CONFIDENTIAL

CLINICAL TRIAL PROTOCOL

Phase I/II study of humanized 3F8 bispecific antibody (Hu3F8 BsAb) in patients with relapsed/refractory neuroblastoma, osteosarcoma, and other GD2(+) solid tumors

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Approval of Clinical Trial Protocol

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1 PROTOCOL SUMMARY AND/OR SCHEMA

This phase I/II trial will assess the toxicity and pharmacokinetics (PK) of the humanized anti-GD2 x anti-CD3 bispecific antibody (hu3F8-BsAb) in phase I and the anti-tumor activity of hu3F8-BsAb in phase II. In phase I, dosages of hu3F8-BsAb follow a dose-finding design in order to determine the maximum tolerated dose (MTD) in cohorts of patients with relapsed/refractory neuroblastoma (NB), osteosarcoma, or other GD2(+) solid tumors. The recommended phase II dose (RP2D) will be established. Hu3F8-BsAb PK will be also evaluated. In phase II, patients with relapsed/refractory NB (Group 1) and osteosarcoma (Group 2) will be treated with hu3F8-BsAb at the RP2D and anti-tumor activity will be assessed.

2 OBJECTIVES AND SCIENTIFIC AIMS

2.1 Phase I

Primary Objective:

- To establish the safety of hu3F8-BsAb, and determine the maximum tolerated dose (MTD) and the recommended phase II dose (RP2D).

Secondary Objectives:

- To study the pharmacokinetics of hu3F8-BsAb.
- To evaluate human anti-human antibody (HAHA).
- Assess the anti-tumor activity and overall response rates of hu3F8-BsAb in patients with relapsed or refractory NB, osteosarcoma and other GD2(+) tumors.

Exploratory Objectives:

- To study immunological effects of hu3F8-BsAb
- To study the effects of hu3F8-BsAb on bone marrow (BM) RNA
- To study the effects of stool microbiome of the activity of hu3F8-BsAb.

2.2 Phase II

Primary Objectives:

- In Group 1: Assess the overall response rates of hu3F8-BsAb in patients with relapsed or refractory NB.

In Group 2: Assess the progression free survival (PFS) in patients with relapsed or refractory osteosarcoma at 4 months from the first hu3F8-BsAb treatment.

Secondary Objectives:

- To evaluate the duration of complete remission in groups 1 and 2.
- In Group 2: Assess the overall response rates of hu3F8-BsAb in patients with relapsed or refractory osteosarcoma.
- To continue to assess the safety/toxicity of hu3F8-BsAb.
- To evaluate HAHA.

Exploratory Objectives:

- To assess overall survival (OS) and PFS after treatment with hu3F8-BsAb.
- To study immunological effects of hu3F8-BsAb
- To study the effects of hu3F8-BsAb on BM DNA and RNA (Neuroblastoma patients only).
- To study the effects of stool microbiome of the activity of hu3F8-BsAb.

3 BACKGROUND AND RATIONALE

3.1 Disease background

NB is the most common extracranial solid tumor of childhood; approximately 700 children are diagnosed each year in the United States. 50-60% of patients present with an unresectable primary tumor and metastasis in BM.[1] Intensive chemotherapy with or without myeloablative therapy, surgery, radiation, and most recently, anti-GD2 immunotherapy have improved event free survival rates in patients with metastatic disease diagnosed >18months of age to approximately 40%.[2-4] Somatic MYCN amplification is associated with a poorer prognosis. In addition, treatment for relapsed NB remains even more inadequate. A recent review of outcomes after relapse from the International Neuroblastoma Risk Group Project found that 5 year post-relapse overall survival for stage 4 NB was only 8%. Stage 4 patients with MYCN amplification did worse, with a 5-year post-relapse overall survival (PRS) of only 4%. Thus, a search for more effective therapies for stage 4 patients and relapsed patients is warranted.[5-7]

Osteosarcoma is a primary bone malignancy that occurs predominantly in children and adolescents with about 400 newly diagnoses each year in the United States. Patients with localized disease have 65-70 % long-term relapse free survival with systemic chemotherapy (combination methotrexate, doxorubicin, and cisplatin [MAP]) and aggressive surgical resection,[8] whereas >80% of patients with detectable metastases at diagnosis have relapse after contemporary therapy.[9] When osteosarcoma recurs, the outcome is dismal. Complete surgical remission is one of the important favorable prognostic factors in recurrent osteosarcoma; 5-year PRS of patients who had complete resection of recurrence was 39% whereas and 3-year PRS of those who did not have complete surgery was 0%.[10-13] Multiple clinical trials of novel agents including ixabepilone, oxaliplatin, trasutuzumab, IGF-1R inhibitors, and mTOR inhibitors have been conducted but no improvement of survival was achieved.[14-18] A recent

retrospective analysis of seven phase II trials conducted by Children's Oncology Group (COG) and its predecessor groups showed that PFS at 4 months from time of enrollment in patients with relapsed measurable disease was 12%.[19]

Currently, the limits of treatment intensity have been reached in up-front therapies in NB and osteosarcoma, leaving limited options for cytotoxic therapies upon relapse; antibody based immunotherapy is therefore particularly appealing in this heavily pre-treated population given its lack of significant acute hematologic toxicity and absence of long term side effects.

3.2 Rationale for anti-GD2 antibody

3.2.1 GD2 as an ideal target for antibody-based therapy in NB and other solid tumors

GD2 is an adhesion molecule abundant on NB and other solid tumors. Specifically, GD2 is expressed by 100% of NB,[20] 88% of osteosarcomas,[21] >50% of melanomas,[22] 70% of desmoplastic small round cell tumors (DSRCT)[23], and 93% of soft tissue sarcomas including liposarcoma, fibrosarcoma, malignant fibrous histiocytoma, leiomyosarcoma, and spindle cell sarcoma,[24] as well as brain tumors.[25] It is rarely expressed in normal tissues except neurons and skin cells. This antigen is genetically stable and relatively inert on the cell membrane. GD2 expression is rarely lost, even after relapse in patients previously treated with anti-GD2 therapy.[26,27]

3.2.2 Experience with 3F8: anti-GD2 murine monoclonal antibody in high risk NB

[redacted]

[redacted]

3.2.3 Experience with humanized 3F8 (hu3F8) in relapsed/refractory HR NB

[redacted]

[redacted]

3.3 Rationale for anti-GD2 bispecific antibody

3.3.1 Bispecific antibody to engage T cells

[redacted]

[redacted]

3.3.2 Humanized 3F8 bispecific antibody

[redacted]

[redacted]

[redacted]

3.4 Pre-clinical studies of hu3F8-BsAb

[redacted]

3.4.1 In vitro cytotoxicity

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

3.4.2 Anti-tumor effect in mouse xenograft models

[redacted]

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[redacted]

3.4.3 Pre-clinical toxicology

[redacted]

[redacted]

3.4.4 Pre-clinical pharmacokinetics (PK)

[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]

3.5 Rationale for phase I study of hu3F8-BsAb

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3.6 Significance and implications

[redacted]

[redacted] OVERVIEW OF STUDY DESIGN/INTERVENTION

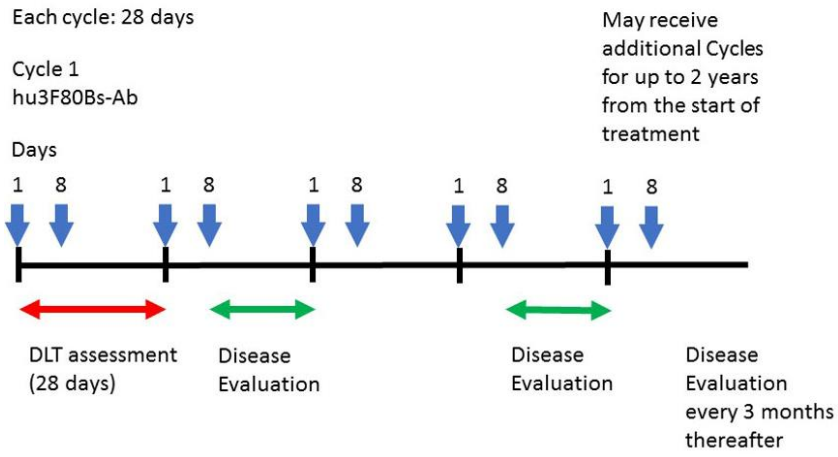
4.1 Design

4.1.1 Phase I

Initially the phase I study will follow a dose-escalation escalation schema with 1 patient assigned per dose until the first ≥Grade 2 toxicity (except for Grade 2 sinus bradycardia, sinus tachycardia, urticaria, fever, nausea, vomiting, diarrhea, urticaria, headache, paresthesia, electrolyte disturbances, pain) is observed, then the subsequent dose allocation will follow the Continual Reassessment Method (CRM).[75,76]a. Eligible patients will include relapsed or refractory NB, osteosarcoma, and GD2 (+) solid tumors. In each cycle, hu3F8-BsAb is given on Days 1 and 8. PK is assessed by blood draws. Patients are monitored for DLT until Day 28. Patients with DLT will come off treatment. HAHA titer will be measured after each cycle. Extent of disease evaluation will be performed after the second dose of cycle 2 and cycle 4, then every 3 months thereafter. A total of 4 cycles will be given. If patients experience no life-threatening toxicity clearly attributable to hu3F8-BsAb, do not develop progression of disease (PD) and continue to meet all eligibility criteria, they have the option of receiving additional cycles up to 2 years from the start of hu3F8-BsAb treatment. Patients may continue therapy beyond 4 cycles even if they achieve remission, see study design Figure 6.

The duration of each subsequent cycle is 28 days with hu3F8-BsAb being given on Days 1 and 8. MTD is defined as a maximum dose level at which ≤ 1 of 6 patients experiencing DLT. The RP2D will be decided based on the safety data in the phase I and can be MTD or lower. If MTD is not reached, RP2D may be determined on the basis of PK studies. If no DLT is observed, the RP2D will be determined as a minimum dose that gives the highest hu3F8-BsAb serum levels based on the PK data. For example, the RP2D of hu3F8 monoclonal antibody was decided as 9 mg/kg/cycle because the serum hu3F8 levels reached a plateau beyond 8.4 mg/kg/cycle with no DLT (see Section 3.2.3). After cycle 2, intra-patient dose escalation will be permitted i.e. patients can receive the highest dose that has been evaluated and proven safe by DLT assessment.

Figure 6 Study design



4.1.2 Phase II

After RP2D is determined, a phase II study will be carried out to assess the anti-tumor activity of hu3F8-BsAb. Two groups of patients will be studied: Group 1 will consist of patients with relapsed or refractory NB and Group 2 of relapsed or refractory measurable osteosarcoma. Hu3F8-BsAb is given on Days 1 and 8 of each cycle. The duration of each cycle is 28 days. HAHA titer will be measured after each cycle. Extent of disease evaluation (EOD) will be performed after the second dose of cycle 2 and cycle 4, then every 3 months thereafter. A total of 4 cycles will be given, and patients with no life-threatening toxicity clearly attributable to hu3F8-BsAb, no PD, and continuing to meet all eligibility criteria have the option of receiving additional cycles up to 2 years from the start of the hu3F8-BsAb treatment. Patients may continue therapy beyond 4 cycles even if they achieve remission. For Group 1, the overall response rate will be evaluated and is defined as the proportion of patients achieving complete remission (CR) or partial response (PR) based on revised International Neuroblastoma Response Criteria (INRC) as the best response at the end of cycle 2 and cycle 4. (See Section 12). For Group 2, 4-month PFS will be assessed and is defined as the proportion of patients who have not developed PD at the end of 4 months from the first dose of Hu3F8-BsAb. In addition, we will determine the number of patients who have CR or PR as the best response at the end of cycle 2 and cycle 4. (see Section 12)

4.1.3 Phase I Dose-Escalation Schedule

The phase I dose-escalation schedule schema is summarized in Table 4 below. Dose Level 1 is the starting dose, and the escalation and de-escalation schedule are described in Section 9.1. DLTs are defined in Section 9.1.

Table 4 Dose escalation schedule

Level	Dose on day 1	Dose on day 8	Dosage per cycle
[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]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

4.2 Intervention

Phase I

Hu3F8-BsAb is given IV over ~1-3 hours on Days 1 and 8 for each cycle. In cycle 1, blood is drawn for PK studies as described in Section 9.1.4.

Phase II

Hu3F8-BsAb is given IV over ~1-3 hours on Days 1 and 8 for each cycle.

5 THERAPEUTIC/DIAGNOSTIC AGENTS

The investigational medicinal product is fully humanized bispecific antibody (hu3F8-BsAb).

Hu3F8-BsAb

The Sponsor, Y-mAbs, will provide hu3F8-BsAb 2 mg/mL solution for intravenous infusion. No other medicinal products or auxiliary medicinal products will be provided by Y-mAbs.

Hu3F8-BsAb drug product (DP) is manufactured in compliance with good manufacturing practice (GMP) regulations and no excipients of human or animal origin have been used. Final clinical DP formulation is a solution with 2 mg/mL hu3F8-BsAb buffered with sodium citrate dihydrate, citric acid monohydrate, and sodium chloride, and stabilized with Kolliphor P188 (Poloxamer 188). The final presentation is sterile filtered and filled into 2 mL vials.

Drug dilution and preparation procedure is provided in Appendix B.

6 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

6.1.1 Diagnosis

Phase I

- Patients must have either (1) a diagnosis of NB as defined by international criteria,[77] i.e., histopathology (confirmed by the MSKCC Department of Pathology) or BM metastases plus high urine catecholamine levels, or (2) high grade osteosarcoma verified by histopathology (confirmed by the MSKCC Department of Pathology), or (3) other GD2-expressing solid tumor.
- For tumors other than NB and osteosarcoma, only tumors known to be GD2 positive are eligible: melanoma, desmoplastic small round cell tumors, retinoblastoma, medulloblastoma, and soft tissue sarcomas including liposarcoma, fibrosarcoma, malignant fibrous histiocytoma, leiomyosarcoma, and spindle cell sarcoma. Patients with medulloblastoma are eligible only if they have metastatic disease outside the CNS (e.g. in the bone marrow)
- NB patients must have chemorefractory (e.g. refractory to standard induction chemotherapy including cyclophosphamide, vincristine, cisplatin, etoposide) or relapsed high-risk (HR) neuroblastoma. HR NB is defined as *MYCN*-amplified stage 3/4/4S of any age, or *MYCN*-nonamplified stage 4 in patients > 18 months of age at diagnosis.
- Osteosarcoma patients must have relapsed or refractory osteosarcoma after receiving standard systemic chemotherapy (e.g. combination methotrexate, doxorubicin, and cisplatin [MAP]).
- For non-NB and non-osteosarcoma tumors known to be GD2(+), patients must have relapsed or refractory disease that is resistant to standard therapy.

Phase II

- **Group 1:**
  - NB patients must have chemo refractory or relapsed HR NB. HR NB is defined as *MYCN*-amplified stage 3/4/4S of any age, or *MYCN*-nonamplified stage 4 in patients > 18 months of age at diagnosis.
  - The diagnosis of NB must be defined by international criteria[77] i.e., histopathology (confirmed by the MSKCC Department of Pathology) or BM metastases plus high urine catecholamine levels.
- **Group 2:**
  - Patients must have a diagnosis of high grade osteosarcoma defined by histopathology (confirmed by the MSKCC Department of Pathology).

- Patients must have relapsed or refractory osteosarcoma after receiving standard systemic chemotherapy (e.g. combination methotrexate, doxorubicin, and cisplatin [MAP]).

All criteria below are common to both phase I and phase II:

6.1.2 Disease status

- For NB patients, patients must have measurable or evaluable disease (e.g. abnormal findings in computed tomography (CT), magnetic resonance imaging (MRI), metaiodobenzylguanidine (MIBG) scan, or positron emission tomography (PET)) OR morphologic evidence of disease in bone marrow.
- For osteosarcoma or other GD2(+) solid tumor patients, patients must have measurable disease. (See Section 12.4 for a definition of measurable disease)

6.1.3 Other criteria

- Patients must be ≥ 1 year of age and < 18 years of age.
- Patients with prior exposure to anti-GD2 antibodies must have a negative HAHA antibody titer .
- Adequate hematopoietic function defined as:
  - Absolute neutrophil count ≥500/ul
  - Absolute lymphocyte count ≥500/ul
  - Platelet count ≥25,000/ul
- Negative serum pregnancy test in women of child-bearing potential.
- Women of child-bearing potential must be willing to practice an effective method of birth control while on treatment.
- Signed informed consent indicating awareness of the investigational nature of this program.

6.2 Subject Exclusion Criteria

- Patients who are in complete remission.
- Existing severe major organ dysfunction. i.e. renal, cardiac, hepatic, neurologic, pulmonary, or gastrointestinal toxicity ≥ Grade 3 except for hearing loss, alopecia, anorexia, nausea, hyperbilirubinemia or hypomagnesemia from TPN, which may be Grade 3.
- Hematologic and active CNS malignancies including CNS metastasis.
- Active life-threatening infection.
- Pregnant women or women who are breast-feeding.

- Inability to comply with protocol requirements.
- History of autoimmune disease with potential CNS involvement or a current autoimmune disease.
- Chemotherapy or immunotherapy within three weeks prior to study enrollment. T-cell based immunotherapies (e.g. CAR-modified T cells, checkpoint inhibitors) should have been completed >6 weeks prior to treatment with hu3F8-BsAb.

7 RECRUITMENT PLAN

Patients will be offered participation in this study by their attending physician in the Department of Pediatrics at Memorial Sloan Kettering Cancer Center ***only***. Patients will be offered the opportunity to participate if they have relapsed or refractory high-risk NB, osteosarcoma, or GD2 (+) tumor as defined in Section 6.1. No patient will be identified by chart review or direct advertising. The consenting physician will be responsible for explaining the study, obtaining written informed consent and registering the patient on study. Patients of both genders and all ethnic backgrounds are eligible for this study.

Up to 30 patients will enroll in Phase I and up to 64 patients will enroll in Phase II.

8 PRETREATMENT EVALUATION

Pretreatment evaluations should be completed within 30 days of start of treatment. Echocardiogram and electrocardiogram can be completed within 45 days prior to treatment. All pretreatment evaluation should be performed after the completion of prior chemotherapy or immunotherapy.

1. Complete history and physical examination.
2. Blood pressure measurement (standing, sitting and lying down).
3. Complete blood count, serum creatinine, blood urea nitrogen, serum aspartate aminotransferase, serum alanine aminotransferase, serum alkaline phosphatase, serum total bilirubin, albumin, sodium, potassium, chloride, bicarbonate, magnesium, phosphate, ferritin, and C-reactive protein (CRP).
4. Echocardiogram (ECHO)
5. Electrocardiogram
6. Phase I only: C3 and CH50
7. Phase I only: Urinalysis.
8. Serum for analysis of HAHA if applicable (i.e., for patients previously treated with anti-GD2 antibodies). If the patient was previously treated with an antibody, any HAHA result since last antibody treatment can be used.

9. Blood for baseline cytokine studies (including IFN-γ, IL-6, IL-8, IL-10).[78,79]
10. Blood for baseline lymphocyte subsets (including CD3, CD4, CD8, Foxp3, CD127, CD45RA, CCR7) and T cell activation/exhaustion markers (including CD25 and PD-1).
11. For NB patients, BM aspirates and biopsies from bilateral iliac crests for morphology and detection of minimal residual disease.
12. Imaging by computed tomography (CT) or magnetic resonance imaging (MRI) of primary tumor site and/or known site(s) of relapse (if applicable), or PET scan, Bone scan, and/or MIBG scan (if the patient has neuroblastoma), or other diagnostic imaging as appropriate for specific disease.
13. Pregnancy test, if applicable, within 2 weeks of treatment.

9 TREATMENT/INTERVENTION PLAN

9.1 Phase I

9.1.1 General Outline

[redacted]

[redacted]

[redacted]

[redacted]During phase I approximately 0.5 ml of the first Bs-Ab infusion will be collected about half-way through the infusion and assayed in Neuroblastoma research laboratory to determine quantity of hu3F8-BsAb in the final infusion product. In cycle 1, blood is drawn for PK studies as described in Section 9.1.4. Patients are admitted for dose 1 of cycle 1 of treatment and will be inpatient for approximately 4 days (minimum 48 hours) though the duration of stay may change if clinically indicated. Subsequent doses can be administered inpatient or outpatient. Patients are monitored for DLT clinically with physical examination, CBC and complete chemistry until Day 28. Patients with DLT will come off treatment HAHA titer is measured after each cycle. HAHA draws require about 5mL of serum. If HAHA develops, cycles are deferred for up to 4 months until HAHA titer until <1300 U/ml. Extent of disease evaluation will be performed after the second dose of hu3F8-BsAb in cycle 2 and cycle 4, every 3 months thereafter. (Table 6) A total of 4 cycles will be given.

Patients with no life-threatening toxicity clearly attributable to hu3F8-BsAb, no PD, and continuing to meet all eligibility criteria (with subsequently explained exceptions) have the option of receiving additional cycles up to 2 years from the start of the hu3F8-BsAb treatment. Inclusion criteria that no longer apply for patients to receive subsequent cycles are: disease status, since patients achieving remission may continue; and prior T cell immunotherapy timing restrictions, since all patients will have received Cycle 1 of hu3F8-BsAb. Even patients who achieve remission may continue therapy beyond

4 cycles. DLT and dose-schedule are described in Section 9.1.2 and 9.1.3. After cycle 2, intra-patient dose escalation will be permitted i.e. patients can receive the highest dose that has been evaluated and proven safe by DLT assessment. Patients will be admitted for at least 48 hours for their first escalated dose.

The treatment and evaluation schedule may require minor adjustment as clinically indicated or due to circumstance (e.g. due to PDH closure for holidays or due to inclement weather).

9.1.2 Dose-Escalation Plan and Dose Limiting Toxicity (DLT)

9.1.2.1 Dose-Escalation Plan

This is a Phase I trial to determine the MTD of hu3F8-BsAb that leads to DLT in approximately 15% of patients. Hu3F8-BsAb is dosed based on body weight (BW) rather than body surface area (BSA). Unlike chemotherapy for which clearance ultimately depends upon cardiac output which is dependent upon BSA, antibody distribution and clearance depend on total blood volume which is determined not by BSA, but by BW. Dose escalation will start at dose level 1, 0.0045 mcg/kg/dose (0.009 mcg/kg/cycle). Dose-escalation will be performed in two stages. In the first stage, the trial will accrue 1 patient per dose until the first ≥ Grade 2 toxicity (except for Grade 2 sinus bradycardia, sinus tachycardia, fever, nausea, vomiting, diarrhea, urticaria, headache, paresthesia, electrolyte disturbances, pain) is encountered. The subsequent dose allocation will follow the Continual Reassessment Method (CRM). CRM assumes a simple model for the probability of a DLT as a function of dose, and uses the occurrence of toxicities in the patients enrolled in the trial to sequentially determine which dose to administer to a new patient. Patients will be assigned at each dose one at a time. New subjects will be allocated a dose as suggested by the CRM algorithm based on the toxicities of previously accrued patients. If DLT data are not available for the previously accrued 1 or 2 patients, the CRM will proceed with the dose as calculated with available toxicities/data, however if there are 3 enrolled patients with incomplete /outstanding DLT data, accrual will be paused. DLT data from at most 2 patients can be pending but no more than 2 patients are allowed to have incomplete DLT data. To protect patient safety, a dose escalation of more than one dose level is not permitted. We will examine 12 dose levels. (Table 4) Our initial estimates of DLT probabilities are: **0.001, 0.005, 0.01, 0.025, 0.05, 0.075, 0.10, 0.15, 0.25, 0.35, 0.45, 0.50** for doses 1-12, respectively. Thus, our a priori belief is that dose level 8 is the MTD. We assume that the dose- toxicity follows a hyperbolic tangent model P(DLT=yes at dose d)=((tanh d+1)/2)^a, where a is the unknown parameter that we need to estimate in order to determine which dose is the MTD.A value of a=1.0 indicate that our prior beliefs were correct; while a value of a less than (greater than) 1.0 indicates that the combinations are more (less) toxic than believed. To reflect the uncertainty in our prior probability estimates, we assume it follows an exponential distribution (prior distribution) with mean 1.0. Dose escalation will be guided by the model after the occurrence of the first DLT. At that time, the above initial estimates of DLT might be revisited before model initiation to reflect the current knowledge on the safety profile and dose levels based on the observed data. We will enroll 30 evaluable patients. All patients who are enrolled in the study and receive their assigned dose are considered evaluable for toxicity. Additional subjects will be enrolled to replace any subjects who are enrolled, but do not receive treatment. In the

unlikely event that patient stops the drug due to HAHA before completing the first cycle, this patient will be considered inevaluable and will be replaced. If life-threatening toxicity occurs in any patient, further accrual at that dosage will be stopped pending review by the Principal Investigator (PI) or Co-PI. Toxicity at each dosage level is evaluated during the 28 days following the first dose of hu3F8-BsAb. No more than two patients will receive their first dose of hu3F8-BsAb within a 24 hour period. If no DLT is observed up to dose level 12 during the initial patient per dose escalation (e.g. no MTD), the RP2D will be determined as a minimum dose that gives the highest hu3F8-BsAb serum levels based on the PK data.

9.1.2.2 Dose Limiting Toxicities

Toxicity will be monitored using CTCAE version 4.0. DLT only during cycle 1, i.e. Days 1 through 28. Allowance will be made for the expected toxicities of hu3F8 from which hu3F8-BsAb was derived. (See Section 11.2). As stated above, in the first stage (1 patient per dose-level) dose-escalation will be performed until ≥Grade 2 related toxicity (except for Grade 2 sinus bradycardia, sinus tachycardia, urticaria, fever, nausea, vomiting, diarrhea, urticaria, headache, paresthesia, electrolyte disturbances, pain) is encountered, then the subsequent dose allocation will follow the CRM.[75,76]

During the CRM phase, any ≥Grade 3 toxicity attributable to hu3F8-BsAb will be considered dose-limiting with the following **exceptions**:

- Grade 3 cytokine release syndrome (CRS) improving to ≤Grade 1 CRS within ≤72 hours after starting systemic steroids (See Section 11.3 for a grading system and algorithm)
- Grade 3 pain improving to ≤Grade 2 within ≤72 hours of its onset. Grade 3 pain associated with myalgia is an expected clinical toxicity associated with cytokine release syndrome (CRS).
- Grade 3 neutropenia or anemia
- Grade 3 thrombocytopenia without clinically significant bleeding.
- Grade 4 lymphopenia
- Grade 3 elevations of AST, ALT if they return to <Grade 3 by day 28
- Catheter related infections
- DLT of hypertension is defined as persistent hypertension higher than 99th percentile for >48 hours.

The following Grade 3 toxicities are related to infusion of monoclonal antibodies and will not be considered DLT if they resolve to <Grade 3 within 24 hours:

- Allergic reactions controlled with supportive care measures
- Vasovagal reaction
- Sinus bradycardia

- Sinus tachycardia
- Urticaria (improved to at least Grade 2)
- Fever

The following Grade 3 toxicities will not be considered a DLT if they resolve within 48 hours:

- Nausea
- Vomiting
- Diarrhea
- Peripheral neuropathy (paresthesia)
- Electrolyte disturbances

In all other cases, DLT is defined as Grade 3 or greater toxicities occurring during cycle 1 with the exception of toxicities clearly related to disease activity or co-interventions.

9.1.3 Criteria for continuing on therapy beyond cycle 1.

If patients experience DLT as described above, they will discontinue treatment. Patients will need to fulfill all eligibility criteria before each cycle. However, patients can continue treatment without delay if they have ≤grade 4 lymphopenia. Cycles 2-4 may be delayed due to toxicities other than DLT for a maximum of four weeks i.e. maximum duration between dose 1 of the prior cycle and dose 1 of the next cycle can be ≤56 days. If HAHA titer of >1300 U/ml develops, cycles beyond cycle 1 may be delayed for up to 120 days. However, if the duration between cycles is >28 days, patients will be required to undergo extent of disease evaluation to ensure that there is no progressive disease.

9.1.4 Pharmacokinetic (PK) studies

Blood (Table 5) will be drawn at various time points to study PK of hu3F8-BsAb. At each timepoint about 2 mL of blood will be collected for PK studies. For further details, see the laboratory manual.

Table 5 PK schedule- Cycle 1 and Dose-Escalated Cycles

Day	Time points
1	Prior to infusion of hu3F8-BsAb infusion
1	~ 5 mins, 3 hrs, ~ 6-8 hrs after the end of hu3F8-BsAb infusion
2	~24 hrs
3	~48 hrs
4	~72 hrs
5	~96 hrs
6	~120 hrs

Day	Time points
7	~144 hrs
8	~168 hrs (prior to hu3F8-BsAb injection) **If dose 2 of hu3F8-BsAb cannot be given on Day 8, a PK sample should still be drawn**
8	~ 5 mins after the end of hu3F8-BsAb infusion dose 2
9	~24 hrs
10	~48 hrs
11	~72 hrs
12	~96 hrs
13	~120 hrs
14	~144 hrs
15	~168 hrs
Approximately 22	~336 hrs
Approximately 28	~480 hrs

In addition, based on our prior studies demonstrating that Cmax is highly correlative with AUC for hu3F8, patients treated both in phase I and II will undergo a single blood draw ~ 5 minutes after each hu3F8-BsAb infusion for determining Cmax for repeat dosing. We will also draw blood for Ctrough prior to the second dose of hu3F8-BsAb, prior to each hu3F8-BsAb infusion in Phase I and prior to each cycle in Phase II. Each blood draw for Cmax and Ctrough will be about 2ml. Blood for HAHA testing can be used for Ctrough prior to each cycle (see 9.3).

9.1.5 Prohibited medication phase I

No simultaneous systemic anti-cancer therapy is permitted while on study, however local radiotherapy or surgical resection of the tumor is allowed >28 days after first dose of hu3F8-BsAb in phase I provided at least one non-irradiated tumor site is available for evaluation.

9.2 Phase II

9.2.1 General outline

Hu3F8-BsAb at R2PD determined in the phase I study is infused IV over ~1-3 hours on Days 1 and 8 of each cycle. A total of 4 cycles will be given. The duration of each cycle is approximately 28 days. However, cycles may be delayed for up to 56 days if >grade 2 toxicity is encountered. In order to receive subsequent cycles, patients should continue to meet all eligibility criteria, should not have experienced life-threatening toxicity clearly attributable to hu3F8-BsAb, and should not have developed PD. HAHA titer will be measured after each cycle. If HAHA develops, cycles are deferred for up to 120 days until HAHA titer until <1300 U/ml. Extent of disease evaluation will be performed after the second dose of hu3F8-BsAb in cycle 2 and cycle 4, then every 3 months thereafter. After cycle 4, patients have the option of receiving therapy for up to 2 years provided that they have not experienced

life-threatening toxicity clearly attributable to hu3F8-BsAb, not developed PD, continue to meet all eligibility criteria and HAHA titer is <1300U/ml. After cycle 4, duration between cycles can range from 28-120 days. Patients may continue therapy beyond 1 cycle even if they achieve remission. Patients will undergo physical examination, CBC and complete chemistry and immune correlates will be tested as described in Section 10.

The treatment and evaluation schedule may require minor adjustment as clinically indicated or due to circumstance (e.g. due to PDH closure for holidays or due to inclement weather).

9.2.2 Prohibited medication and surgery phase II

No simultaneous systemic anti-cancer therapy is permitted while on study, however local radiotherapy is allowed at any time provided at least one non-irradiated tumor site is available for evaluation. Patients may undergo surgery to any site prior to first extent of disease evaluation provided there another site of evaluable disease remains; patients may be rendered NED by surgery after their first EOD evaluation.

All interventions below are common to both phase I and phase II

9.3 HAHA testing

Blood (~5ml per draw) drawn before each cycle for HAHA testing will be measured using ELISA. Anti-idiotype antibodies (Ab3, Ab3') will be also tested with the same blood samples.[81]

This blood sample may also be tested for HAMA. HAMA positivity is allowed and will not affect patients' eligibility for treatment.

9.4 Immunological effects of hu3F8-BsAb

Lymphocyte subsets (including CD3, CD4, CD8, Foxp3, CD127, CD45RA, CCR7) and T cell activation/exhaustion markers (including CD25, PD-1) will be tested before and after the hu3F8-BsAb treatment (See Section 10). Each draw will collect about 16 mL of serum. Analysis will be performed in the MSK Immune Monitoring Core Facility. CBC should be checked with blood collected on the same day. Cytokine levels (including IFN-γ, IL-6, IL-8, IL-10) will be measured before and after the hu3F8-BsAb treatment during cycle 1 of phase I, and analyzed in the MSK Immune Monitoring Core Facility. During subsequent cycles of phase I or in phase II, these cytokine levels will be measured if CRS is clinically suspected. Each draw for cytokine levels will collect about 4mL of serum.

9.5 Bone marrow (BM) RNA

For NB patients, tumor RNA will be used for transcriptome and microRNA profiling. Tumor RNA in BM from blood will be used for minimal residual disease (MRD) testing, to be completed in the Cheung Lab at MSK. The samples will not have any associated patient-specific identifying information, but will be linked to clinical information such as patient age, gender, tumor stage, tumor recurrence status,

and tumor site. Frozen tumors will be tested for tissue antigens and gangliosides (including but not limited to GM2, GD2, GD3].

9.6 Stool microbiome

Based on emerging data on the impact of stool microbiome on function of immunotherapeutic agents, a stool sample will be collected on all patients prior to first dose of hu3F8-BsAb, where feasible. Sequencing of stool microbiota will be performed and correlated with clinical and immunological response.

10 EVALUATION DURING TREATMENT/INTERVENTION

10.1 History and physical exam

- Phase I cycle 1: History and physical exams will be completed every day for the first 4 days, on Day 8, and weekly thereafter until Day 28.
- For subsequent cycles in Phase I, and Phase II: Before each dose of hu3F8-BsAb and within 72 hours of treatment.

10.2 Patients are monitored by pulse oximeter during hu3F8-BsAb infusion and have blood pressure taken no later than 30 minutes after the end of infusion of hu3F8-BsAb. In cycle 1 and for all inpatient treatment, blood pressure measurement (standing, sitting and lying down) will be taken pre infusion, once daily throughout their hospital stay and at discharge. For treatment administered outpatient, blood pressure measurement (standing, sitting and lying down) will be taken pre infusion and at discharge.

10.3 CBC

- Phase I cycle 1: CBC will be done approximately on Days 2, 5, 8 (prior to the second dose of hu3F8-BsAb), and approximately Days 15 and 28.
- For subsequent cycles in Phase I, and Phase II: Before each dose of hu3F8-BsAb. (Pre-treatment CBC can be used for pre-cycle 1 CBC.)

10.4 C3 and CH50

- Phase I only: C3 and CH50 will be tested on Day 2 during cycle 1.

10.5 Urinalysis

- Phase I only: In cycle 1, urinalysis will be tested prior to each dose of hu3F8-BsAb and approximately one week after the second dose. (Pre-treatment urinalysis can be used for pre-cycle1 test.)

10.6 Liver and kidney function blood tests (ALT, AST, alkaline phosphatase, albumin, bilirubin, BUN and creatinine), and serum electrolytes (sodium, potassium, chloride, bicarbonate, magnesium, and phosphate).

- Phase I cycle 1: Before each dose of hu3F8-BsAb, within 72 hours after each dose of hu3F8-BsAb, and on approximately Day 15. (Pre-treatment test can be used for pre-cycle1 test.)
- For subsequent cycles in Phase I, and Phase II: Before each dose of hu3F8-BsAb. (Pre-treatment test can be used for pre-cycle1 test.)

10.7 Pregnancy test within 2 weeks before each cycle, if applicable.

10.8 Phase I only: Electrocardiogram will be done post the first infusion of hu3F8-BsAb on Day 1 and prior to the second dose hu3F8-BsAb on Day 8 during cycle 1. This must be done on the same day as the dose, but does not need to be immediately post or immediately prior.

10.9 Phase I only: Pharmacokinetic studies will be done as described in Section 9.1.4

10.10 Phase I and II: Cmax and Ctrough will be measured as described in Section 9.1.4

10.11 Ferritin and C-reactive protein

- Phase I cycle 1: On Day 8 (prior to the second dose of hu3F8-BsAb) and approximately on Day 28.
- For subsequent cycles in phase I, and phase II: If CRS is suspected based on clinical symptoms. (See Section 11.3)

10.12 LDH

- Phase I cycle 1: On Day 8 (prior to the second dose of hu3F8-BsAb) and approximately on Day 28.
- For subsequent cycles in phase I, and phase II: If CRS is suspected based on clinical symptoms. (See Section 11.3)

10.13 Blood will be obtained before each cycle of hu3F8-BsAb to assay for HAHA titers, then approximately every 3 months for 24 months from first hu3F8-BsAb dose, and then approximately every 6 months for up to 5 years from first hu3F8 dose or until patient is off study, whichever is earlier. This blood sample may also be tested for HAMA.

10.14 Blood will be tested for cytokine studies including IFN-γ, IL-6, IL-8, IL-10.

- Phase I cycle 1: cytokine studies should be tested prior to and ~ 6-8 hrs after the first and second infusion of hu3F8-BsAb, and on Days 2, 3, 4, 9, 10, and 11.
- For subsequent cycles in Phase I and Phase II: If CRS is suspected based on clinical symptoms. (See Section 11.3)

10.15 Lymphocytes from heparinized blood will be collected for lymphocyte subset markers (including CD3, CD4, CD8, Foxp3, CD127, CD45RA, CCR7) and T cell activation/exhaustion markers (including CD25 and PD-1).

- Phase I Cycle 1: On Days 2, 8 (prior to hu3F8-BsAb), 9, 10 and 11, and approximately Days 15 and 28.
- For subsequent cycles in phase I, and phase II: Before each dose of hu3F8-BsAb.

10.16 For patients with a diagnosis of neuroblastoma, the following studies will be performed any time after the second dose of hu3F8-BsAb in cycle 2 and cycle 4, and then approximately every 3 months while on study:

- BM aspirates and biopsies from bilateral iliac crests for standard histochemical studies, as well as for immunocytology and other molecular markers of disseminated NB.
- Imaging by computed tomography (CT) or magnetic resonance imaging (MRI) of primary tumor site, plus metaiodobenzylguanidine (MIBG) scan or positron emission tomography (PET).

10.17 For patients with osteosarcoma or GD2(+) tumors, the following studies will be performed any time after the second dose of hu3F8-BsAb in cycle 2 and cycle 4*, and then approximately every 3 months while on study:

- Disease evaluations with imaging by computed tomography (CT) or magnetic resonance imaging (MRI) of primary tumor site and/or known site(s) of relapse (if applicable), with/without positron emission tomography (PET) or bone scan, or other diagnostic imaging as appropriate for specific disease.

  *Cycle 4 scans should occur at approximately 4 months (+/- 1 month) in order for scans to be used to evaluate the 4 month disease control rate (see section 14.2.1.2).

10.18 Pain Assessment (Phase I only): Pain scores will be assessed using the Face, Legs, Arms, Cry, Consolability (FLACC) scale[82-84] and/or the numeric scale[85], as appropriate (see Appendix A for pain scales). Pain scores will be evaluated at the following time points on days with hu3F8-BsAb treatment: (a) once prior to commencement of any study drug administration, (b) at least once during the acute pain episode when rescue pain medication doses are required (for hu3F8 this is towards the end of the 30 minute infusion or soon after completion of antibody infusion) and (c) ~1 hour after the end of infusion or prior to discharge from the Pediatric Day Hospital. Pain scores will be assessed for all treatment cycles. Opioid requirements, rather than pain score, will be used to establish safety for the Phase I primary objective (Section 2.1). Pain scores will be used to assist clinical judgment (e.g. decision of pain medication use, observation of pain relief) and for documentation.

10.19 Phase I and II: stool sample collection as described in section 9.6.

All research blood (as identified below) will be completed where feasible. Patients who are 10 kilograms or less in weight will only have research bloods drawn at the discretion of the Principal Investigator.

Table 6 Phase I

Tests	Pre-treatment	During Treatment	During Follow-up
Complete history and physical	✓	Cycle 1: Daily from Day 1 through Day 4, on Day 8, then weekly thereafter until Day 28 Subsequent Cycles: Before each dose of hu3F8-BsAb and within 72 hours of treatment	-
Pharmacokinetic studies (research blood)	✓	See Section 9.1 Cycle 1: As shown in Table 5 Subsequent Cycles: Before each dose of hu3F8-BsAb, ~5 minutes after hu3F8-BsAb infusion	
Complete blood count	✓	Cycle 1: Approximately Days 2, 5, 8 (prior to the second dose of hu3F8-BsAb), and approximately Days 15 and 28. Subsequent Cycles: Before each dose of hu3F8-BsAb	-
Liver and Renal function tests (ALT, AST, alkaline phosphatase, albumin, bilirubin, BUN and creatinine) and serum electrolytes (sodium, potassium, chloride, bicarbonate, magnesium, and phosphate)	✓	Cycle 1: Before each dose of hu3F8-BsAb, within 72 hours after each dose of hu3F8-BsAb and approximately Day 15 Subsequent Cycles: Before each dose of hu3F8-BsAb	-
C3 & CH50	✓	Cycle 1 Only: Day 2	-
LDH	✓	Cycle 1: Day 8 and approximately Day 28 Subsequent Cycles: If CRS is suspected based on clinical symptoms	
Ferritin and C-reactive protein	✓	Cycle 1: Day 8 and approximately Day 28 Subsequent Cycles: If CRS is suspected based on clinical symptoms	-
SpO2 and Blood pressure measurement	Blood pressure measurement (standing, sitting, lying down)	SpO2: During hu3F8-BsAb BP: No later than 30 minutes after the end of hu3F8-BsAb infusion Cycle 1: Blood pressure measurement (standing, sitting, lying down) pre infusion, once daily throughout hospital stay and at discharge. For outpatient treatment: pre infusion and at discharge	-

Tests	Pre-treatment	During Treatment	During Follow-up
Blood for HAHA	✓	Before each cycle	Approximately q3 months for 24 months from first hu3F8-BsAb dose and then q6 months for up to 5 years from first dose or until patient is off study, whichever is earlier
Urinalysis	✓	Cycle 1: Prior to each dose of hu3F8-BsAb and approximately one week after the second dose	-
Pregnancy test, if applicable	✓	Within 2 weeks before each cycle	-
Electrocardiogram	✓	Cycle 1: Post-infusion of the first dose and prior to the second dose on Day 8	-
ECHO	✓	-	-
Cytokine studies (research blood)	✓	Cycle 1: prior to and ~ 6-8 hrs after the first and second hu3F8-BsAb dose, and on Days 2, 3, 4, 9, 10 and 11 Subsequent Cycles: If CRS is suspected based on clinical symptoms	-
Lymphocyte subsets, T cell activation/exhaustion markers (research blood)	✓	Cycle 1: on Days 2, 8 (prior to hu3F8-BsAb), 9, 10, and 11, and approximately on Days 15 and 28 Subsequent Cycles: Before each dose of hu3F8-BsAb	-
Bone marrow studies*	✓	Any time after the second dose in cycle 2 and cycle 4 and approximately every 3 months thereafter	-
CT/MRI, MIBG scan* with/without PET, bone scan	✓	Any time after the second dose in cycle 2 and cycle 4 and approximately every 3 months thereafter	-
Pain Assessment	✓	On days of hu3F8-BsAb treatment: prior to hu3F8-BsAb administration, during acute pain episode, and ~1 hour after the end of infusion or prior to discharge from the PDH	-
Stool microbiome	✓, where feasible	-	-

*only for patients with neuroblastoma

Table 7 Phase II

Tests	Pre-treatment	During Treatment	During Follow-Up
Complete history and physical	✓	Before each dose of hu3F8-BsAb and within 72 hours of treatment	-
Complete blood count	✓	Before each dose of hu3F8-BsAb	-

Tests	Pre-treatment	During Treatment	During Follow-Up
Liver and Renal function tests (ALT, AST, alkaline phosphatase, albumin, bilirubin, BUN and creatinine) and serum electrolytes (sodium, potassium, chloride, bicarbonate, magnesium, and phosphate)	✓	Before each dose of hu3F8-BsAb	-
LDH	✓	If CRS is suspected based on clinical symptoms	
Ferritin and CRP	✓	If CRS is suspected based on clinical symptoms	-
SpO2 and Blood pressure measurement	Blood pressure measurement (standing, sitting, lying down)	SpO2: During hu3F8-BsAb infusion BP: No later than 30 minutes after the end of hu3F8-BsAb infusion Cycle 1: Blood pressure measurement (standing, sitting, lying down) pre infusion, once daily throughout hospital stay and at discharge. For outpatient treatment: pre infusion and at discharge	-
Blood for HAHA	✓(if applicable)	Before each cycle	Approximately q3 months for 24 months and then q6 months for up to 5 years from first hu3F8 dose or until patient is off study, whichever is earlier
Pregnancy test, if applicable	✓	Within 2 weeks before each cycle	-
Electrocardiogram	✓	-	-
ECHO	✓	-	-
Cytokine studies (research)	✓	If CRS is suspected based on clinical symptoms	-
Lymphocyte subsets, T cell activation/exhaustion markers (research)	✓	Before each dose of hu3F8-BsAb	-
Bone marrow studies*	✓	After the second dose of cycle 2 and cycle 4 and approximately every 3 months thereafter	-
CT/MRI, MIBG scan* with/without PET, bone scan	✓	After the second dose of cycle 2 and cycle 4 and approximately every 3 months thereafter	-
Stool microbiome	✓, where feasible	-	-

*only for patients with neuroblastoma

11 TOXICITIES/SIDE EFFECTS

11.1 Information regarding the definition and reporting of adverse events, including serious adverse events, is provided in Section 17.2.

11.2 Hu3F8-BsAb is expected to be associated with the known side effects of m3F8 and hu3F8

Common side effects: pain, paresthesia, hypertension, hypotension, sympathetic neuropathy, presyncope, syncope, tachycardia, urticaria, pruritus, flushing, fever, somnolence, nausea, and emesis.

Less common side effects: diarrhea, serum sickness, hyponatremia, airway constriction, peripheral neuropathy, impaired accommodation of the eye, poor reactivity of pupils to light, transaminitis.

Rare but serious side effects: anaphylaxis, posterior reversible encephalopathy syndrome (PRES)

11.3 Cytokine release syndrome (CRS) has been reported in patients with B cell leukemia or lymphoma treated by anti-CD19 T cell immunotherapies (e.g. blinatumomab, anti-CD19 CAR-modified T cells). CRS correlated with anti-tumor efficacy, and the incidence, severity, and onset of CRS depend on therapeutic agents, cancer type, tumor burden, and patient's immune system.[64,65,67,79,86-89] Severe cytokine release syndrome is characterized by fever (≥ 3days), elevation of serum cytokines, and clinical toxicities (hypotension, hypoxia, neurological symptoms). In NB patients, 19 patients were treated by anti-GD2 CAR-modified T cells therapy in a phase I study and severe CRS was not observed.[90] In adults, severe CRS is characterized by hypotension, respiratory distress and cardiac changes including arrhythmias, acute coronary syndrome, and impaired LV function, and neurological symptoms such as seizures. Instead of grading CRS according to CTCAE 4.0, CRS will be graded using the CRS revised grading system by Lee et al.[91] described below in Table 8.

Table 8 CRS revised grading system[91]

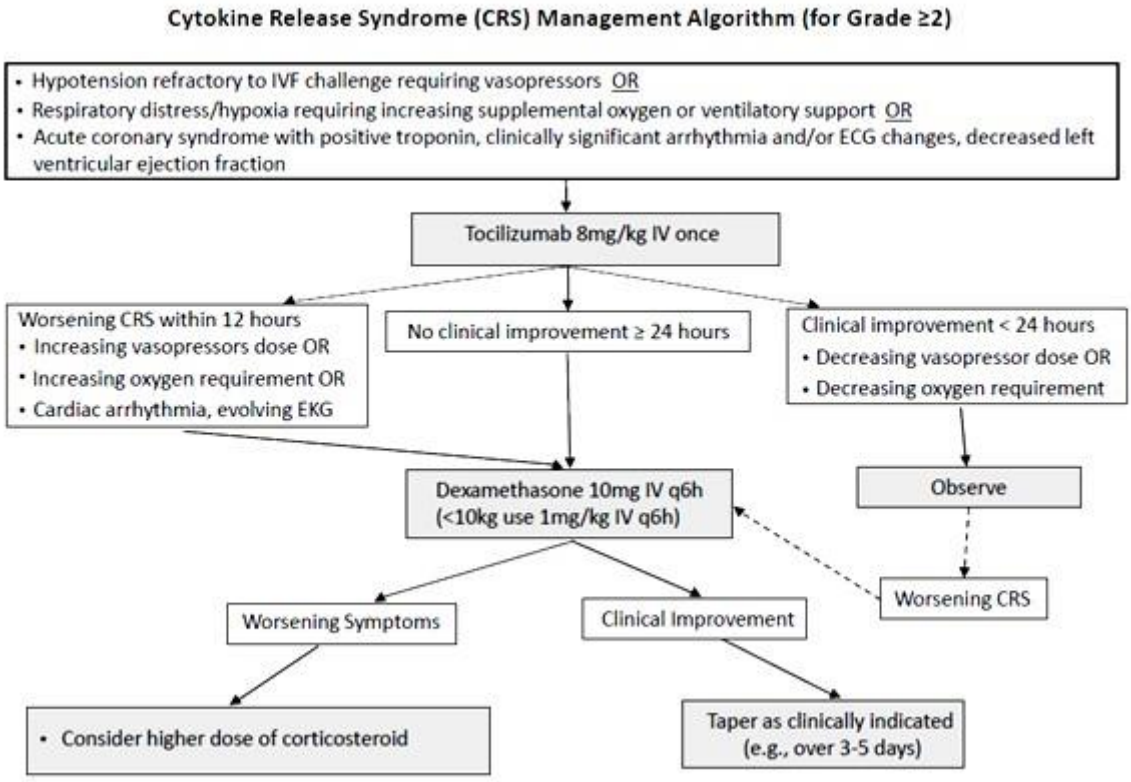
Grade	Toxicity
Grade 1	Symptoms are not life-threatening and require symptomatic treatment only, e.g. fever, nausea, fatigue, headache, myalgias, malaise
Grade 2	Symptoms require and respond to moderate intervention Oxygen requirement < 40% or Hypotension responsive to fluids or low dose of one vasopressor or Grade 2 organ toxicity

Grade	Toxicity
Grade 3	Symptoms require and respond to aggressive intervention Oxygen requirement ≥ 40% or Hypotension requiring high dose for≥ 3 hours * or multiple vasopressors or Grade 3 organ toxicity or Grade 4 transaminitis
Grade 4	Life-threatening symptoms Requirement to ventilator support or Grade 4 organ toxicity (excluding transaminitis)
Grade 5	Death

*High dose vasopressors are defined as follows: dopamine > 10 ug/kg/min; norepinephrine > 0.1 ug/kg/min; epinephrine> 0.1 ug/kg/min; or dobutamine> 10 ug/kg/min.

11.3.1 Management of Cytokine release syndrome

The following algorithm will be used if severe cytokine release syndrome occurs.

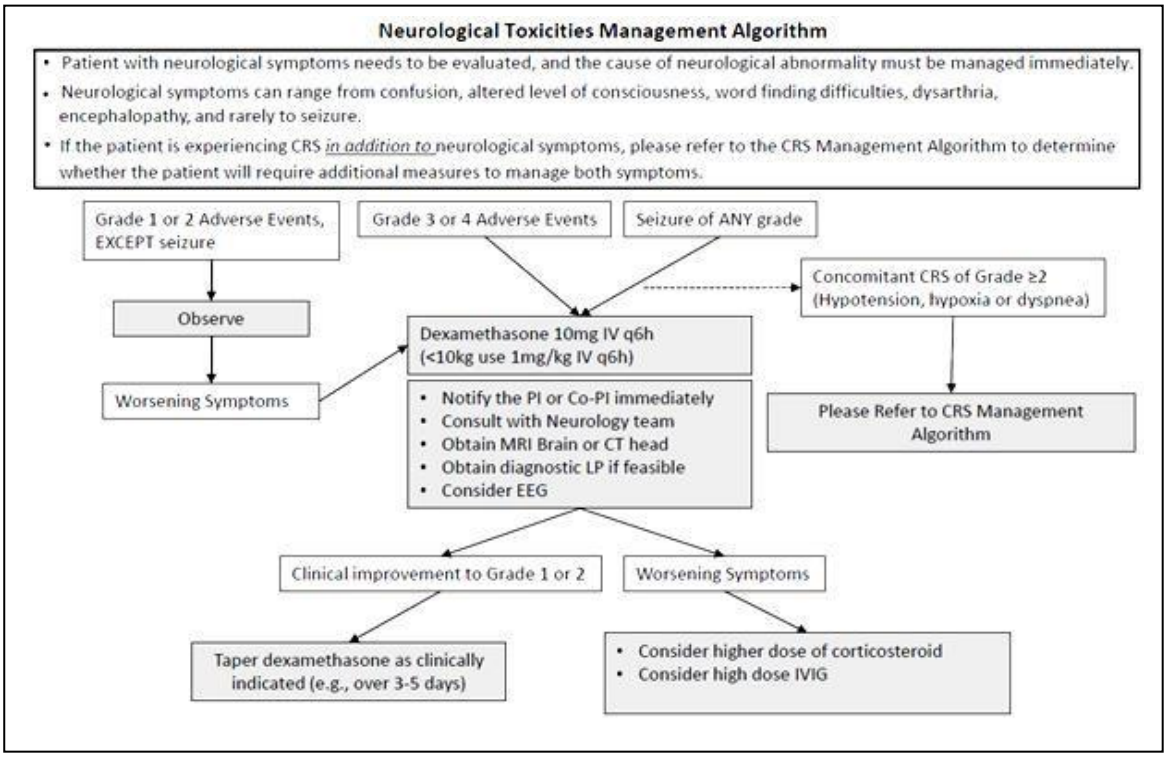


11.4 Neurotoxicity is another side effect of blinatumomab or anti-CD19 CAR-modified T cells. It was rare (< 5%) in children compared to adult patients in blinatumomab clinical studies.[67] Pathophysiology of the neurological toxicity is not clear; it could be related to CRS or could be associated with leukemic cells in CNS, hence it is unclear whether this is restricted to

CD19 targeting immunotherapies or not.[64,65] In the phase I study of anti-GD2 CAR-modified T cells mentioned in Section 11.3, no patients developed neurological symptoms.[90] In addition to CRS, patients treated with hu3F8-BsAb may develop neurological toxicities due to adverse events of opiate analgesics, posterior reversible encephalopathy syndrome (PRES) which has been described with m3F8 and hu3F8.[64-66,79] Patients who develop abnormal neurological symptoms need to be evaluated immediately and appropriate management should be instituted as soon as possible. This can include discontinuation of opiates, naloxone, management of hypertension and head imaging.

11.4.1 Management of Neurological toxicities

The following algorithm will be used for the management of neurotoxicity.



Grade 4 seizure, encephalopathy, depressed level of consciousness, or somnolence will require discontinuation of the hu3F8-BsAb. Except in the case of paresthesia, patients experiencing any grade 3 neurotoxicity (e.g. depressed level of consciousness, encephalopathy or somnolence) and patients with uncontrolled seizures will be considered as having DLT and not receive subsequent doses of hu3F8-BsAb. If parasthesia resolves to <grade 3 within 48 hours it will not be considered a DLT and patient may continue to receive protocol treatment if all eligibility criteria are met. In addition, if patients experience seizures, at least 72 hours should elapse before the next dose of hu3F8-BsAb is administered.

11.5 Management of Orthostatic Hypotension

[redacted]

11.6 If treatment is stopped due to toxicity, treatment may resume once the patient returns to meeting eligibility criteria as described in Section 6.

12 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 Response duration is calculated from first day of treatment with hu3F8-BsAb.

12.2 Patients are considered a response failure under this protocol if progressive disease is evident at any time.

12.3 Disease response for NB will use the revised International NB Response Criteria[92]:

12.3.1 Overall Response:

Overall response will be defined by combining response of the individual components (i.e. soft tissue, bone, and bone marrow disease) (See 12.3.2-12.3.4).

- Complete response/remission (CR): All components meet criteria for CR.
- Partial response/remission (PR): PR in as least one component and all other components are either CR, minimal disease (MD) (bone marrow), PR (soft tissue or bone), or not involved (NI)*; no component with progression disease (PD).
- Mixed response (MR): PR or CR in at least one component but at least one other component with stable disease (SD); no component with PD.
- Stable disease (SD): SD in one component with no better than SD or NI* in any other component; no component with PD.
- Progressive disease (PD): Any component with PD.

  *NI – Site not involved at study entry and remains uninvolved.

12.3.2 Primary (soft tissue) Tumor Response:

- CR: <10mm residual soft tissue at primary site AND Complete resolution of MIBG or FDG-PET uptake (for MIBG-nonavid tumors) at primary site.
- PR: ≥30% decrease in longest diameter of primary site AND MIBG or FDG-PET uptake at primary site stable, improved, or resolved.
- PD: >20% increase in longest diameter taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study) AND Minimum absolute increase of 5mm in longest dimension.
- SD: Neither sufficient shrinkage for PR or sufficient increase for PD at the primary site.

12.3.3 Tumor Response at Metastatic Soft Tissue and Bone Sites:

- CR: Resolution of all sites of disease, defined as: Nonprimary target and nontarget lesions measure <10mm AND Lymph nodes identified as target lesions decrease to a short axis <10mm AND MIBG uptake or FDG-PET uptake (for MIBG-nonavid tumors) of nonprimary lesions resolves completely.
- PR: ≥30% decrease in sum of diameters of nonprimary target lesions compared with baseline AND all of the following:

  Nontarget lesions may be stable or smaller in size AND No new lesions AND ≥50% reduction in MIBG absolute bone score (relative MIBG bone score ≥ 0.1 to ≤ 0.5) or ≥50% reduction in number of FDG-PET-avid bone lesion
- PD: Any of the following:

  Any new soft tissue lesion detected by CT/MRI that is also MIBG avid or FDG-PET avid

  Any new soft tissue lesion seen on anatomic imaging that is biopsied and confirmed to be neuroblastoma or ganglioneuroblastoma

  Any new bone site that is MIBG avid

  A new bone site that is FDG-PET avif (for MIBG-nonavig tumors) AND has CT/MRI findings consistent with tumor OR has been conferment histologically to be neuroblastoma or ganglioneuroblastoma

  >20% increase in longest diameter taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study) AND minimum absolute increase of 5mm in sum diameters of target soft tissue lesions

  Relative MIBG score ≥1.2

- SD: Neither sufficient shrinkage for PR or sufficient increase for PD of nonprimary lesions.

12.3.4 Bone Marrow Metastatic Response:

- CR: Bone marrow with no tumor infiltration on reassessment, independent of baseline tumor involvement.
- PD: Any of the following:

  Bone marrow without tumor infiltration that becomes >5% tumor infiltration on reassessment OR
- Bone marrow with rumor infiltration that increases by > two-fold and has >20% tumor infiltration on reassessment.
- MD: Any of the following:

  Bone marrow with ≤5% tumor infiltration and remains >0 to ≤5% tumor infiltration on reassessment OR

  Bone marrow with no tumor infiltration that has ≤5% tumor infiltration on reassessment OR

  Bone marrow with >20% tumor infiltration that has >0 to ≤5% tumor infiltration on reassessment
- SD: Bone marrow with tumor infiltration that remains positive with >5% tumor infiltration on reassessment but does not meet CR, MD, or PD criteria.

12.4 Disease response for non-neuroblastoma tumors will use the Response Evaluation Criteria in Solid Tumor (RECIST) Version 1.1.

12.4.1 Measurable Disease:

The presence of at least one lesion that can be accurately measure in at least one dimension with the longest diameter at least 20 mm or lesions at least 10 mm on spiral scan is considered measurable disease.

12.4.2 Response Criteria

- Complete Response (CR): Disappearance of all clinical and radiological evidence of target lesions.
- Partial Response (PR): A greater than or equal to 30% decrease in the sum of the longest diameter of all index lesions in reference to the baseline sum of the longest diameters of the index lesions.
- Stable Disease (SD): Response that is neither sufficient decrease to qualify as PR nor sufficient increase to qualify as Progressive Disease (PD).

- Progressive Disease (PD): A greater than or equal to 20% increase in the sum of the longest diameters of all index ("target") lesions, taking as reference the smallest sum of the longest diameters recorded at or following baseline OR the appearance of one or more new site of disease.

12.5 Pseudoprogression (tumor growth from immune response rather than true disease progression) can be seen in early time points after T-cell mediated immunotherapy. If pseudoprogression is suspected, patients will be followed clinically and/or by scans about 4 weeks later to determine if patient has true PD

12.6 Adequacy of trial: All patients who fulfill the eligibility requirements and complete the first cycle of hu3F8-BsAb will have an adequate trial and are considered evaluable for protocol.

In phase I, patients will be considered evaluable if patients develop progressive disease after completing protocol treatment during cycle 1 but do not receive any other treatment before Day 28 of the DLT observation period.

13 CRITERIA FOR REMOVAL FROM STUDY

13.1 Patients come off treatment if there is PD at any time. Best clinical judgment will be used to determine whether a patient must undergo tests between cycles to make sure disease is not progressing. Patients are followed for toxicities for 30 days from last treatment.

13.2 Patients come off treatment if they have DLT (during cycle 1 of phase I) or life-threatening toxicity clearly attributable to hu3F8-BsAb. Patients in the first stage of phase I (one patient per dose level cohort) who experience ≤ grade 2 toxicity, will not come off treatment but can continue at the same dose if they do not experience DLT and if toxicity subsides to <grade 2 within 35 days of dose 1 of Hu3F8-BsAb.

13.3 Patients who become pregnant during the study must be withdrawn from treatment

13.4 The investigators will make every reasonable effort to keep each patient in the study until all planned treatments and assessments have been performed. The investigators may discontinue study drug treatment for the following reasons:

- Adverse events, including unacceptable toxicity or exacerbation of underlying disease, associated with study drug administration and necessitating discontinuation of treatment. Patients who are coming off treatment due to adverse events will be treated and followed according to established, acceptable medical practice. All pertinent information concerning the outcome of such treatment will be entered in the MSKCC institutional Clinical Research Database (CRBD). Patients will be followed until resolution or stabilization of the adverse event.

13.5 Off Study Criteria:

- Thirty days after the last dose of study drug for patients coming off treatment due to DP
- For patients coming off treatment due to adverse events, patients will be followed until the date the event returns to baseline or <Grade 3 (whichever is higher) or the date patients start another treatment, whichever is earlier.
- Death
- Lost to follow-up
- **Withdrawal of consent**. The patient's desire to withdraw from the study may occur at any time. The investigator should carefully consider whether the patient's withdrawal of consent is due to an adverse event, and if so, record the adverse event as the reason for withdrawal.
- **Withdrawal by the physician** for clinical reasons not related to study drug treatment in the absence of an adverse event.
- **Violation of the study protocol**, including failure to return for required treatments or assessments. Patients who fail to return for treatments will be included into the study assessment if they receive at least one dose of hu3F8-BsAb.

14 BIOSTATISTICS

14.1 Phase I

14.1.1 Primary endpoint

The main objective of the study is to establish the maximum tolerated dosage (MTD) of hu3F8-BsAb. The MTD will be defined as the dose whose toxicity rate does not exceed an acceptable threshold of toxicity of 15%. DLT is defined in Section 9.1. In the first stage, the trial will accrue 1 patient per dose until the first ≥ Grade 2 related toxicity (except for Grade 2 sinus bradycardia, sinus tachycardia, fever, nausea, vomiting, diarrhea, urticaria, headache, paresthesia, electrolyte disturbances, pain) is encountered. The subsequent dose allocation will follow the CRM. CRM assumes a simple model for the probability of a DLT as a function of dose, and uses the occurrence of toxicities in the patients enrolled in the trial to sequentially determine which dose to administer to a new patient. Patients will be assigned at each dose one at a time. New subjects will be allocated a dose as suggested by the CRM algorithm based on the toxicities of previously accrued patients. If the DLT data are not available for the previously accrued 1 or 2 patients, the CRM will proceed with the dose as calculated with available toxicities/data, however if there are 3 enrolled patients with incomplete /outstanding DLT data the accrual will be paused. DLT data from at most 2 patients can be pending but no more than 2 patients are allowed to have DLT data not complete. To protect patient safety, a dose escalation of more than

one dose level is not permitted. If no DLT is observed, the RP2D will be determined as a minimum dose that gives the highest hu3F8-BsAb serum levels based on the PK data.

We will examine 12 dose levels as described in Table 4 (Section 9.1). Our initial estimates of DLT probabilities are: 0.001, 0.005, 0.01, 0.025, 0.05, 0.075, 0.10, 0.15, 0.25, 0.35, 0.45, 0.50 for doses 1-12, respectively. Thus, our a priori belief is that dose level 8 is the MTD. The starting dose level will be dose level 1. We assume that the dose-toxicity follows a hyperbolic tangent model P(DLT=yes at dose d)=((tanh d+1)/2)^a, where a is the unknown parameter that we need to estimate in order to determine which dose is the MTD.A value of a=1.0 indicates that our prior beliefs were correct; while a value of a less than (greater than) 1.0 indicates that the combinations are more (less) toxic than believed. To reflect the uncertainty in our prior probability estimates, we assume it follows an exponential distribution (prior distribution) with mean 1.0. Dose escalation will be guided by the model after the occurrence of the first DLT. At that time, the above initial estimates of DLT might be revisited before model initiation to reflect the current knowledge on the safety profile and dose levels based on the observed data.

We will enroll 30 evaluable patients; We expect to enroll 10-15 patients/year, and it is expected that the trial will be open to accrual for 36 months. All patients who are enrolled in the study and receive at least one dose of hu3F8-BsAb are considered evaluable for toxicity. Additional subjects will be enrolled to replace any subjects who are enrolled, but do not receive any treatment. An amendment in the dose levels was done after the inclusion of 5 patients to dose levels 1 to 4; however, the modifications only impacted dose level 5 and higher, therefore the 5 first patients are still part of the CRM and will be analyzed as planned. The amendment does not modify the operating characteristics of the CRM.

14.1.1.1 Operating characteristics

Through 1000 simulated trials with the above parameters we expect the method to behave in the following way, assuming three different hypothetical scenarios for the true toxicity rates at each dose level:

Table 9 Hypothetical True Toxicity Rates

Dose levels	1	2	3	4	5	6	7	8	9	10	11	12
Scenario 1	0.01	0.02	0.05	0.15	0.25	0.35	0.35	0.40	0.45	0.50	0.50	0.50
Scenario 2	0.001	0.005	0.01	0.02	0.03	0.05	0.10	0.15	0.25	0.35	0.45	0.60
Scenario 3	0.001	0.005	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10

Table 10 Percent of simulated trials that selected each dose under each scenario

Dose levels	1	2	3	4	5	6	7	8	9	10	11	12
Scenario 1	0.1	1.5	15.2	47.2	26.3	5.4	3.2	0.9	0.2	0.0	0.0	0.0
Scenario 2	0.0	0.0	0.0	0.2	1.0	5.4	18.5	42.5	26.3	5.5	0.5	0.1
Scenario 3	0.0	0.0	0.0	0.2	0.4	1.2	2.9	10.7	14.7	15.7	14.4	39.8

Table 11 Percent of patients treated at each dose under each scenario

Dose levels	1	2	3	4	5	6	7	8	9	10	11	12
Scenario 1	3.9	7.2	19.5	30.8	20.8	8.7	5.1	2.9	0.8	0.2	0.06	0.0
Scenario 2	3.4	4.3	6.7	6.6	7.8	9.9	14.7	23.2	16.3	5.3	1.6	0.2
Scenario 3	3.4	4.4	6.7	6.5	6.9	7.2	8.6	11.8	11.9	9.8	8.7	14.0

Table 12 Average toxicities across trials at each dose

Dose levels	1	2	3	4	5	6	7	8	9	10	11	12
Scenario 1	0.0	0.0	0.3	1.4	1.5	1.0	0.5	0.3	0.1	0.0	0.0	0.0
Scenario 2	0.0	0.0	0.0	0.0	0.1	0.1	0.4	1.0	1.2	0.6	0.2	0.0
Scenario 3	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.4

14.1.1.2 Dose Allocation in CRDB Section for the Protocol

Patient Registration and the CRDB CRM module are run independently. Once new patients are registered, the CRDB CRM module can be run by members of the protocol research team to generate the CRM report which includes the recommended dose level for the newly registered patient.

During the first stage, one patient is accrued at a time starting at the first dose level and followed for DLT before the next patient is accrued. Patients must be followed for 28 days to determine if they had a DLT. If no DLT is seen, then the next patient is accrued at the next higher dose level. This process

will continue until a DLT is seen. Once a DLT is seen, the study team should notify the CRDB Helpline and the study statistician in order to have the protocol switched to the model-based stage. The dose level of subsequent patients will be assigned by the CRM module. The CRM program uses information specified in the Biostatistics section of the protocol and the summarized toxicity data of previously treated patients as displayed in the CRM report to determine the next dose level to which the patient will be assigned. Only eligible and evaluable patients are used in determining the next dose level. Patients accrued during the model-based stage must also be followed for 28 days to determine if they had a DLT. Since the protocol allows 2 patients to have incomplete toxicity data, then the CRM algorithm can still generate a dose assignment even if toxicity/DLT data are missing for up to 2 patients who were last registered to the protocol. If toxicity data is missing on more than 2 patients or other required data is missing, the CRM module will not provide the next dose assignment and a message indicating missing data will be generated.

The CRM report includes summary and detailed toxicity information and the recommended next dose level (determined by the CRM program). The report will normally be generated by the CRC; however other members of the research team can be given access to run the CRM module. The dose level for new patients is not automatically saved. The PI must check the report to determine if all toxicity data are correct and up-to-date. If the data are correct, the PI will acknowledge this and sign the last page of the report which displays the recommended dose assignment. The last page of the report also contains space to fill in the MRN and name of the newly registered patient who is to receive this dose assignment. The CRC will enter the dose level in CTMS after a patient is registered.

If any data are incomplete or incorrect, the PI will inform the CRC. The CRC will correct the data and indicate to the PI when this is done. The CRDB CRM module will be run again to determine the next patient's dose level. The report will be generated and the PI will be notified to approve the assignment. The dose level will then be entered into CTMS.

Although both children and adults are eligible for enrollment on this study, we do not anticipate any significant differences in target specific toxicity between adults and children based on our experience of over 780 children under age 12 and over 100 patients over age 12 (including >50 over age 18) in the past 20 years with m3F8 and hu3F8. Additionally, there are no reported significant differences in the toxicity of blinatumomab in children compared to adults; 65 thus children and adults will be enrolled and evaluated simultaneously on each dose level.

14.1.2 Secondary endpoints

14.1.2.1 Pharmacokinetics of hu3F8-BsAb

PK will be measured by serial blood sampling during cycle 1 as listed in Table 5. PK analysis will be carried out by noncompartmental analysis of the serum concentration-time data. The following variables are determined: area under the serum concentration-time curve (AUC), the maximum serum concentration (C_{max}), the time to reach the maximum concentration (t_{max}), and the terminal half-life ($t_{1/2}$). The variables AUC and $C_{max(ss)}$ are dose normalized in order to calculate clearance (C_{Lss}), volume

of distribution (V_{ss}) at steady state, the accumulation index for AUC ($R_{A,AUC}$), C_{max} ($R_{A,Cmax}$), apparent terminal half-life, and mean residence time.

14.1.2.2 To assess the activity of hu3F8-BsAb against NB, osteosarcoma, and other GD2(+) tumors and to assess HAHA response

For NB, anti-tumor activity will be measured by revised INRC. For osteosarcoma and other GD2(+) tumors, the response and progression will be evaluated in this study using the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1. as described in Section 12.[93] The proportion of NB, osteosarcoma, and other GD2(+) tumor patients responding to therapy will be determined for each disease type. HAHA will be measured as previously described and the proportion of patients developing HAHA after hu3F8-BsAb therapy will be determined. The correlation between HAHA response and overall response (for neuroblastoma), disease control (for osteosarcoma), overall survival, and progression free survival will be assessed. The correlation between HAHA response and overall response rate (for neuroblastoma) or disease control rate (for osteosarcoma) will be tested using chi-square test. Kaplan-Meier method will be used for estimating overall survival and progression-free survival. Log-rank test will be used for assessing the correlation between HAHA response and the survivals (e.g. OS, PFS) in univariable analysis. Cox-Regression will be used for multivariable analysis for variables including known prognostic factors and HAMA response if $p<0.05$ in the univariable analysis. This information will be summarized separately by cancer type.

14.1.2.3 To study stool microbiome

Sequencing of stool microbiota will be performed and correlated with clinical and immunological response. Given the heterogeneity of the diagnosis and stage in this study, this objective is exploratory no formal statistical comparisons will be made.

14.2 Phase II

14.2.1 Primary endpoint

14.2.1.1 For Group 1 (relapsed or refractory NB)

The objective is to improve overall response rate (ORR) defined as the proportion of patients achieving CR or PR based on revised INRC as the best response at the end of cycle 2 and cycle 4. The phase II study of ch14.18 + GM-CSF for relapsed/refractory NB (POG 9347) demonstrated overall response rate of 17. 8% in a total 28 patients.[94] Our phase II study of 3F8 + GM-CSF for refractory NB had ORR of 28.8% (13 CR+PR out of total 45).[95] Based on these clinical trial results we decided that the historical ORR of refractory/relapsed NB by antibody therapy is < 25 %.

Our null hypothesis is that the hu3F8-BsAb treatment has ORR < 25%. The alternative hypothesis is that the ORR after the hu3F8-BsAb treatment is 50%.

We will accrue 30 patients. At the end of the trial 95% one-sided confidence interval for ORR will be computed using Wilson's method. If the confidence interval does not include the null ORR rate of 25% we will consider this treatment successful. The sample size is based on confidence interval half-width

0.15 and desirable ORR rate of 50%. Based on simulation if the true rate is 50% the CI will not include 25% with probability 90%.

Patients who received at least 1 dose of hu3F8-BsAb will be included into the assessment even if they are removed from the study before completing cycle 4 due to any reasons. We expect to enroll 10-15 patients/year, therefore accrual of this group will be completed in 2-3 years.

14.2.1.2 For Group 2 (relapsed or refractory osteosarcoma)

The objective is to improve 4-month PFS. A retrospective analysis of seven phase II trials conducted by Children's Oncology Group (COG) and its predecessor groups demonstrated that PFS at 4 months from time of enrollment in patients with relapsed measurable disease was 12% (95% CI: 6% to 19%).[19]

Our null hypothesis is that the hu3F8-BsAb treatment has 4-month PFS <12% The alternative hypothesis is that the 4-month PFS after hu3F8-BsAb treatment is 40%. We will accrue 18 patients. At the end of the trial 95% one-sided confidence interval for 4 months PFS will be computed using Wilson's method. If the confidence interval does not include the null rate of 12% we will consider this treatment successful. The sample size is based on confidence interval half-width 0.190 and desirable 4 months PFS rate of 40%. Based on simulation if the true rate is 40% the CI will not include 12% with probability 90%.

Patients who received at least 1 dose of hu3F8-BsAb will be included into the assessment even if they are removed from the study before completing cycle 4 due to any reasons. We expect to enroll 5-10 patients/year, therefore accrual of this group will be completed in 2-4 years.

14.2.2 Secondary endpoints

14.2.2.1 Evaluation of the duration of complete remission

Duration of complete remission (CR) is defined only for patients who achieve CR after hu3F8-BsAb and will be calculated from the time of remission. Duration of CR will be estimated using the Kaplan-Meier method.

14.2.2.2 For Group 2 (relapsed or refractory osteosarcoma): To assess the overall response rate

Overall response rate (ORR) is defined as the proportion of patients achieving CR or PR based on RECIST v1.1 as the best response at the end of cycle 2 and cycle 4. A retrospective analysis of seven phase II trials conducted by Children's Oncology Group (COG) and its predecessor groups demonstrated that 2 out of 87 evaluable patients with relapsed osteosarcoma had CR or PR after treatment of the trial.[19] Hence, we decided that the historical ORR is <3%. We are not making a hypothesis of the ORR in patients treated by hu3F8-BsAb because this is not the primary objective of this study, however, we will compare the ORR in this study with the historical data.

14.2.2.3 To assess the toxicity of hu3F8-BsAb and to assess HAHA response

Toxicity of hu3F8-BsAb will be monitored using CTCAE version 4.0 and CRS revised grading system. (See Section 11) HAHA will be measured as previously described and the proportion of patients developing HAHA after hu3F8-BsAb therapy will be determined.

14.3 Exploratory endpoints

14.3.1 For Phase II: To assess overall survival and progression free survival

Overall survival (OS) is defined as the time from the study enrollment to the time of death or last follow up. Progression free survival (PFS) is defined as the time from the study enrollment to the time of disease progression or last follow up. OS and PFS will be estimated using Kaplan-Meier method.

Exploratory endpoints below are common to both Phase I and Phase II. They will be assessed separately for Phase I and Phase II and by disease group.

14.3.2 To study immunological effects of hu3F8-BsAb

Biologic correlate studies include (1) host immunity, (2) host T cell cytotoxic capacity, and (3) T cell homing into the tumor. The parameters measured for each patient under "host immunity" include: the induction of HAHA, the induction of Ab3, the induction of Ab3'. Under "T cell cytotoxic capacity", the following measurements will be made: (1) cytokine profiles including IFN-γ, IL-6, IL-8, IL-10 [78,79], (2) changes in lymphocyte populations and their differentiation including CD3, CD4, CD8, Foxp3, CD127, CD45RA, CCR7, and (3) activation and exhaustion markers on T cells including CD25 and PD-1. "T cell homing" will be tested by immunohistochemistry of tumor samples if available. Host immunity, T cell cytotoxic capacities, and T cell homing will be tested for correlation with hu3F8-BsAb dose, as well as for association with patient response and survival, using Benjamini-Hochberg adjustment for multiple comparisons. However, given the heterogeneity of patients and disease stage, and the limited number of patients, multivariable statistical analysis is not planned. Nevertheless, these comparisons will provide insights into biologic effects of hu3F8-BsAb and parameters that may predict response and survival. The establishment of the feasibility of these critical assays will facilitate future phase I/II explorations of combination with other cytokines orbiologics.

14.3.3 To study tumor DNA, RNA

For neuroblastoma patients only: tumor DNA and RNA matched to normal RNA, will be archived to profile tumor gene aberrations and gene expressions as well as to test correlation with MRD. This information will become relevant when combined with genome and expression data from current and future studies of hu3F8-BsAb. Given the heterogeneity of the diagnosis and stage in this study, this objective is exploratory no formal statistical comparisons will be made.

14.3.4 To study stool microbiome

Sequencing of stool microbiota will be performed and correlated with clinical and immunological response. Given the heterogeneity of the diagnosis and stage in this study, this objective is exploratory no formal statistical comparisons will be made.

15 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming that the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed and inform the Sponsor for review and approval of eligibility of the patient.

15.2 Randomization

Not applicable

16 DATA MANAGEMENT ISSUES

A Clinical Research Coordinator (CRC) will be assigned to the study. The responsibilities of the CRC include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.1 Quality Assurance

Registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extext and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action

Random-sample data quality and protocol compliance audits will be conducted by the study team.

16.2 Data and Safety Monitoring

A Data Monitoring Committee (DMC) is established to assure patient safety and will function independently of all other individuals associated with the conduct of the trial, including site investigators participating in the trial. The DMC will consist of a minimum of 2 physicians whose expertise covers relevant specialties and, where applicable, a statistician.

During the conduct of Phase I the DMC will evaluate available safety information and recommend trial continuation or termination. If continuation is recommended, the DMC will recommend to the sponsor whether the dose should be escalated, de-escalated, or held at the same level based on the CRM.

At the completion of Phase I, the DMC will review relevant data and provide a recommendation regarding the MTD and RP2D.

During the conduct of Part 2, DMC meetings will be held on a regular basis to review cumulative safety data and evaluate whether the trial should be modified, stopped, or continue unchanged. The Sponsor Safety Committee will evaluate the recommendations from the DMC after each DMC meeting. Any significant finding/recommendation from the DMC and endorsed by the Sponsor Safety Committee will be communicated to the regulatory authorities and Institutional Review Board (IRB)/Ethics Committee (EC) as appropriate, and to the sites.

Responsibilities, procedures, content of the DMC packages, and workflow of the DMC are specified in the DMC Charter.

17 PROTECTION OF HUMAN SUBJECTS

The investigator agrees to conduct this study in accordance with the International Conference on Harmonization (ICH) principles of Good Clinical Practice and with the Declaration of Helsinki (1989). The investigator will conduct all aspects of this study in accordance with all national, state, and local laws of the applicable regulatory agencies.

- Most patients will be children, adolescents, and young adults because of the nature of these tumors. Patients of both sexes and all ethnic backgrounds are eligible for this study. Alternative treatments are available and will be discussed with patient or legal guardian.
- Consent Process: Participation in this trial is voluntary. All patients will be required to sign a statement of informed consent, which must conform to MSKCC IRB guidelines.
- Benefits: It is not known whether this treatment will improve the overall survival of the patient.
- Risks: The potential risks of this therapy as described in Section 11 of this protocol may outweigh the potential benefits in an individual patient. The potential risks are related to adverse effects that could be induced by administration of hu3F8-BsAb.

- Costs: Patients are responsible for the costs of physician visits and usual laboratory tests, hospitalizations, inpatient and outpatient care. Patients are not responsible for the cost of hu3F8-BsAb, and the costs of the research bloods: HAHA, cytokine studies, and studies on lymphocytes. Patients If there is an injury as a result of this research study, emergency care, hospitalization, and outpatient care will be made available by Memorial Hospital and billed to the patient as part of the medical expenses. No money will be provided by Memorial Hospital as compensation for research-related injury. Patients will not be paid for taking part in this study.
- Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential.

17.1 Privacy

MSK's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals/entities described in the Research Authorization form. A Research Authorization form must be approved by the IRB and Privacy Board (IRB/PB).

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with others at the time of study publication.

17.2 Adverse Event (SAE) Reporting

17.2.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a patient administered a pharmaceutical product which does not necessarily have a causal relationship with the treatment.

An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether or not considered related to the product.

An AE includes:

- A clinically significant worsening of a concomitant illness.
- A laboratory abnormality which is clinically significant, i.e. an abnormality that suggests a disease and/or organ toxicity and is of a severity that requires active management. Active management includes interventional treatment or further investigations, for example change of medicine dose or more frequent follow-up due to the abnormality.

Pre-existing condition, (i.e., a disorder present before the AE reporting period started and noted on the medical history/physical examination form) should not be reported as an AE unless the condition worsens, or episodes increase in frequency during the AE reporting period.

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. A medical condition for which an unscheduled procedure was performed should, however, be reported if it meets the definition of an AE. For example, an acute appendicitis should be reported as the AE and not the appendectomy.

17.2.2 Definition of Serious Adverse Events

Each AE is to be classified by the Investigator as either serious or non-serious. This classification of the seriousness of the AE determines the reporting procedures to be followed. An AE that meets one or more of the following criteria/outcomes is classified as serious:

- Is fatal or life-threatening[1]
- Requires inpatient hospitalization or prolongation of existing hospitalization[2]
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Medically important event[3]

[1]: The term "life-threatening" in the definition of "serious" refers to an event in which the patient, in view of either the Investigator or Sponsor, was at risk of death at the time of the event; it does not refer to an event, that hypothetically might have caused death if it were more severe. Death alone is not considered an AE; it is an outcome of an AE.

Reports of death should be accompanied by the corresponding AE term for the event that led to death. However, sudden death or death due to unexplainable cause should be reported as an SAE, while follow-up is pursued to determine the cause.

[2]: Hospitalization is defined as admission to a hospital/inpatient (irrespective of the duration of physical stay) or is not admitted to a hospital/not an inpatient but stays at the hospital for treatment or observation for more than 24 hours. Events leading to hospitalizations for the following reasons should not be reported as SAEs:

- Trial-related purposes, not associated with any deterioration in condition
- Social reasons in the absence of any deterioration in the patient's general condition
- Elective surgery or other scheduled hospitalization periods that were planned before the patient was included in this trial.

[3]: Medical and scientific judgment must be exercised in deciding whether an AE is believed to be "medically important". Medically important events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

17.2.3 Definition of Non-Serious Adverse Events

A non-serious AE is any AE which does not fulfil the definition of an SAE.

17.2.4 Adverse Event Reporting Period

17.2.4.1 Non-Serious Events

Non-serious AEs should be reported from the day of first IMP (Hu3F8 BsAb) administration until 30 days after the last IMP administration. Non-serious AEs occurring between signing the ICF and the first IMP administration must be recorded as medical history.

17.2.4.2 Serious Adverse Events

Serious AEs should be reported from signing the ICF until 30 days after the last IMP administration.

17.2.4.3 Adverse Events during Follow-up

During the follow-up period SAEs at least possibly related to IMP and new onset of secondary malignancies, regardless of causality, should be reported until end of trial.

17.2.4.4 Adverse Events with Onset after End-of-Trial

If the Investigator becomes aware of an SAE after end of trial with a suspected causal relationship to the IMPs, it should immediately be reported to Sponsor.

17.2.5 Recording of Adverse Events

All events meeting the definition of an AE must be collected and reported in the eCRF. SAEs and Dose Limiting Toxicities (DLTs) should be reported both in the eCRF and on the Clinical AE Report form.

During each contact with the trial site staff, the patient must be asked about AEs, for example by asking: "Have you experienced any problems since the last contact?" All AEs, observed by the Investigator or patient, must be reported by the Investigator.

17.2.5.1 Diagnosis

The Investigator should report the diagnosis, if available. If no diagnosis is available, the Investigator should record each sign and symptom as individual AEs using separate AE forms. For a cytokine release syndrome (CRS) event, both the diagnosis and the CRS related symptoms should be documented as individual events and graded according to Lee et. al. 2014 and CTCAE 4.0, respectively.

17.2.5.2 Onset Date and Time

Start date for an (S)AE is the date of occurrence of the first symptom. The onset of time should be reported for all events if known, as a minimum the time should be entered if the event starts on a dosing day of IMP or if the duration of the event is less than 24 hours.

17.2.5.3 End Date and Time

The end date should be filled in if the outcome of an AE is fatal, recovered/resolved or recovered/resolved with sequelae. The end time should be entered for all events for which start time

should be entered i.e. if the event starts on a dosing day of or if the duration of the event is less than 24 hours.

17.2.5.4 Severity

For all AEs, apart from cytokine release syndrome, the Investigator will use the Common Terminology Criteria for Adverse Events (CTCAE) version *4.0* to describe the severity of the AE. AEs of cytokine release syndrome will be graded using the CRS revised grading system by Lee et al.[91] described in Table 8 in Section 11.3. If the severity changes over the course of the event, the grade assigned by the Investigator should be the most severe, that occurred during the AE period.

17.2.5.5 Outcome

Investigator must judge outcome of the AE by the following terms:

- Death
- Not Recovered
- Recovered
- Recovered or Resolved with Sequelae
- Recovering or Resolving
- Unknown *

*Should only be used if patient is lost to follow-up.

17.2.5.6 Relationship to Investigational Medicinal Product

The Investigator must assess whether the event is related to the IMP (Hu3F8 BsAb). A suspected adverse drug reaction is defined as one in which there is a reasonable possibility that the IMP caused the AE. Relatedness must be assessed and reported from the first time the AE is being reported. When assessing the causal relationship of an AE to an IMP, the following should be taken into consideration:

<u>Not related (unlikely)</u>

The AE is not related to the IMP which means the AE:

- Does not follow a reasonable temporal sequence from IMP administration
- Is readily explained by the patient's clinical state or by other modes of therapy administered to the patient
- Is clearly not related to the IMP

<u>Possibly Related</u>

- The AE follows a reasonable temporal sequence from IMP administration but could have been produced by the patient's clinical state, medical history, or the trial procedures/conditions.

Alternative etiology should be provided for all AEs assessed as possibly related to IMP.

Probably Related

The AE is probably related to the IMP, which means the AE:

- Follows a reasonable temporal sequence from IMP administration
- Abates spontaneously upon discontinuation of the IMP (de-challenge) without any curative treatment
- Is confirmed by reappearance of the same reaction on repeat exposure (re-challenge) (if applicable)
- Cannot be reasonably explained by the known characteristics of the patient's clinical state or medical history

Definite

The AE is clearly related to the IMP, with no other possible alternative etiologies

17.2.5.7 Action Taken with Investigational Medicinal Product

The action taken with the IMP should be noted as:

- Dose Increased
- Dose Not Changed
- Dose Decreased
- Administration Interrupted
- Treatment Discontinued
- Not applicable*
- Unknown
- Hospitalized

-

*: should be used if the AE occurs before first treatment, IMP has been discontinued for other reasons or after end of treatment.

17.2.6 Events Requiring Immediate Reporting

The following events require reporting to Sponsor within 24 hours of knowledge (for details see Section 17.2.7):

- SAE
- DLT
- Pregnancy

17.2.6.1 Pregnancy

Any pregnancy, including partner pregnancy, that occurs during trial participation must be reported to Sponsor within 24 hours of knowledge using the pregnancy form. Pregnant trial patients must be discontinued from IMP treatment immediately (see Section 13). The pregnancy must be followed up to determine outcome and status of mother and child. The child must be followed at least to the age of one month. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE.

17.2.7 Initial Reporting of AEs

SAEs/DLTs:
The paper Clinical AE Report form must be reported from site to Sponsor within 24 hours of the Investigator's first knowledge of the event. The paper Clinical AE Report form is to be sent to the designated drug safety provider.

All AEs:
The eCRF AE form should be updated in accordance with agreed data entry timelines.

17.2.8 Contact details for reporting

Completed paper Clinical AE Report forms and paper pregnancy forms must immediately be reported to:

safetymailbox@ymabs.com
In emergency situations, the completed Clinical AE Report forms or pregnancy forms can be faxed to:
FAX +45 7879 6060

17.3 Follow-Up on AEs

SAE/DLTs:

- New Follow-up information available at site must be reported within 24 hours of knowledge.
- Follow-up information requested from Sponsor must be replied to within three working days. The eCRF AE form should be updated in accordance to agreed data entry timelines
- If an ongoing SAE changes in intensity, relationship to IMP or as new information becomes available for the event, the paper Clinical AE Report Form should be completed and sent to the designated drug safety provider within 24 hours of the change in assessment.
- Grade 3 or higher non-serious AEs that are considered treatment related and all SAEs (including DLTs) should be followed on a regular basis, according to the Investigator's clinical judgment, until the event has been resolved or until the Investigator can assess it as chronic or stable. This includes follow-up after end of treatment.

Non SAEs:

- Non-serious AEs should be followed until they are either resolved, returned to baseline, or until the end of trial for the patient, whichever comes first.

17.4 Reporting of SUSARs

Sponsor will ensure that all relevant information about Suspected Unexpected Serious Adverse Reactions (SUSARs) is reported to regulatory authorities in accordance with regulatory requirements.

The Contract Research Organization (CRO) appointed by Sponsor will notify Investigators of SUSARs in accordance with local requirements. Furthermore, Investigators will be informed of any trial related SAEs that may warrant a change in any trial procedure. The CRO appointed by Sponsor will inform the Institutional Review Boards (IRBs) of SUSARs in accordance with local requirement, unless locally this is an obligation of the Investigator.

The Sponsor assessment of expectedness for the IMP (Hu3F8 BsAb) will be performed according to the current version of the Investigator's Brochure.

17.5 Communication of Significant Safety Issues

In the event of any significant safety related issues, the Sponsor will decide upon immediate action to be taken and will communicate to regulatory authorities, Investigators, IRB, and patients as needed within regulatory timelines.

18 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion

in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20 APPENDICES

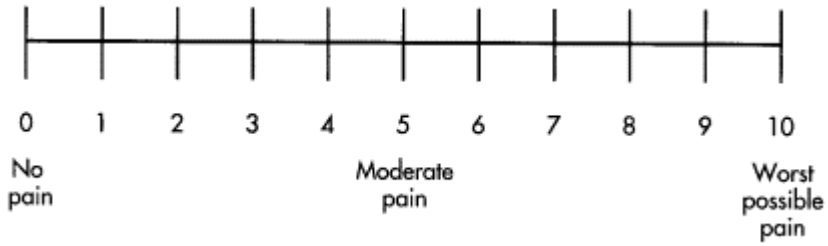
Appendix A Pain Scales

FLACC SCALE

CATEGORIES	SCORING		
	0	1	2
FACE	No particular expression or smile	Occasional grimace or frown, withdrawn, disinterested.	Frequent to constant quivering chin, clenched jaw.
LEGS	Normal position or relaxed.	Uneasy, restless, tense.	Kicking, or legs drawn up.
ACTIVITY	Lying quietly, normal position moves easily.	Squirming, shifting back and forth, tense.	Arched, rigid or jerking.
CRY	No cry, (awake or asleep)	Moans or whimpers; occasional complaint	Crying steadily, screams or sobs, frequent complaints.
CONSOLABILITY	Content, relaxed.	Reassured by occasional touching hugging or being talked to, distractible.	Difficulty to console or comfort

Manworren RC, Hynan LS. Clinical validation of FLACC: preverbal patient pain scale. Pediatr Nurs 2003;29:140-6.
Malviya S, Voepel-Lewis T, Burke C, Merkel S, Tait AR. The revised FLACC observational pain tool: improved reliability and validity for pain assessment in children with cognitive impairment. Paediatr Anaesth 2006;16:258-65.
Merkel SI, Voepel-Lewis T, Shayevitz JR, Malviya S. The FLACC: a behavioral scale for scoring postoperative pain in young children. Pediatr Nurs 1997;23:293-7.

Numeric Pain Rating Scale

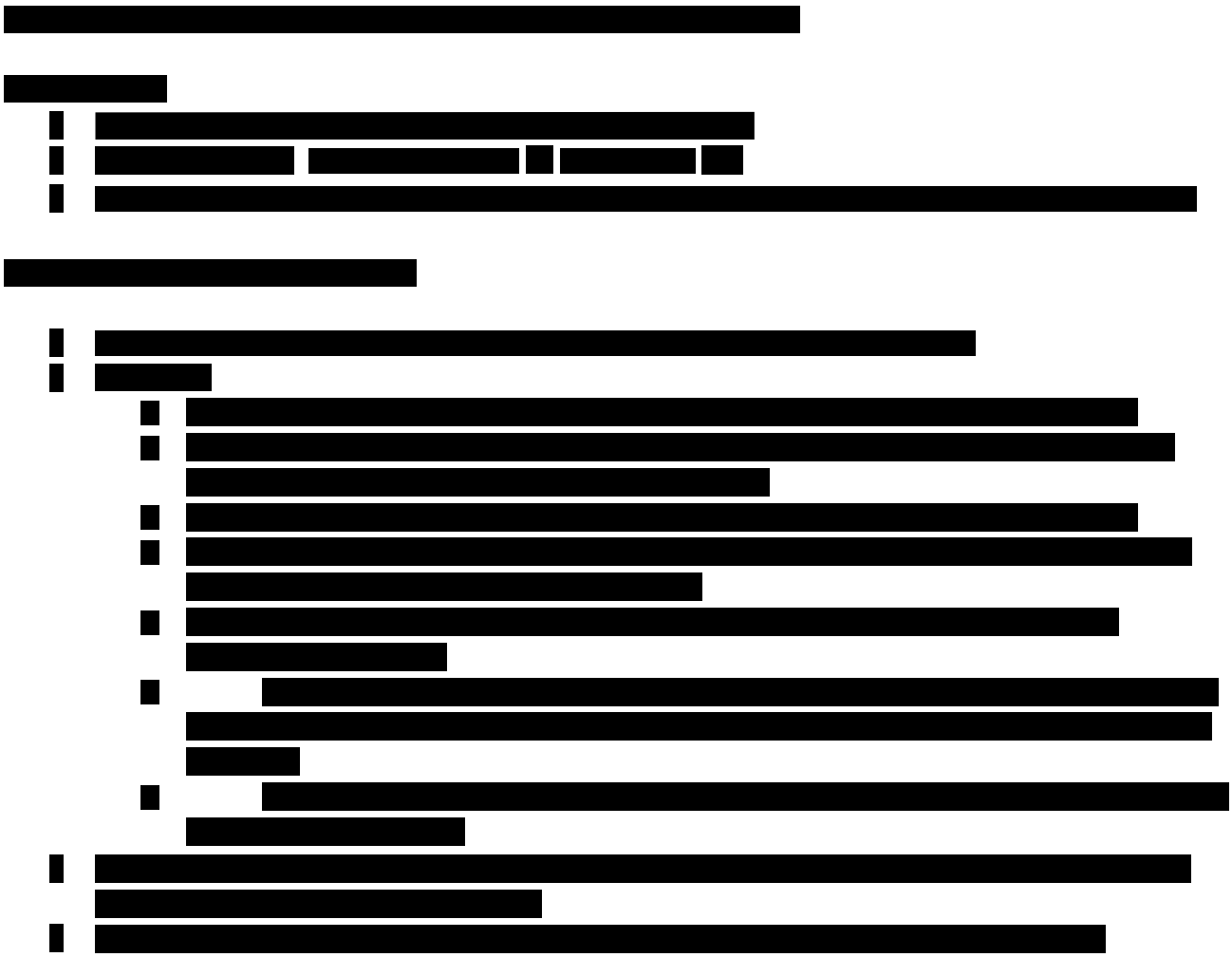


Faces Pain Rating Scale

Wong DL, Baker CM. Pain in children: comparison of assessment scales. Pediatr Nurs 1988;14:9-17.

Appendix B

Memorial Sloan Kettering Cancer Center
Pediatric Pharmacy
IRB #18-034
Humanized 3F8 Bispecific Antibody (Hu3F8-BsAb) Standard
Operating Procedure for Dose Preparation



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Approval	[redacted] 14-Jan-2022 08:14:24 GMT+0000

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