl J Med. 1962 Dec 20; 267:1273-8*

Of Note: Larger volumes approximating at least 10% of the CSF volume, isovolumetric delivery, with the patient remaining prone after the procedure may facilitate drug distribution. These procedures have not been validated in clinical trials. They are allowed but not mandated for patients on COG studies.

Intrathecal cytarabine mixed in NS, lactated Ringer's injection, or Elliot's B solution is stable for 24 hours at 25ºC but contains no preservative and should be administered as soon as possible after preparation.

9.2.5 Supplier

Commercially available from various manufacturers. See package insert for further information.

9.3 **Useful Links and Contacts**

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP Associate Registration and IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov

10.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

10.1 Criteria for Removal from Protocol Therapy

a) Clinical (including physical examination or serum tumor markers) or radiographic

evidence of progressive disease (See Section 12).
b) Have not achieved at least a PR following 2 successive treatment cycles.
c) Adverse Events requiring removal from protocol therapy (See Section 13).
d) Development of a DLT following 1 dose reduction.
e) An infusion stop or delay of more than 2 weeks due to AE or more than 2 discontinuations per cycle due to AE (does not apply for prolongation of the scheduled treatment free period of a cycle).
f) Refusal of protocol therapy by patient/parent/guardian
g) Non-compliance that in the opinion of the investigator does not allow for ongoing participation.
h) Completion of 6 cycles of therapy.
i) Physician determines it is not in the patient's best interest.
j) Repeated eligibility laboratory studies (CBC with differential, bilirubin, ALT (SGPT) or serum creatinine) are outside the parameters required for eligibility prior to the start of flotetuzumab (See Section 8.1).
k) Study is terminated by Sponsor.
l) Pregnancy

Patients who are removed from protocol therapy during Cycle 1 should continue to have the required observations in Section 8.1 until the originally planned end of the cycle or until all adverse events have resolved per Section 13.4.4, whichever happens LATER. The only exception is with documentation of the patient's withdrawal of consent. Patients who are removed from protocol therapy in subsequent cycles should have the necessary observations to ensure adequate clinical care.

Patients who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below). Ongoing adverse events, or adverse events that emerge after the patient is removed from protocol therapy, but within 30 days of the last dose of investigational agent, must be followed and reported via RAVE and CTEP-AERS (if applicable). Follow-up data will be required unless consent is withdrawn.

10.2 Off Study Criteria

a) Thirty days after the last dose of the investigational agent.
b) The patient does not receive protocol treatment after study enrollment.
c) Death
d) Lost to follow-up
e) Withdrawal of consent for any required observations or data submission.
f) Enrollment onto another COG therapeutic (anti-cancer) study

11.0 STATISTICAL AND ETHICAL CONSIDERATIONS

11.1 Sample Size and Study Duration

This is a Phase 1 study involving a dose escalation cohort using a rolling six design with a primary aim of identification of safe and tolerable dosing of the CD123 x CD3 DART, flotetuzumab, in children between < 21 years of age with AML in second or greater relapse, refractory AML after two or more chemotherapy cycles, or first relapse after primary chemotherapy-refractory disease. Prior hematopoietic stem cell transplantation (HSCT) is permitted.

If DL1 is safe and tolerable based on standard immunotherapy DLT assessments, an additional cohort of patients will dose-escalate to flotetuzumab at 700 nanograms/kg/day (DL2). If fewer than 2 DLTs occur at DL2, this will be considered the RP2D. If there are $\geq$ 2 DLTs at DL2, DL1 will be considered the MTD of flotetuzumab monotherapy. If flotetuzumab monotherapy at DL1 is not tolerable, dose de-escalation will occur to 300 nanograms/kg/day (DL-1). If DL-1 is deemed tolerable, this dosing will be the MTD. Further dose de-escalation beyond DL-1 will not be permitted and will limit further exploration of flotetuzumab in this patient population. Bone marrow evaluation will be performed with blood count recovery from Cycle 1 therapy as an exploratory measurement of response. Patients with at least stable disease after Cycle 1 may continue to Cycle 2. Patients with at least a PR following Cycle 2 may receive subsequent cycles of flotetuzumab for a total of up to 6 cycles.

A minimum of 4 evaluable patients and a maximum of 18 will be required for determination of the MTD/RP2D. Once the MTD or RP2D has been defined, up to 6 additional patients with AML in second or greater relapse, refractory AML after two or more chemotherapy cycles, first relapse after primary chemotherapy-refractory disease, or first relapse after HSCT without restrictions on heme evaluability may be enrolled to acquire PK data in a representative number of young patients (i.e. patients < 12 years old). Review of the enrollment rate into previous COG new agent studies indicates that 1-2 patients per month are available, which will permit completion of the study within 27 months. A maximum of 27 patients is anticipated allowing for three dose levels, PK expansion, and 10% attrition. In the unlikely event that each dose level is expanded to 12 patients, an absolute maximum of 47 patients may be required allowing for 10% inevaluability, and this would be completed within 47 months.

11.2 Definitions

11.2.1 Evaluable For Adverse Events

Any patient who receives at least one dose of the study drug(s) or who experiences a dose-limiting toxicity is considered evaluable for Adverse Events. In addition, for the dose-escalation portion during Cycle 1, patients must receive at least 80% of the prescribed dose per protocol guidelines and must have the appropriate toxicity monitoring studies performed to be considered evaluable for dose limiting toxicity. Patients who do not have DLT and are not considered evaluable for toxicity will be replaced.

11.2.2 Maximum Tolerated Dose

- The MTD will be the maximum dose at which fewer than one-third of patients experience DLT (See Section 5.5) during Cycle 1 of therapy.

- If two or more of a cohort of up to six patients experience DLT at a given dose level, then the MTD has been exceeded and dose escalation will be stopped. If review of the DLTs suggests that they would potentially be acceptable (e.g., because of rapid reversibility, different classes of Adverse Effects) or are potentially attributable to disease rather than therapy, then expansion of the cohort to 12 patients to better assess tolerability of this dose level will be considered assuming the following conditions are met:
  - One of the DLTs does not appear to be dose-related
  - The Adverse Effects are readily reversible

- The study chair, DVL statistician, DVL committee chair or vice chair, IND sponsor, and CTEP all agree that expansion of the cohort is acceptable

If fewer than 1/3 of patients in the expanded cohort experience dose-limiting toxicities, the dose escalation can proceed.

- The DLTs observed in the pharmacokinetic (PK) expansion cohort will be counted towards the total number of DLTs observed at the MTD during the dose escalation portion of the study. If ≥ 1/3 of the cohort of patients at the MTD (during the dose escalation plus the PK expansion) experience DLT then the MTD will be exceeded.

11.3 Dose Escalation and Determination of MTD

The rolling six phase 1 trial design will be used for the conduct of this study.[35] Two to six patients can be concurrently enrolled onto a dose level, dependent upon (1) the number of patients enrolled at the current dose level, (2) the number of patients who have experienced DLT at the current dose level, and (3) the number of patients entered but with tolerability data pending at the current dose level. However, enrollment will be staggered so that no more than 1 patient initiates enrollment every 7 days for dose levels greater than DL1. Accrual is suspended when a cohort of six has enrolled or when the study endpoints have been met.

Dose level assignment is based on the number of participants currently enrolled in the cohort, the number of DLTs observed, and the number of participants at risk for developing a DLT (i.e., participants enrolled but who are not yet assessable for toxicity). For example, when three participants are enrolled onto a dose cohort, if toxicity data is available for all three when the fourth participant entered and there are no DLTs, the dose is escalated and the fourth participant is enrolled to the subsequent dose level. If data is not yet available for one or more of the first three participants and no DLT has been observed, or if one DLT has been observed, the new participant is entered at the same dose level. Lastly, if two or more DLTs have been observed, the dose level is de-escalated. This process is repeated for participants five and six. In place of suspending accrual after every three participants, accrual is only suspended when a cohort of six is filled. When participants are inevaluable for toxicity, they are replaced with the next available participant if escalation or de-escalation rules have not been fulfilled at the time the next available participant is enrolled onto the study.

The following table provides the decision rules for enrolling a patient at (i) the current dose level (ii) at an escalated dose level, (iii) at a de-escalated dose level, or whether the study is suspended to accrual:

# Pts Enrolled	# Pts with DLT	# Pts without DLT	# Pts with Data Pending	*Decision*
2	0 or 1	0, 1 or 2	0, 1 or 2	Same dose level

2	2	0	0	De-escalate*
3	0	0, 1 or 2	1, 2 or 3	Same dose level
3	1	0, 1 or 2	0, 1 or 2	Same dose level
3	0	3	0	Escalate**
3	≥ 2	0 or 1	0 or 1	De-escalate*
4	0	0, 1, 2 or 3	1, 2, 3 or 4	Same dose level
4	1	0, 1, 2 or 3	0, 1, 2 or 3	Same dose level
4	0	4	0	Escalate**
4	≥ 2	0, 1 or 2	0, 1 or 2	De-escalate*
5	0	0, 1, 2, 3 or 4	1, 2, 3, 4 or 5	Same dose level
5	1	0, 1, 2, 3 or 4	0, 1, 2, 3 or 4	Same dose level
5	0	5	0	Escalate**
5	≥ 2	0, 1, 2 or 3	0, 1, 2 or 3	De-escalate*
6	0	0, 1, 2, 3, or 4	2, 3, 4, 5 or 6	Suspend
6	1	0, 1, 2, 3 or 4	0, 1, 2, 3 or 4	Suspend
6	0 or 1	5 or 6	0 or 1	Escalate**
6	≥ 2	0, 1, 2, 3 or 4	0, 1, 2, 3 or 4	De-escalate*

* If six patients already entered at next lower dose level, the MTD has been defined.
**If final dose level has been reached, the recommended dose has been reached.

If two or more of a cohort of up to six patients experience DLT at a given dose level, then the MTD has been exceeded and dose escalation will be stopped (see Section 11.2.2 for exception to rule).

In addition to determination of the MTD, a descriptive summary of all toxicities will be reported.

11.4 Inclusion of Children, Women and Minorities

PLANNED ENROLLMENT REPORT					
Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	0	0	0	0	0
Asian	0	1	0	0	1
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	3	3	0	0	6
White	14	20	3	3	40
More Than One Race	0	0	0	0	0
Total	17	24	3	3	47

The study is open to all participants regardless of gender or ethnicity. Review of accrual to past COG studies of new agents demonstrates the accrual of both genders and all NIH-identified ethnicities to such studies. Efforts will be made to extend the accrual to a representative population, but in a Phase 1 trial which will accrue a limited number of patients, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

11.5 Pharmacokinetic and Correlative Studies and Response Analysis

A descriptive analysis of pharmacokinetic (PK) parameters of flotetuzumab will be performed to define systemic exposure, drug clearance, and other pharmacokinetic parameters. The PK parameters will be summarized with simple summary statistics, including means, medians, ranges, and standard deviations (if numbers and distribution permit).

While the primary aim of this study is to evaluate the toxicity of flotetuzumab, patients will have disease evaluations performed as indicated in Section 8.1. Disease response will be assessed according to the revised AML International Working Group (IWG) criteria[37] and will be reported descriptively.

CD123 expression will be analyzed in an exploratory fashion, both using a binary scale and using a continuous scale to evaluate whether there are correlations between CD123 expression and antitumor effects. Anti-drug antibody analyses will be measured by MacroGenics. Biomarkers, including those identified in the secondary objectives, will be evaluated for association with cytogenetic/molecular features and outcome.

All these analyses will be descriptive and exploratory and hypothesis-generating in nature.

12.0 EVALUATION CRITERIA

12.1 Common Terminology Criteria for Adverse Events (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

12.2 Response Criteria for Patients with Leukemia

Patients must have received $\geq$ 1 cycle of flotetuzumab, be evaluable for DLT, and have had $\geq$ 1 bone marrow evaluation(s) following treatment. Response will be defined as best response after up to 4 cycles. The AML response criteria are derived from the revised AML International Working Group (IWG) Criteria.[38] It is anticipated that patients who achieve morphologic remission with study treatment may proceed to subsequent allogeneic HSCT at the treating physician's discretion.

12.2.1 Complete Remission (CR)

Attainment of an M1 bone marrow (< 5% blasts) with no evidence of circulating blasts or extramedullary disease and with recovery of peripheral blood counts (ANC $\geq 1000/mm^3$ and platelet count $\geq$ 100,000/mm^3). Occasionally, rare peripheral blood blasts may be identified during marrow regeneration; however, the marrow must be M1 status by morphology and without Auer rods to be considered CR. Flow cytometry may also be useful to distinguish between leukemia and a regenerating bone marrow. There is no requirement for bone marrow cellularity.

12.2.2 CR with Partial Recovery of Platelet Count (CRp)

Attainment of an M1 bone marrow (< 5% blasts), no evidence of circulating blasts or extramedullary disease, and recovery of ANC $\geq 1000/mm^3$ and platelet transfusion independence (defined as no platelet transfusions in 1 week).

12.2.3 CR with incomplete blood count recovery (CRi)

Attainment of an M1 bone marrow (< 5% blasts), no evidence of circulating blasts or extramedullary disease and with ANC < $1000/mm^3$ or platelet count < 100,000/mm^3 without platelet transfusion independence (defined as no platelet transfusions in 1 week).

12.2.4 Partial Response (PR)

A decrease of at least 50% in the percentage of blasts to 5% to 25% in the bone marrow aspirate. Bone marrow must have adequate cellularity (e.g., > 15%) to determine response. PR status will not be included in calculation of response to the regimen. A repeat bone marrow aspiration after several weeks may be required to distinguish between a PR and increased blasts caused by bone marrow regeneration. A value of < 5% blasts may also be considered a PR if Auer rods are present.

12.2.5 Progressive Disease (PD)

Increase in the absolute number of circulating (in peripheral blood) or bone marrow blasts of at least 50% from nadir or development of extramedullary disease.

12.2.6 Stable Disease (SD)

Criteria for CR, CRp, CRi, CRc, PR, or progressive disease (PD) not met.

12.2.7 Relapse

Morphologic relapse after CR/CRp/CRi is defined as a reappearance of leukemic blasts in the peripheral blood or $\geq$ 5% blasts in the bone marrow not attributable to any other cause (e.g., bone marrow regeneration after consolidation therapy). In the setting of recent treatment, if there are no circulating blasts and the bone marrow contains 5% to 20% blasts, a repeat bone marrow performed at least a week later is necessary to distinguish relapse from bone marrow regeneration. Should flow cytometric analyses suggest relapse (by the reappearance of a similar immunophenotype to the original leukemia) in the presence of < 5% blasts, or $\geq$ 5% blasts in a regenerating marrow, a repeat bone marrow(s) performed at least a week later is necessary to confirm relapse by morphologic methods. In such instances the date of recurrence is defined as the first date that more than 5% blasts were observed in the marrow. The

reappearance or development of cytologically proven extramedullary disease also indicates relapse. Molecular and/or genetic relapse is characterized by reappearance of a cytogenetics or molecular abnormality.

12.2.8 Unevaluable

Aplastic or severely hypocellular marrow with any blast percentage. In this instance, marrow evaluation should be repeated weekly until response determination can be made through at least Day 49.

12.2.9 Bone Marrow Classification:

M1 is < 5% blasts
M2 is 5-25% blasts
M3 is > 25% blasts

12.3 CNS Leukemia at Diagnosis

CNS 1:	In cerebral spinal fluid (CSF), absence of blasts on cytospin preparation, regardless of the number of WBCs.
CNS 2:	In CSF, presence < 5/μL WBCs and cytospin positive for blasts, or ≥ 5/μL WBCs but negative by Steinherz/Bleyer algorithm:
CNS 2a:	< 10/μL RBCs; < 5/μL WBCs and cytospin positive for blasts;
CNS 2b:	≥ 10/μL RBCs; < 5/μL WBCs and cytospin positive for blasts;
CNS 2c:	≥ 10/μL RBCs; ≥ 5/μL WBCs and cytospin positive for blasts, but negative by Steinherz/Bleyer algorithm (see below).
CNS 3:	In CSF, presence of ≥ 5/μL WBCs and cytospin positive for blasts and/or clinical signs of CNS leukemia:
CNS 3a:	< 10/μL RBCs; ≥ 5/μL WBCs and cytospin positive for blasts;
CNS 3b:	≥ 10/μL RBCs, ≥ 5/μL WBCs and positive by Steinherz/Bleyer algorithm (see below);
CNS 3c:	Clinical signs of CNS leukemia (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome).

Method of Evaluating Initial Traumatic Lumbar Punctures:

If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic and contains ≥ 5 WBC/μL and blasts, the following algorithm should be used to distinguish between CNS 2 and CNS 3 disease:

$$\frac{\text{CSF WBC}}{\text{CSF RBC}} > 2X \frac{\text{Blood WBC}}{\text{Blood RBC}}$$

A patient with CSF WBC ≥ 5/μL blasts, whose CSF WBC/RBC is 2X greater than the blood WBC/RBC ratio, has CNS disease at diagnosis. Example: CSF WBC = 60/μL; CSF RBC = 1500/μL; blood WBC = 46000/μL; blood RBC = 3.0×10^6/μL:

$$\frac{60}{1{,}500} = 0.04 > 2X \frac{46{,}000}{3.0 \times 10^6} = 0.015$$

13.0 ADVERSE EVENT REPORTING REQUIREMENTS

Adverse event data collection and reporting which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. (Please follow directions for routine reporting provided in the Case Report Forms for this protocol). Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care. The following sections provide information about expedited reporting.

Reporting requirements may include the following considerations: 1) whether the patient has received an investigational or commercial agent; 2) whether the adverse event is considered serious; 3) the grade (severity); and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

An investigational agent is a protocol drug administered under an Investigational New Drug Application (IND). In some instances, the investigational agent may be available commercially, but is actually being tested for indications not included in the approved package label.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. The NCI, rather than a commercial distributor, may on some occasions distribute commercial agents for a trial.

13.1 Steps to Determine If an Adverse Event Is To Be Reported In an Expedited Manner

Step 1: Identify the type of adverse event using the NCI CTCAE version 5.0. The descriptions and grading scales found in the revised CTCAE version 5.0 will be used for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website (http://ctep.cancer.gov).

Step 2: Grade the adverse event using the NCI CTCAE.

Step 3: Review Table A in this section to determine if:

- the adverse event is considered serious;
- there are any protocol-specific requirements for expedited reporting of specific adverse events that require special monitoring; and/or
- there are any protocol-specific exceptions to the reporting requirements.

NOTE: This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported according to the instructions in the table below. Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite.

Table A: Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	7 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found below under the section entitled "Additional Instructions or Exceptions".
Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

- The NCI defines hospitalization for expedited AE reporting purposes as an inpatient hospital stay equal to or greater than 24 hours. Hospitalization is used as an indicator of the seriousness of the AE and should ONLY be used for situations where the AE truly fits this definition and NOT for hospitalizations associated with less serious events (i.e., a hospital visit where a patient is admitted for observation or minor treatment such as hydration and released in less than 24 hours). Furthermore, hospitalization for pharmacokinetic sampling is not an AE and therefore is not to be reported either as a routine AE or in an expedited report.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or

prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

Additional Instructions or Exceptions to CTEP-AERS Expedited Reporting Requirements for Phase 1 Trials Utilizing an Agent under a CTEP-IND or Non-CTEP IND:

- Any death that occurs more than 30 days after the last dose of treatment with an investigational agent which can be attributed (possibly, probably, or definitely) to the agent and is not clearly due to progressive disease must be reported via CTEP-AERS for an agent under a CTEP or non-CTEP IND agent per the timelines outlined in the table above.

- Myelosuppression, (Grade 1 through Grade 4 adverse events as defined in the table below), does not require expedited reporting, unless it is associated with hospitalization.

Category	Adverse Events
INVESTIGATIONS	Platelet count decreased
INVESTIGATIONS	White blood cell decreased
INVESTIGATIONS	Neutrophil count decreased
INVESTIGATIONS	Lymphocyte count decreased
BLOOD/LYMPHATICS DISORDERS	Anemia

As referenced in the CTEP Adverse Events Reporting Requirements, an AE that resolves and then recurs during a subsequent cycle does not require CTEP-AERS reporting unless (1) the Grade increases; or (2) hospitalization is associated with the recurring AE.

13.2 When to Report an Event in an Expedited Manner

- Some adverse events require notification **within 24 hours** (refer to Table A) to NCI via the web at http://ctep.cancer.gov (telephone CTEP at: **301-897-7497** within 24 hours of becoming aware of the event if the CTEP-AERS 24-Hour Notification web-based application is unavailable) and by telephone call to the Study Chair. Once internet connectivity is restored, a 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

- When the adverse event requires expedited reporting, submit the report **within 5 or 7 calendar days** of learning of the event (refer to Table A).

- Expedited AE reporting for this study must only use CTEP-AERS (Adverse Event Expedited Reporting System), accessed via the CTEP home page https://eapps-ctep.nci.nih.gov/ctepaers.

13.3 Expedited Reporting Methods

13.3.1 CTEP-AERS Reporting

To report adverse events in an expedited fashion use the NCI's Adverse Event Expedited Reporting System (CTEP-AERS) that can be found at http://ctep.cancer.gov.

A CTEP-AERS report must be submitted electronically via the CTEP-AERS Web-based application located at https://eapps-ctep.nci.nih.gov/ctepaers/. If prompted to enter a sponsor email address, please type in: PEPCTNAERS@childrensoncologygroup.org.

Send supporting documentation to the COG PEP-CTN by fax (fax# 310-640-9193) and by email to the Study Assigned Research Coordinator. **ALWAYS include the ticket number on all faxed and emailed documents**.

13.4 Definition of Onset and Resolution of Adverse Events

Note: These guidelines below are for reporting adverse events on the COG PEP-CTN case report forms and do not alter the guidelines for CTEP-AERS reporting.

13.4.1 If an adverse event occurs more than once in a course (cycle) of therapy only the most severe grade of the event should be reported.

13.4.2 If an adverse event progresses through several grades during one course of therapy, only the most severe grade should be reported.

13.4.3 The duration of the AE is defined as the duration of the highest (most severe) grade of the Adverse Effects.

13.4.4 The resolution date of the AE is defined as the date at which the AE returns to baseline or less than or equal to Grade 1, whichever level is higher (note that the resolution date may therefore be different from the date at which the grade of the AE decreased from its highest grade). If the AE does not return to baseline the resolution date should be recorded as "ongoing."

13.4.5 An adverse event that persists from one course to another should only be reported once unless the grade becomes more severe in a subsequent course. An adverse event which resolves and then recurs during a different course, must be reported each course it recurs.

13.5 Other Recipients of Adverse Event Reports

13.5.1 Events that do not meet the criteria for CTEP-AERS reporting (Section 13.2) should be reported at the end of each cycle using the forms provided in the CRF packet (See Section 14.1).

13.5.2 COG will forward reports and supporting documentation to the Study Chair, to the FDA (when COG holds the IND) and to the pharmaceutical company (for industry sponsored trials).

13.5.3 Adverse events determined to be reportable must also be reported according to the local policy and procedures to the Institutional Review Board responsible for oversight of the patient.

13.6 Secondary Malignancy:

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

1) Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
2) Myelodysplastic syndrome (MDS)
3) Treatment-related secondary malignancy.

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

Second Malignancy:

A *second malignancy* is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

13.7 Reporting Pregnancy, Fetal Death, and Death Neonatal

When submitting CTEP-AERS reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the Pregnancy Information Form should be completed and emailed to the Study Assigned Research Coordinator along with any additional medical information along with any additional medical information. The potential risk of exposure of the fetus to the investigational agent should be documented in the "Description of Event" section of the CTEP-AERS report.

13.7.1 Pregnancy

- Patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic. For this reason, pregnancy occurring on study or within 6 months following the last dose of study therapy should be reported in an expedited manner via CTEP-AERS as **Grade 3** ***"Pregnancy, puerperium and perinatal conditions - Other (Pregnancy)"*** under the ***"Pregnancy, puerperium and perinatal conditions"*** **System Organ Class** (SOC).

- Pregnancy should be followed until the outcome is known. If the baby is born with a birth defect or anomaly, then a second CTEP-AERS report is required.

13.7.2 Pregnancy Loss (Fetal Death)

- Pregnancy loss is defined in CTCAE as "Death in utero."

- Any pregnancy loss should be reported expeditiously, as **Grade 4** ***"Pregnancy loss"*** under the ***"Pregnancy, puerperium and perinatal conditions"*** **SOC**. Do NOT report a pregnancy loss as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

13.7.3 Death Neonatal

- Neonatal death, defined in CTCAE as ***"Newborn deaths occurring during the first 28 days after birth"*** that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.

- A neonatal death should be reported expeditiously as Grade 4 "Death neonatal" under the "General disorders and administration" SOC **when the death is the result of a patient pregnancy or pregnancy in partners of men on study**

- Do NOT report a neonatal death resulting from a patient pregnancy or pregnancy in partners of men as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

Pregnancy should be followed up until the outcome of the pregnancy is known at intervals deemed appropriate by her physicians. The "Pregnancy Information Form" should be used for all necessary follow-ups. This form is available at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportForm.pdf.

14.0 RECORDS, REPORTING, AND DATA AND SAFETY MONITORING PLAN

14.1 Categories of Research Records

Research records for this study can be divided into three categories:

1. Non-computerized Information: Therapy Delivery Maps, Pathology Reports, Surgical Reports. These forms are uploaded into RAVE.

2. Reference Labs, Biopathology Reviews, and Imaging Center data: These data accompany submissions to these centers, which forward their data electronically to the PEP-CTN Operations and Data/Statistics Center.

3. Computerized Information Electronically Submitted: All other data will be entered in RAVE with the aid of schedules and worksheets (essentially paper copies of the OPEN and RAVE screens) provided in the case report form (CRF) packet.

See separate CRF Packet, which includes submission schedule.

14.2 CDUS

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis. Reports are due January 31, April 30, July 31 and October 31. This is not a responsibility of institutions participating in this trial. Instructions for submitting data using the CDUS can be found on the CTEP web site: http://ctep.cancer.gov/reporting/cdus.html

14.3 Data and Safety Monitoring Plan

Data and safety is ensured by several integrated components including the COG PEP-

CTN Data and Safety Monitoring Committee.

14.3.1 Data and Safety Monitoring Committee

This study will be monitored in accordance with the COG PEP-CTN policy for data and safety monitoring of Phase 1 and 2 studies. In brief, the role of the COG PEP-CTN Data and Safety Monitoring Committee is to protect the interests of patients and the scientific integrity for all Phase 1 and 2 studies. The DSMC consists of a chair; a statistician external to COG; one external member; one consumer representative; the lead statistician of the PEP-CTN scientific committee; and a member from the NCI. The DSMC meets at least every 6 months to review current study results, as well as data available to the DSMC from other related studies. Approximately 6 weeks before each meeting of the Phase 1 and 2 DSMC, study chairs will be responsible for working with the study statistician to prepare study reports for review by the DSMC. The DSMC will provide recommendations to the PEP-CTN Chair and the Group Chair for each study reviewed to change the study or to continue the study unchanged. Data and Safety Committee reports for institutional review boards can be prepared using the public data monitoring report as posted on the COG PEP-CTN Web site.

14.3.2 Monitoring by the Study Chair and Developmental Therapeutics Leadership

The study chair will monitor the study regularly and enter evaluations of patients' eligibility, evaluability, and dose limiting toxicities into the study database. In addition, study data and the study chair's evaluations will be reviewed by the PEP-CTN Chair, Vice Chair and Statistician on a weekly conference call.

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APPENDIX I: PERFORMANCE STATUS SCALES/SCORES

Karnofsky		Lansky	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

APPENDIX II: CORRELATIVE STUDIES GUIDE

Correlative Study	Appx.	Volume per Sample	Total Volume
Pharmacokinetics analysis (peripheral blood)	VI	2 – 3 ml	20 – 57 ml
Immunogenicity study (peripheral blood)	VII	2 – 3 ml	6 – 9 ml
Baseline CD123 site density quantification (bone marrow)	VIII	5 ml	5 ml
NGS MRD (bone marrow)	IX	10 – 15 ml	70 – 105 ml
CD3 T-lymphocyte quantification (peripheral blood)	X	2 – 3 ml	20 – 30 ml
Cytokine analysis (peripheral blood)	XI	2 – 3 ml	4 – 12 ml
Tumor microenvironment profiling (bone marrow)	XII	10 – 15 ml	10 – 15 ml
Total Blood + Bone Marrow Volume			**135 – 233 ml**

APPENDIX III: TOXICITY-SPECIFIC GRADING

Bilirubin

Grade 1:	> ULN- ≤ 1.5 x ULN
Grade 2:	> 1.5 x ULN - 3.0 x ULN
Grade 3:	> 3.0 x ULN - 10.0 x ULN
Grade 4:	> 10.0 x ULN

ALT: For the purpose of this study, the ULN for SGPT is 45 U/L regardless of baseline.

Grade 1:	> 45 U/L - ≤ 135 U/L
Grade 2:	136 U/L - 225 U/L
Grade 3:	226 U/L - 900 U/L
Grade 4:	> 900 U/L

AST: For the purpose of this study, the ULN for SGOT is 50 U/L regardless of baseline.

Grade 1:	> 50 U/L - ≤ 150 U/L
Grade 2:	151 U/L - 250 U/L
Grade 3:	251 U/L - 1000 U/L
Grade 4:	> 1000 U/L

GGT:

Grade 1:	> ULN- 2.5 x ULN
Grade 2:	> 2.5 x ULN - 5.0 x ULN
Grade 3:	> 5.0 x ULN - 20.0 x ULN
Grade 4:	> 20.0 x ULN

APPENDIX IV-A: THERAPY DELIVERY MAP FOR CYCLE 1, DOSE LEVEL 1

Therapy Delivery Map – Cycle 1, Dose Level 1 This Therapy Delivery Map (TDM) relates to Cycle 1. Each cycle lasts 29 days.	_____ Patient COG ID number _____ DOB

Criteria to start each cycle are in Section 5.2. Extensive treatment details are in Section 5.1.
This TDM is on 4 pages. Flotetuzumab therapy ends on Day 29 given its continuous infusion administration.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
IT Cytarabine (ARAC) (all patients)	Intrathecal (IT)	Age-based dosing: **Age (yrs) Dose** < 1 20 mg 1-1.99 30 mg 2-2.99 50 mg ≥ 3 70 mg	-6 to 0	Patients should receive a single dose of IT cytarabine administered prior to the start of protocol therapy.
Flotetuzumab	Intravenous (IV)	**Day Dose** 1 30 nanograms/kg/day 2 60 nanograms/kg/day 3 100 nanograms/kg/day 4 200 nanograms/kg/day 5 300 nanograms/kg/day 6 400 nanograms/kg/day 7-28 500 nanograms/kg/day	1 – 28	See Section 5.1 for premedications recommendations. See Section 6 for dose modifications for adverse events.

Ht _____cm **Wt _____kg** **BSA _____m^2** **Dose Level _____nanograms/kg/day**

Date Due	Date Given	Day	IT ARAC _____ mg	Flotetuzumab 30 nanograms/kg/day ng	Flotetuzumab 60 nanograms/kg/day ng	Flotetuzumab 100 nanograms/kg/day ng	Flotetuzumab 200 nanograms/kg/day ng	Flotetuzumab 300 nanograms/kg/day ng	Flotetuzumab 400 nanograms/kg/day ng	Flotetuzumab 500 nanograms/kg/day ng	Studies	
			Enter calculated dose above and actual dose administered below									
		Pre-	_____ mg								a – d, f – q, u – x	
		1		_____ ng							e, r – t	a, b, d, f, g
		2			_____ ng							
		3				_____ ng					e	
		4					_____ ng				e	
		5						_____ ng			e	
		6							_____ ng		e	
		7								_____ ng	e	
		8								_____ ng	e, r, s	a, b, d, f, g
		9								_____ ng		
		10								_____ ng		
		11								_____ ng		
		12								_____ ng	e	
		13								_____ ng		
		14								_____ ng		
		15								_____ ng	r, e	a, b, d, f, g
		16								_____ ng		
		17								_____ ng		
		18								_____ ng		
		19								_____ ng	e	
		20								_____ ng		
		21								_____ ng		
		22								_____ ng	e, r	a, b, d,
		23								_____ ng		
		24								_____ ng		

		25									_____ ng		f, g
		26									_____ ng		
		27									_____ ng	e	
		28									_____ ng		
		29										e, i, j, q, r, v, w, y	

Required Observations in Cycle 1

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below.

a. History. Prior to Cycle 1 and every week during Cycle 1.
b. Physical exam (including VS). Prior to Cycle 1 and every week during Cycle 1.
c. Performance Status. Prior to Cycle 1.
d. CBC/diff/platelets. Prior to Cycle 1 and twice weekly during Cycle 1. If patients have Grade 4 neutropenia then CBCs should be checked at least every other day until recovery to Grade 3 or until meeting the criteria for dose limiting toxicity.
e. Pharmacokinetics. Samples to be collected from all patients on Cycle 1, Day 1 (pre-infusion), Days 3 – 7 (30 minutes prior to syringe change and dose escalation), Day 8 (24 hours after Day 7 syringe change and final dose escalation), and Days 12, 15, 19, 22, 27, and 29 (at any time) if patient follows the dosing schedule exactly as outlined in Section 5.1. For patients that require dose interruptions during Cycle 1, PK samples should be drawn according to when the patient reaches certain dose levels (see Appendix VI-A for details). Blood samples will also be collected 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after the end of infusion on the patient's last day of protocol therapy. See Section 8.2 for details.
f. Electrolytes including Ca^{++}. Prior to Cycle 1, twice weekly (every 3 to 4 days) during Week 1 of Cycle 1, and then weekly for the duration of Cycle 1.
g. Creatinine, ALT, bilirubin, and AST. Prior to Cycle 1 and weekly during Cycle 1.
h. Albumin. Prior to Cycle 1.
i. Imaging (CT or MRI) of chloroma. Only to be performed if clinically indicated. Prior to Cycle 1 and at the end of Cycle 1 (if present at baseline).
j. Bone marrow aspirate. Prior to Cycle 1 and at the end of Cycle 1.
k. Urinalysis. Prior to Cycle 1.
l. Pregnancy test. Prior to Cycle 1 for women of childbearing potential.
m. ECHO or gated radionuclide study. Prior to Cycle 1.

n. EKG. Prior to Cycle 1.

o. Neurologic exam. Prior to Cycle 1.

p. Ht/Wt/BSA. Prior to Cycle 1.

q. NGS MRD comparison with flow cytometry MRD. In consenting patients only at the time of bone marrow evaluation. See Section 8.5 for details.

r. CD3 T-lymphocyte quantification. In consenting patients, blood samples will be collected on Cycle 1 Days 1, 8, 15, 22, and 29. See Section 8.6 for details.

s. Cytokine production measurement. In consenting patients, blood samples will be collected on Cycle 1, Day 1 (prior to flotetuzumab infusion), and on Cycle 1, Day 8. Additionally, if patients experience IRR/CRS, blood samples will be collected from consenting patients at the time of IRR/CRS and at the time of IRR/CRS resolution. See Section 8.7 for details.

t. Immunogenicity study. One sample to be collected from all patients on Cycle 1, Day 1 (pre-infusion). See Section 8.3 for details.

u. Tumor microenvironment profiling. Prior to Cycle 1. See Section 8.8 for details.

v. Clot section/biopsy. Prior to Cycle 1 and at the end of Cycle 1. To be performed in conjunction with the bone marrow aspiration.

w. CD123 site density analysis. In consenting patients prior to Cycle 1. To be performed on the collected bone marrow aspirate. See Section 8.4 for details.

x. Cytogenetics and FISH. Prior to Cycle 1. To be performed on the collected bone marrow aspirate. It is recommended that cytogenetics/FISH be performed by a COG-approved laboratory.

y. Flow cytometry with MRD determination. At the end of Cycle 1 with bone marrow evaluation.

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

APPENDIX IV-B: THERAPY DELIVERY MAP FOR CYCLE 1, DOSE LEVEL -1

Therapy Delivery Map – Cycle 1, Dose Level -1 This Therapy Delivery Map (TDM) relates to Cycle 1. Each cycle lasts 29 days.	_____ Patient COG ID number _____ DOB

Criteria to start each cycle are in Section 5.2. Extensive treatment details are in Section 5.1.
This TDM is on 4 pages. Flotetuzumab therapy ends on Day 29 given continuous infusion administration.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
IT Cytarabine (ARAC) (all patients)	Intrathecal (IT)	Age-based dosing: **Age (yrs) Dose** < 1 20 mg 1-1.99 30 mg 2-2.99 50 mg ≥ 3 70 mg	-6 to 0	Patients should receive a single dose of IT cytarabine administered prior to the start of protocol therapy.
Flotetuzumab	Intravenous (IV)	**Day Dose** 1 30 nanograms/kg/day 2-3 60 nanograms/kg/day 4-5 100 nanograms/kg/day 6-7 200 nanograms/kg/day 8-28 300 nanograms/kg/day	1 – 28	See Section 5.1 for premedications recommendations. See Section 6 for dose modifications for adverse events.

Ht _____cm Wt _____kg BSA _____m^2 Dose Level _____nanograms/kg/day

Date Due	Date Given	Day	IT ARAC _____ mg	Flotetuzumab 30 nanograms/kg/day _____ ng	Flotetuzumab 60 nanograms/kg/day _____ ng	Flotetuzumab 100 nanograms/kg/day _____ ng	Flotetuzumab 200 nanograms/kg/day _____ ng	Flotetuzumab 300 nanograms/kg/day _____ ng	Studies	
			Enter calculated dose above and actual dose administered below							
		Pre-	_____ mg						a – d, f – q, u – x	
		1		_____ ng					e, r	a, b,

										– t	d, f, g
		2			_____ ng						
		3			_____ ng						
		4				_____ ng				e	
		5				_____ ng					
		6					_____ ng			e	
		7					_____ ng				
		8						_____ ng		e, r, s	a, b, d, f, g
		9						_____ ng		e	
		10						_____ ng			
		11						_____ ng			
		12						_____ ng			
		13						_____ ng		e	
		14						_____ ng			
		15						_____ ng		r	a, b, d, f, g
		16						_____ ng			
		17						_____ ng		e	
		18						_____ ng			
		19						_____ ng			
		20						_____ ng			
		21						_____ ng		e	
		22						_____ ng		r	a, b, d, f, g
		23						_____ ng			
		24						_____ ng			
		25						_____ ng		e	
		26						_____ ng			
		27						_____ ng			
		28						_____ ng			
		29								e, i, j, q, r, v, w, y	

Required Observations in Cycle 1

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below.

a. History. Prior to Cycle 1 and every week during Cycle 1.
b. Physical exam (including VS). Prior to Cycle 1 and every week during Cycle 1.
c. Performance Status. Prior to Cycle 1.
d. CBC/diff/platelets. Prior to Cycle 1 and twice weekly during Cycle 1. If patients have Grade 4 neutropenia then CBCs should be checked at least every other day until recovery to Grade 3 or until meeting the criteria for dose limiting toxicity.
e. Pharmacokinetics. Samples to be collected from all patients on Cycle 1, Day 1 (pre-infusion), Days 4, 6, and 8 (30 minutes prior to syringe change and dose escalation), Day 9 (24 hours after Day 8 syringe change and final dose escalation) and Days 13, 17, 21, 25, and 29 (at any time) if patient follows the dosing schedule exactly as outlined in Section 5.1. For patients that require dose interruptions during Cycle 1, PK samples should be drawn according to when the patient reaches certain dose levels (see Appendix VI-B for details). Blood samples will also be collected 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after the end of infusion on the patient's last day of protocol therapy. See Section 8.2 for details.
f. Electrolytes including Ca^{++}. Prior to Cycle 1, twice weekly (every 3 to 4 days) during Week 1 of Cycle 1, and then weekly for the duration of Cycle 1.
g. Creatinine, ALT, bilirubin, and AST. Prior to Cycle 1 and weekly during Cycle 1.
h. Albumin. Prior to Cycle 1.
i. Imaging (CT or MRI) of chloroma. Only to be performed if clinically indicated. Prior to Cycle 1 and at the end of Cycle 1 (if present at baseline).
j. Bone marrow aspirate. Prior to Cycle 1 and at the end of Cycle 1.
k. Urinalysis. Prior to Cycle 1.
l. Pregnancy test. Prior to Cycle 1 for women of childbearing potential.
m. ECHO or gated radionuclide study. Prior to Cycle 1.
n. EKG. Prior to Cycle 1.
o. Neurologic exam. Prior to Cycle 1.
p. Ht/Wt/BSA. Prior to Cycle 1.
q. NGS MRD comparison with flow cytometry MRD. In consenting patients only at the time of bone marrow evaluation. See Section 8.5 for details.
r. CD3 T-lymphocyte quantification. In consenting patients, blood samples will be collected on Cycle 1 Days 1, 8, 15, 22, and 29. See Section 8.6 for details.

s. Cytokine production measurement. In consenting patients, blood samples will be collected on Cycle 1, Day 1 (prior to flotetuzumab infusion) and on Cycle 1, Day 8. Additionally, if patients experience IRR/CRS, blood samples will be collected from consenting patients at the time of IRR/CRS and at the time of IRR/CRS resolution. See Section 8.7 for details.

t. Immunogenicity study. One sample to be collected from all patients on Cycle 1, Day 1 (pre-infusion). See Section 8.3 for details.

u. Tumor microenvironment profiling. Prior to Cycle 1. See Section 8.8 for details.

v. Clot section/biopsy. Prior to Cycle 1 and at the end of Cycle 1. To be performed in conjunction with the bone marrow aspiration.

w. CD123 site density analysis. In consenting patients prior to Cycle 1. To be performed on the collected bone marrow aspirate. See Section 8.4 for details.

x. Cytogenetics and FISH. Prior to Cycle 1. To be performed on the collected bone marrow aspirate. It is recommended that cytogenetics/FISH be performed by a COG-approved laboratory.

y. Flow cytometry with MRD determination. At the end of Cycle 1 with bone marrow evaluation.

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

APPENDIX IV-C: THERAPY DELIVERY MAP FOR CYCLE 1, DOSE LEVEL 2

Therapy Delivery Map – Cycle 1, Dose Level 2 This Therapy Delivery Map (TDM) relates to Cycle 1. Each cycle lasts 29 days.	_____ Patient COG ID number _____ DOB

Criteria to start each cycle are in Section 5.2. Extensive treatment details are in Section 5.1.
This TDM is on 4 pages. Flotetuzumab therapy ends on Day 29 given continuous infusion administration.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
IT Cytarabine (ARAC) (all patients)	Intrathecal (IT)	Age-based dosing: **Age (yrs) Dose** < 1 20 mg 1-1.99 30 mg 2-2.99 50 mg ≥ 3 70 mg	-6 to 0	Patients should receive a single dose of IT cytarabine administered prior to the start of protocol therapy.
Flotetuzumab	Intravenous (IV)	**Day Dose** 1 30 nanograms/kg/day 2 100 nanograms/kg/day 3 200 nanograms/kg/day 4 300 nanograms/kg/day 5 400 nanograms/kg/day 6 500 nanograms/kg/day 7 600 nanograms/kg/day 8-28 700 nanograms/kg/day	1 – 28	See Section 5.1 for premedications recommendations. See Section 6 for dose modifications for adverse events.

THIS PROTOCOL IS FOR RESEARCH PURPOSES ONLY, SEE PAGE 1 FOR USAGE POLICY

Ht _____cm Wt _____kg BSA _____m^2 Dose Level _____nanograms/kg/day

Date Due	Date Given	Day	IT ARAC _____ mg	Flotetuzumab 30 nanograms/kg/day ng	Flotetuzumab 100 nanograms/kg/day ng	Flotetuzumab 200 nanograms/kg/day ng	Flotetuzumab 300 nanograms/kg/day ng	Flotetuzumab 400 nanograms/kg/day ng	Flotetuzumab 500 nanograms/kg/day ng	Flotetuzumab 600 nanograms/kg/day ng	Flotetuzumab 700 nanograms/kg/day ng	Studies	
			Enter calculated dose above and actual dose administered below										
		Pre-	_____ mg									a – d, f – q, u – x	
		1		_____ ng								r – t	a, b, d, f, g
		2			_____ ng								
		3				_____ ng						e	
		4					_____ ng					e	
		5						_____ ng				e	
		6							_____ ng			e	
		7								_____ ng		e	
		8									_____ ng	e, r, s	a, b, d, f, g
		9									_____ ng	e	
		10									_____ ng		
		11									_____ ng		
		12									_____ ng		
		13									_____ ng	e	
		14									_____ ng		
		15									_____ ng	r	a, b, d, f, g
		16									_____ ng		
		17									_____ ng	e	
		18									_____ ng		
		19									_____ ng		
		20									_____ ng		
		21									_____ ng	e	
		22									_____ ng	r	a, b, d, f, g
		23									_____ ng		
		24									_____ ng		
		25									_____ ng	e	
		26									_____ ng		
		27									_____ ng		
		28									_____ ng		
		29										e, i, j, q, r, v, w, y	

Required Observations in Cycle 1

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below.

a. History. Prior to Cycle 1 and every week during Cycle 1.
b. Physical exam (including VS). Prior to Cycle 1 and every week during Cycle 1.
c. Performance Status. Prior to Cycle 1.
d. CBC/diff/platelets. Prior to Cycle 1 and twice weekly during Cycle 1. If patients have Grade 4 neutropenia then CBCs should be checked at least every other day until recovery to Grade 3 or until meeting the criteria for dose limiting toxicity.
e. Pharmacokinetics. Samples to be collected from all patients on Cycle 1, Day 1 (pre-infusion), Days 3 – 8 (30 minutes prior to syringe change and dose escalation), Day 9 (24 hours after Day 8 syringe change and final dose escalation) and Days 13, 17, 21, 25, and 29 (at any time) if patient follows the dosing schedule exactly as outlined in Section 5.1. For patients that require dose interruptions during Cycle 1, PK samples should be drawn according to when the patient reaches certain dose levels (see Appendix VI-C for details). Blood samples will also be collected 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after the end of infusion on the patient's last day of protocol therapy. See Section 8.2 for details.
f. Electrolytes including Ca^{++}. Prior to Cycle 1, twice weekly (every 3 to 4 days) during Week 1 of Cycle 1, and then weekly for the duration of Cycle 1.
g. Creatinine, ALT, bilirubin, and AST. Prior to Cycle 1 and weekly during Cycle 1.
h. Albumin. Prior to Cycle 1.
i. Imaging (CT or MRI) of chloroma. Only to be performed if clinically indicated. Prior to Cycle 1 and at the end of Cycle 1 (if present at baseline).
j. Bone marrow aspirate. Prior to Cycle 1 and at the end of Cycle 1.
k. Urinalysis. Prior to Cycle 1.
l. Pregnancy test. Prior to Cycle 1 for women of childbearing potential.
m. ECHO or gated radionuclide study. Prior to Cycle 1.
n. EKG. Prior to Cycle 1.
o. Neurologic exam. Prior to Cycle 1.
p. Ht/Wt/BSA. Prior to Cycle 1.
q. NGS MRD comparison with flow cytometry MRD. In consenting patients only at the time of bone marrow evaluation. See Section 8.5 for details.
r. CD3 T-lymphocyte quantification. In consenting patients, blood samples will be collected on Cycle 1 Days 1, 8, 15, 22, and 29. See Section 8.6 for details.

s. Cytokine production measurement. In consenting patients, blood samples will be collected on Cycle 1, Day 1 (prior to flotetuzumab infusion) and on Cycle 1, Day 8. Additionally, if patients experience IRR/CRS, blood samples will be collected from consenting patients at the time of IRR/CRS and at the time of IRR/CRS resolution. See Section 8.7 for details.

t. Immunogenicity study. One sample to be collected from all patients on Cycle 1, Day 1 (pre-infusion). See Section 8.3 for details.

u. Tumor microenvironment profiling. Prior to Cycle 1. See Section 8.8 for details.

v. Clot section/biopsy. Prior to Cycle 1 and at the end of Cycle 1. To be performed in conjunction with the bone marrow aspiration.

w. CD123 site density analysis. In consenting patients prior to Cycle 1. To be performed on the collected bone marrow aspirate. See Section 8.4 for details.

x. Cytogenetics and FISH. Prior to Cycle 1. To be performed on the collected bone marrow aspirate. It is recommended that cytogenetics/FISH be performed by a COG-approved laboratory.

y. Flow cytometry with MRD determination. At the end of Cycle 1 with bone marrow evaluation.

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

APPENDIX V: THERAPY DELIVERY MAP FOR CYCLES 2+

Therapy Delivery Map – Cycles 2+ This Therapy Delivery Map (TDM) relates to Cycles 2+. Each cycle lasts 29 days.	_____ Patient COG ID number _____ DOB

Criteria to start each cycle are in Section 5.2. Extensive treatment details are in Section 5.1.
This TDM is on 4 pages. Flotetuzumab therapy ends on Day 29 given continuous infusion administration.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
IT Cytarabine (ARAC) (all patients)	Intrathecal (IT)	Age-based dosing: **Age (yrs) Dose** < 1 20 mg 1-1.99 30 mg 2-2.99 50 mg ≥ 3 70 mg	1	Subsequent doses and timing may be administered at the discretion of the treating physician.
Flotetuzumab	Intravenous (IV)	At Cycle 1 target dose	1-28	See Section 5.1 for premedications recommendations. See Section 6 for dose modifications for adverse events.

NOTE: If a treatment-free interval > 3 days occurs between any 2 cycles, the subsequent cycle will require step-up dosing. If a treatment-free interval > 3 days occurs between any 2 cycles, the Study Chair must be notified and the subsequent cycle dosing schedule must be discussed.

Ht _____cm **Wt _____kg** **BSA _____m^2** **Dose Level _____nanograms/kg/day**

Date Due	Date Given	Day	IT ARAC _____ mg	Flotetuzumab at Cycle 1 Target Dose nanograms/kg/day	Studies	
			Enter calculated dose above and actual dose administered below			
		Pre-			a, b, d – g, i – k, m	
		1	_____ mg	_____ ng	l, o	
		2		_____ ng		
		3		_____ ng		
		4		_____ ng		c
		5		_____ ng		
		6		_____ ng		
		7		_____ ng		
		8		_____ ng		
		9		_____ ng		
		10		_____ ng		
		11		_____ ng		c
		12		_____ ng		
		13		_____ ng		
		14		_____ ng		
		15		_____ ng		
		16		_____ ng		
		17		_____ ng		
		18		_____ ng		c
		19		_____ ng		
		20		_____ ng		
		21		_____ ng		
		22		_____ ng		c
		23		_____ ng		

		24		_____ ng		
		25		_____ ng		
		26		_____ ng		
		27		_____ ng		
		28		_____ ng		
		29			h, n, p, q, r	

Required Observations for Cycles 2+

a. History. Prior to subsequent cycles.
b. Physical exam (including VS). Prior to subsequent cycles.
c. CBC/diff/platelets. Weekly. If patients develop Grade 4 neutropenia then CBCs should be checked at least every 3 to 4 days until recovery to Grade 3 or until meeting the criteria for dose limiting toxicity.
d. Ht/Wt/BSA. Prior to subsequent cycles.
e. Electrolytes including Ca^{++}. Prior to subsequent cycles.
f. Creatinine, ALT, bilirubin, and AST. Prior to subsequent cycles.
g. Albumin. Prior to subsequent cycles.
h. Imaging (CT or MRI) of chloroma. Only to be performed if clinically indicated. At the end of every cycle (if present on previous study).
i. ECHO or gated radionuclide study. Prior to subsequent cycles.
j. EKG. Prior to subsequent cycles.
k. Neurologic exam. Prior to subsequent cycles.
l. CD3 T-lymphocyte quantification. In consenting patients, samples to be collected on Day 1 of Cycles 2+. See Section 8.6 for details.
m. Cytokine production measurement. If patients experience IRR/CRS, blood samples are to be collected from consenting patients at the time of IRR/CRS and at the time of IRR/CRS resolution (if applicable). See Section 8.7 for details.
n. Bone marrow aspirate. At the end of every cycle.
o. Immunogenicity study. Samples to be collected from all patients on Cycle 3, Day 1 (pre-infusion), and on Cycle 5, Day 1 (pre-infusion). See Section 8.3 for details.
p. Pharmacokinetics. Blood samples will be collected 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after the end of infusion on the patient's last day of

protocol therapy. See Section 8.2 for details.

q. NGS MRD comparison with flow cytometry MRD. In consenting patients only at the time of bone marrow evaluation. See Section 8.5 for details.

r. Flow cytometry with MRD determination. At the end of each cycle with bone marrow evaluation.

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

APPENDIX VI-A: PHARMACOKINETIC STUDY FORM FOR PATIENTS ON DL1

COG Pt ID # _____ Cycle 1, Day 1 Date: _____

Please do not write patient names on this form or on samples.

Patient Weight: _____kg

Flotetuzumab Dose Level: _____nanograms/kg/day Flotetuzumab Total Daily Dose: _____nanograms

Blood samples (2 – 3 ml) will be collected in SST tubes at the following time points during Cycle 1 (± 10 min) for patients that do NOT require dose interruptions: Day 1 (prior to infusion), Days 3 – 7 (30 minutes prior to syringe change and dose escalation), Day 8 (24 hours after Day 7 syringe change and final dose escalation) and on Days 12, 15, 19, 22, 27, and 29 (at any time). For patients that require dose interruptions during Cycle 1, PK samples should be drawn according to when the patient reaches certain dose levels (see chart below). **NOTE:** Patients ≤ 23 kg should only have 2 ml of blood drawn per sample.

Additional blood samples will also be collected at the following time points on the patient's last day of protocol therapy (± 10 min): 30 minutes, 1 hour, 2, hours, 4 hours, 8 hours, and 24 hours after the end of infusion.

Record the exact time the samples are drawn during Cycle 1, as well as the exact time infusion begins and ends on Days 1 and 29 (respectively), and the exact time of syringe change on days in which PK samples are collected. Also record the exact time the sample is drawn on the patient's last day of protocol therapy, the exact time infusion ends on the patient's last day of protocol therapy, the patient's last cycle number, and the day in the 29 day cycle in which the patient stopped protocol therapy.

Cycle 1					
Blood Sample No.	**Planned Day of Collection***	**Actual Day of Collection**	**Scheduled Collection Time**	**Actual Date Sample Collected**	**Actual Time Sample Collected (24-hr clock)**
1	**Day 1**	**Day 1**	Prior to flotetuzumab infusion	___/___/___	____ : ____
Flotetuzumab Infusion on Day 1			**Date:** ___/___/___ **Infusion Start Time:** ____ : ____		
2	**Day 3**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	____ : ____
Syringe Change from 60 to 100 ng/kg/day			**Date:** ___/___/___ **Time of Syringe Change:** ____ : ____		
3	**Day 4**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	____ : ____
Syringe Change from 100 to 200 ng/kg/day			**Date:** ___/___/___ **Time of Syringe Change:** ____ : ____		
4	**Day 5**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	____ : ____
Syringe Change from 200 to 300 ng/kg/day			**Date:** ___/___/___ **Time of Syringe Change:** ____ : ____		
5	**Day 6**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	____ : ____
Syringe Change from 300 to 400 ng/kg/day			**Date:** ___/___/___ **Time of Syringe Change:** ____ : ____		
6	**Day 7**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	____ : ____
Syringe Change from 400 to 500 ng/kg/day			**Date:** ___/___/___ **Time of Syringe Change:** ____ : ____		
7	**Day 8**	**Day 8**	24 hrs after syringe change for final dose escalation	___/___/___	____ : ____
8	**Day 12**	**Day 12**	Any time	___/___/___	____ : ____
9	**Day 15**	**Day 15**	Any time	___/___/___	____ : ____
10	**Day 19**	**Day 19**	Any time	___/___/___	____ : ____
11	**Day 22**	**Day 22**	Any time	___/___/___	____ : ____
12	**Day 27**	**Day 27**	Any time	___/___/___	____ : ____

13	**Day 29**	**Day 29**	Any time	___/___/___	____ : ____
Flotetuzumab Infusion on Day 29 Date:___/___/___ Infusion End Time: ____ : ____					

*Assuming patient follows the dosing schedule in Section 5.1 and requires no dose interruptions.

Signature: _____ Date:_____
(site personnel who collected samples)

Cycle #: _____				
Blood Sample No.	**Time Point**	**Scheduled Collection Time**	**Actual Date Sample Collected**	**Actual Time Sample Collected (24-hr clock)**
Flotetuzumab Infusion on Patient's Last Day of Therapy Date:___/___/___			**Infusion End Time:**	____ : ____
14	**Day __**	30 mins after end of infusion	___/___/___	____ : ____
15	**Day __**	1 hr after end of infusion	___/___/___	____ : ____
16	**Day __**	2 hrs after end of infusion	___/___/___	____ : ____
17	**Day __**	4 hrs after end of infusion	___/___/___	____ : ____
18	**Day __**	8 hrs after end of infusion	___/___/___	____ : ____
19	**Day __**	24 hrs after end of infusion	___/___/___	____ : ____

One copy of this Pharmacokinetic Study Form should be uploaded into RAVE. A second copy should be sent with the samples to the address listed in Section 8.2.6. See Section 8.2 for detailed guidelines for packaging and shipping PK samples.

Signature: _____ Date:_____
(site personnel who collected samples)

APPENDIX VI-B: PHARMACOKINETIC STUDY FORM FOR PATIENTS ON DL-1

COG Pt ID # _____ Cycle 1, Day 1 Date: _____

Please do not write patient names on this form or on samples.

Patient Weight: _____kg

Flotetuzumab Dose Level: _____nanograms/kg/day Flotetuzumab Total Daily Dose: _____nanograms

Blood samples (2 – 3 ml) will be collected in SST tubes at the following time points during Cycle 1 (± 10 min) for patients that do NOT require dose interruptions: Day 1 (prior to infusion), Days 4, 6, and 8 (30 minutes prior to syringe change and dose escalation), Day 9 (24 hours after Day 8 syringe change and final dose escalation) and on Days 13, 17, 21, 25, and 29 (at any time). For patents that require dose interruptions during Cycle 1, PK samples should be drawn according to when the patient reaches certain dose levels (see chart below). **NOTE:** Patients ≤ 23 kg should only have 2 ml of blood drawn per sample.

Additional blood samples will also be collected at the following time points on the patient's last day of protocol therapy (± 10 min): 30 minutes, 1 hour, 2, hours, 4 hours, 8 hours, and 24 hours after the end of infusion.

Record the exact time the sample are drawn during Cycle 1, as well as the exact time infusion begins and ends on Days 1 and 29 (respectively), and the exact time of syringe change on days in which PK samples are collected. Also record the exact time the sample is drawn on the patient's last day of protocol therapy, the exact time infusion ends on the patient's last day of protocol therapy, the patient's last cycle number, and the day in the 29 day cycle in which the patient stopped protocol therapy.

Cycle 1					
Blood Sample No.	**Planned Day of Collection***	**Actual Day of Collection**	**Scheduled Collection Time**	**Actual Date Sample Collected**	**Actual Time Sample Collected (24-hr clock)**
1	**Day 1**	**Day 1**	Prior to flotetuzumab infusion	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Day 1 Date:___/___/___ Infusion Start Time: |__|__| : |__|__|					
2	**Day 4**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	|__|__| : |__|__|
Syringe Change from 60 to 100 ng/kg/day Date:___/___/___ Time of Syringe Change: |__|__| : |__|__|					
3	**Day 6**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	|__|__| : |__|__|
Syringe Change from 100 to 200 ng/kg/day Date:___/___/___ Time of Syringe Change: |__|__| : |__|__|					
4	**Day 8**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	|__|__| : |__|__|
Syringe Change 200 to 300 ng/kg/day Date:___/___/___ Time of Syringe Change: |__|__| : |__|__|					
5	**Day 9**	**Day 9**	24 hrs after Day 8 syringe change and final dose escalation	___/___/___	|__|__| : |__|__|
6	**Day 13**	**Day 13**	Any time	___/___/___	|__|__| : |__|__|
7	**Day 17**	**Day 17**	Any time	___/___/___	|__|__| : |__|__|
8	**Day 21**	**Day 21**	Any time	___/___/___	|__|__| : |__|__|
9	**Day 25**	**Day 25**	Any time	___/___/___	|__|__| : |__|__|
10	**Day 29**	**Day 29**	Any time	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Day 29 Date:___/___/___ Infusion End Time: |__|__| : |__|__|					

*Assuming patient follows the dosing schedule in Section 5.1 and requires no dose interruptions.

(continued next page)

Signature: _____ Date:_____
(site personnel who collected samples)

Cycle #: _____				
Blood Sample No.	**Time Point**	**Scheduled Collection Time**	**Actual Date Sample Collected**	**Actual Time Sample Collected (24-hr clock)**
Flotetuzumab Infusion on Patient's Last Day of Therapy		**Date:___/___/___**	**Infusion End Time:** |__|__| : |__|__|	
11	**Day __**	30 mins after end of infusion	___/___/___	|__|__| : |__|__|
12	**Day __**	1 hr after end of infusion	___/___/___	|__|__| : |__|__|
13	**Day __**	2 hrs after end of infusion	___/___/___	|__|__| : |__|__|
14	**Day __**	4 hrs after end of infusion	___/___/___	|__|__| : |__|__|
15	**Day __**	8 hrs after end of infusion	___/___/___	|__|__| : |__|__|
16	**Day __**	24 hrs after end of infusion	___/___/___	|__|__| : |__|__|

One copy of this Pharmacokinetic Study Form should be uploaded into RAVE. A second copy should be sent with the samples to the address listed in Section 8.2.6. See Section 8.2 for detailed guidelines for packaging and shipping PK samples.

Signature: _____ Date:_____
(site personnel who collected samples)

APPENDIX VI-C: PHARMACOKINETIC STUDY FORM FOR PATIENTS ON DL2

COG Pt ID # _____ Cycle 1, Day 1 Date: _____

Please do not write patient names on this form or on samples.

Patient Weight: _____kg

Flotetuzumab Dose Level: _____nanograms/kg/day Flotetuzumab Total Daily Dose: _____nanograms

Blood samples (2 – 3 ml) will be collected in SST tubes at the following time points during Cycle 1 (± 10 min) for patients that do NOT require dose interruptions: Day 1 (prior to infusion), Days 3 – 8 (30 minutes prior to syringe change and dose escalation), Day 9 (24 hours after Day 7 syringe change and final dose escalation) and on Days 13, 17, 21, 25, and 29 (at any time). For patients that require dose interruptions during Cycle 1, PK samples should be drawn according to when the patient reaches certain dose levels (see chart below). **NOTE:** Patients ≤ 23 kg should only have 2 ml of blood drawn per sample.

Additional blood samples will also be collected at the following time points on the patient's last day of protocol therapy (± 10 min): 30 minutes, 1 hour, 2, hours, 4 hours, 8 hours, and 24 hours after the end of infusion.

Record the exact time the samples are drawn during Cycle 1, as well as the exact time infusion begins and ends on Days 1 and 29 (respectively), and the exact time of syringe change on days in which PK samples are collected. Also record the exact time the sample is drawn on the patient's last day of protocol therapy, the exact time infusion ends on the patient's last day of protocol therapy, the patient's last cycle number, and the day in the 29 day cycle in which the patient stopped protocol therapy.

Cycle 1					
Blood Sample No.	**Planned Day of Collection**	**Actual Day of Collection**	**Scheduled Collection Time**	**Actual Date Sample Collected**	**Actual Time Sample Collected (24-hr clock)**
1	**Day 1**	**Day 1**	Prior to flotetuzumab infusion	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Day 1 Date:___/___/___ Infusion Start Time: |__|__| : |__|__|					
2	**Day 3**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	|__|__| : |__|__|
Syringe Change from 100 to 200 ng/kg/day Date:___/___/___ Time of Syringe Change: |__|__| : |__|__|					
3	**Day 4**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	|__|__| : |__|__|
Syringe Change from 200 to 300 ng/kg/day Date:___/___/___ Time of Syringe Change: |__|__| : |__|__|					
4	**Day 5**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	|__|__| : |__|__|
Syringe Change from 300 to 400 ng/kg/day Date:___/___/___ Time of Syringe Change: |__|__| : |__|__|					
5	**Day 6**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	|__|__| : |__|__|
Syringe Change from 400 to 500 ng/kg/day Date:___/___/___ Time of Syringe Change: |__|__| : |__|__|					
6	**Day 7**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	|__|__| : |__|__|
Syringe Change from 600 to 600 ng/kg/day Date:___/___/___ Time of Syringe Change: |__|__| : |__|__|					
7	**Day 8**	**Day __**	30 mins prior to syringe change and dose escalation	___/___/___	|__|__| : |__|__|
Syringe Change from 700 to 800 ng/kg/day Date:___/___/___ Time of Syringe Change: |__|__| : |__|__|					
8	**Day 9**	**Day 9**	24 hrs after Day 8 syringe change and final dose escalation	___/___/___	|__|__| : |__|__|
9	**Day 13**	**Day 13**	Any time	___/___/___	|__|__| : |__|__|
10	**Day 17**	**Day 17**	Any time	___/___/___	|__|__| : |__|__|
11	**Day 21**	**Day 21**	Any time	___/___/___	|__|__| : |__|__|
12	**Day 25**	**Day 25**	Any time	___/___/___	|__|__| : |__|__|

13	**Day 29**	**Day 29**	Any time	___/___/___	____ : ____
Flotetuzumab Infusion on Day 29 Date:___/___/___ Infusion End Time: ____ : ____					

*Assuming patient follows the dosing schedule in Section 5.1 and requires no dose interruptions.

Signature: _____ Date:_____
(site personnel who collected samples)

Cycle #: _____				
Blood Sample No.	**Time Point**	**Scheduled Collection Time**	**Actual Date Sample Collected**	**Actual Time Sample Collected (24-hr clock)**
Flotetuzumab Infusion on Patient's Last Day of Therapy Date:___/___/___ Infusion End Time: ____ : ____				
14	**Day __**	30 mins after end of infusion	___/___/___	____ : ____
15	**Day __**	1 hr after end of infusion	___/___/___	____ : ____
16	**Day __**	2 hrs after end of infusion	___/___/___	____ : ____
17	**Day__**	4 hrs after end of infusion	___/___/___	____ : ____
18	**Day __**	8 hrs after end of infusion	___/___/___	____ : ____
19	**Day __**	24 hrs after end of infusion	___/___/___	____ : ____

One copy of this Pharmacokinetic Study Form should be uploaded into RAVE. A second copy should be sent with the samples to the address listed in Section 8.2.6. See Section 8.2 for detailed guidelines for packaging and shipping PK samples.

Signature: _____ Date:_____
(site personnel who collected samples)

APPENDIX VII: IMMUNOGENICITY STUDY FORM

COG Pt ID # _____ Cycle 1, Day 1 Date: _____
Please do not write patient names on this form or on samples.

Patient Weight: _____kg

Flotetuzumab Dose Level: _____nanograms/kg/day Flotetuzumab Total Daily Dose: _____nanograms

Blood samples (2 – 3 mL) will be collected from all patients at the following time points: Cycle 1, Day 1 (pre-infusion), Cycle 3, Day 1 (pre-infusion), and Cycle 5, Day 1 (pre-infusion). **NOTE:** Patients ≤ 23 kg should only have 2 mL of blood drawn per sample.

Record the exact time the sample is drawn along with the exact time flotetuzumab infusion begins on Day 1 of Cycles 1, 3, and 5.

Blood Sample No.	Cycle Number	Scheduled Collection Time	Actual Date Sample Collected	Actual Time Sample Collected (24-hr clock)
1	1	Prior to flotetuzumab infusion	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Cycle 1, Day 1 Date:___/___/___ Infusion Start Time: |__|__| : |__|__|				
2	3	Prior to flotetuzumab infusion	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Cycle 3, Day 1 Date:___/___/___ Infusion Start Time: |__|__| : |__|__|				
3	5	Prior to flotetuzumab infusion	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Cycle 5, Day 1 Date:___/___/___ Infusion Start Time: |__|__| : |__|__|				

One copy of this Immunogenicity Study Form should be uploaded into RAVE. A second copy should be sent with the samples to the address listed in Section 8.3.5. See Section 8.3 for detailed guidelines for packaging and shipping immunogenicity samples.

Signature: _____ Date:_____
(site personnel who collected samples)

APPENDIX VIII: BASELINE CD123 SITE DENSITY QUANTIFICATION STUDY FORM

COG Pt ID # _____ Cycle 1, Day 1 Date: _____

Please do not write patient names on this form or on samples.

Patient Weight: _____kg

Flotetuzumab Dose Level: _____nanograms/kg/day Flotetuzumab Total Daily Dose: _____nanograms

Bone marrow samples (3 – 5 ml) will be collected from consenting patients prior to Cycle 1.

Record the exact time the bone marrow sample is collected.

Bone Marrow Sample No.	Scheduled Collection Time	Actual Date Sample Collected	Actual Time Sample Collected (24-hr clock)
1	Prior to Cycle 1	___/___/___	|__|__| : |__|__|

One copy of this Baseline CD123 Site Density Quantification Study Form should be uploaded into RAVE. A second copy should be sent with the samples to the address listed in Section 8.4.5. See Section 8.4 for detailed guidelines for packaging and shipping Baseline CD123 Site Density Quantification samples.

Signature: _____ Date:_____
(site personnel who collected samples)

APPENDIX IX: NGS MRD COMPARISON WITH FLOW CYTOMETRY MRD STUDY FORM

COG Pt ID # _____ Cycle 1, Day 1 Date: _____

Please do not write patient names on this form or on samples.

Patient Weight: _____kg

Flotetuzumab Dose Level: _____nanograms/kg/day Flotetuzumab Total Daily Dose: _____nanograms

Bone marrow samples (10 – 15 ml) will be collected from consenting patients at the time of each bone marrow evaluation.

Record the exact time the bone marrow sample is collected at the end of each cycle.

Bone Marrow Sample No.	Scheduled Collection Time	Actual Date Sample Collected	Actual Time Sample Collected (24-hr clock)
1	Prior to Cycle 1	___/___/___	__|__| : |__|__|
2	At the end of Cycle 1	___/___/___	__|__| : |__|__|
3	At the end of Cycle 2	___/___/___	__|__| : |__|__|
4	At the end of Cycle 3	___/___/___	__|__| : |__|__|
5	At the end of Cycle 4	___/___/___	__|__| : |__|__|
6	At the end of Cycle 5	___/___/___	__|__| : |__|__|
7	At the end of Cycle 6	___/___/___	__|__| : |__|__|

One copy of this NGS MRD Comparison with Flow Cytometry MRD Study Form should be uploaded into RAVE. A second copy should be sent with the samples to the address listed in Section 8.5.6. See Section 8.5 for detailed guidelines for packaging and shipping NGS MRD Comparison with Flow Cytometry MRD samples.

Signature: _____ Date:_____
(site personnel who collected samples)

APPENDIX X: CD3 T-LYMPHOCYTE QUANTIFICATION STUDY FORM

COG Pt ID # _____ Cycle 1, Day 1 Date: _____

Please do not write patient names on this form or on samples.

Patient Weight: _____kg

Flotetuzumab Dose Level: _____nanograms/kg/day Flotetuzumab Total Daily Dose: _____nanograms

Blood samples (2 – 3 ml) will be collected from consenting patients at the following time points during Cycle 1: pre-infusion on Day 1, and on Days 8, 15, 22, and 29 (prior to the end of infusion). For subsequent cycles, blood samples (2 – 3 ml) will be collected on Day 1 prior to infusion. **NOTE:** Patients ≤ 23 kg should only have 2 ml of blood drawn per sample.

Record the exact time the sample is drawn along with the exact time flotetuzumab infusion begins on Day 1 of each cycle. Quantification results will be collected in RAVE per the guidance in the CRF packet.

Cycle 1			
Blood Sample No.	**Scheduled Collection Time**	**Actual Date Sample Collected**	**Actual Time Sample Collected (24-hr clock)**
1	Day 1, pre-infusion	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Day 1 Date: ___/___/___ **Infusion Start Time:** |__|__| : |__|__|			
2	Day 8	___/___/___	|__|__| : |__|__|
3	Day 15	___/___/___	|__|__| : |__|__|
4	Day 22	___/___/___	|__|__| : |__|__|
5	Day 29	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Day 29 Date: ___/___/___ **Infusion End Time:** |__|__| : |__|__|			

Cycles 2+				
Blood Sample No.	**Cycle Number**	**Scheduled Collection Time**	**Actual Date Sample Collected**	**Actual Time Sample Collected (24-hr clock)**
1	**Cycle #** _____	Prior to flotetuzumab infusion	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Day 1 Date: ___/___/___ **Infusion Start Time:** |__|__| : |__|__|				

One copy of this CD3 T-Lymphocyte Quantification Study Form should be uploaded into RAVE.

Signature: _____ Date:_____
(site personnel who collected samples)

APPENDIX XI: CYTOKINE ANALYSIS

COG Pt ID # _____ Cycle 1, Day 1 Date: _____

Please do not write patient names on this form or on samples.

Patient Weight: _____kg

Flotetuzumab Dose Level: _____nanograms/kg/day Flotetuzumab Total Daily Dose: _____nanograms

Blood samples (2 – 3 ml) will be collected from consenting patients at the following time points: prior to Cycle 1, Cycle 1 Day 8, at the time of IRR/CRS (if applicable), and at the time of IRR/CRS resolution (if applicable). **NOTE:** Patients ≤ 23 kg should only have 2 ml of blood drawn per sample.

Record the exact time the sample is drawn along with the exact time flotetuzumab infusion begins on Day 1 of Cycle 1.

Blood Sample No.	Cycle Number	Scheduled Collection Time	Actual Date Sample Collected	Actual Time Sample Collected (24-hr clock)
1	1	Day 1, pre-infusion	___/___/___	|__|__| : |__|__|
Flotetuzumab Infusion on Day 1 Date:___/___/___ Infusion Start Time: |__|__| : |__|__|				
2	1	Day 8	___/___/___	|__|__| : |__|__|
3	Cycle #___	At the time of IRR/CRS[1]	___/___/___	|__|__| : |__|__|
4	Cycle #___	At the time of IRR/CRS resolution[1]	___/___/___	|__|__| : |__|__|

[1]Only applicable for patients who experience IRR/CRS

One copy of this Cytokine Production Measurement Study Form should be uploaded into RAVE. A second copy should be sent with the samples to the address listed in Section 8.7.6. See Section 8.7 for detailed guidelines for packaging and shipping Cytokine Production Measurement samples.

Signature: _____ Date:_____
(site personnel who collected samples)

APPENDIX XII: TUMOR MICROENVIRONMENT STUDY FORM

COG Pt ID # _____ Cycle 1, Day 1 Date: _____

Please do not write patient names on this form or on samples.

Patient Weight: _____kg

Flotetuzumab Dose Level: _____nanograms/kg/day Flotetuzumab Total Daily Dose: _____nanograms

Bone marrow samples (10 – 15 mL) and paraffin-embedded tissue blocks will be collected from consenting patients at the following time point: prior to Cycle 1.

Record the exact time the bone marrow sample is collected.

Blood Sample No.	Cycle Number	Scheduled Collection Time	Actual Date Sample Collected	Actual Time Sample Collected (24-hr clock)
1	Pre-	Prior to Cycle 1	___/___/___	|__|__| : |__|__|

One copy of this Tumor Microenvironment Study Form should be uploaded into RAVE. A second copy should be sent with the samples to the address listed in Section 8.8.6. See Section 8.8 for detailed guidelines for packaging and shipping Tumor Microenvironment study samples.

Signature: _____ Date:_____
(site personnel who collected samples)

APPENDIX XIII: CTEP AND CTSU REGISTRATION PROCEDURES

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN, RAVE, or TRIAD or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

Additional information can be found on the CTEP website at < https://ctep.cancer.gov/investigatorResources/default.htm >. For questions, please contact the RCR ***Help Desk*** by email at < RCRHelpDesk@nih.gov >.

CTSU Registration Procedures

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

IRB Approval:
Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

Requirements For PEPN1812 Site Registration:

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)
- For applicable studies with a radiation and/or imaging (RTI) component, the enrolling site must be aligned to a RTI provider. To manage provider associations access the Provider Association tab on the CTSU website at https://www.ctsu.org/RSS/RTFProviderAssociation, to add or remove associated providers. Sites must be linked to at least one IROC credentialed provider to participate on trials with an RT component. Enrolling sites are responsible for ensuring that the appropriate agreements are in place with their RTI provider, and that appropriate IRB approvals are in place.

Submitting Regulatory Documents:
Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission
When applicable, original documents should be mailed to:
CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103

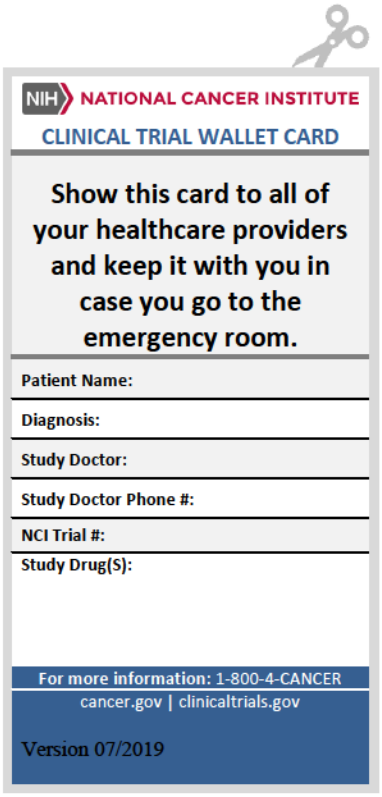
Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

APPENDIX XIV: REQUIREMENTS FOR POTENTIAL SATELLITE PHARMACY SITES

When the registering/treating institution is considering use of a home health care agency as a satellite pharmacy, the following must be assessed by the registering/treating institution in relation to the suitability of the home health care agency:

- Ability to appropriately store (temperature and security) the intact agent vials and/or prepared infusion bags.
- Ability to provide documentation of controlled and monitored temperature storage conditions while the IND agent is in the local infusion center or home health care agency possession.
- Availability of appropriately trained staff to prepare doses in compliance with the protocol in an ISO Class 5 containment device (ideally in an ISO Class 7 room as described in USP), to label infusion bags according to the protocol instructions and to store agent doses under appropriate controlled temperature conditions.
- For home health care agency services, the ability to transport each prepared dose individually to the subject's home under appropriate controlled storage conditions or the ability to assess and confirm that cold-chain management of prepared infusion bags shipped to the subject's home is maintained prior to administration.
- Availability of appropriately trained staff to administer the prepared doses and perform the infusion bag changes according to the protocol.
- Methods for proper disposal of the waste, empty vials, IV bags, etc. are in place.
- Plan for return of unused intact vials to the registering/treating institution is in place.
- Source documentation to confirm agent administration must be maintained by the local infusion center or home health care agency and must be provided to the registering/treating institution for incorporation into the patient's medical/research records and for audit purposes.
- Plan for handling missed doses is in place
- Agent accountability must be maintained via use of the NCI Drug Accountability Record Form (DARF). The originating site must keep a Control DARF and the local infusion center or home health care agency would be required to maintain a Satellite DARF if receiving and storing supplies of intact vials or receiving and storing infusion bags prepared by the registering/treating institution. Maintenance of a Satellite DARF is not required by home health care agency staff for prepared infusions bags shipped to the subject's home.
- The DARF must be provided to the registering/treating institution for record keeping purposes and audits.
- Documentation of IRB coverage for the protocol must be maintained. The IRB of record for the site must be informed that the study subject may receive therapy administered by a non-research site (i.e., the local infusion center or home health care agency).

APPENDIX XV: PATIENT CLINICAL TRIAL WALLET CARD



NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Patient Name:

Diagnosis:

Study Doctor:

Study Doctor Phone #:

NCI Trial #:

Study Drug(S):

For more information: 1-800-4-CANCER

cancer.gov | clinicaltrials.gov

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