Hu3F8/GM-CSF Immunotherapy Plus Isotretinoin for Consolidation of First Remission of Patients with High-Risk Neuroblastoma: A Phase II Study

MSK THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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ACRONYMS and ABBREVIATIONS

ADCC, antibody-dependent cellular cytotoxicity AE, adverse event
ANC, absolute neutrophil count
ASCT, autologous stem-cell transplantation BM, bone marrow
CBC, complete blood count
CMC, complement-mediated cytotoxicity
COG, Children's Oncology Group
CT, computed tomography CR, complete remission
CRC, Clinical Research Coordinator
CRDB, Clinical Research Database
CTCAE, Common Toxicity Criteria for Adverse Events
DSM, Data and Safety Monitoring
DSMB, Data and Safety Monitoring Board
DSMC, Data and Safety Monitoring Committee
GM-CSF, granulocyte-macrophage colony-stimulating factor
HACA, human anti-chimeric
antibody HAHA, human anti-human antibody / HAMA, human anti mouse antibody
HR-NB, high-risk neuroblastoma
ICH, International Conference of Harmonization
IL-2, interleukin-2
IME, important medical events
IND, investigational new drug
IRB/PB, Institutional Review Board/Privacy Board
iv, intravenous
KIR, killer immunoglobulin-like receptor
LAR, legally authorized representative
LFTs, liver function tests
mAb, monoclonal antibody MIBG, metaiodobenzylguanidine
MRD, minimal residual disease
MRI, magnetic resonance imaging
MSKCC, Memorial Sloan Kettering Cancer Center
NB, neuroblastoma
NCI, National Cancer Institute
NIH, National Institutes of Health
OS, overall survival
PBMC, peripheral blood mononuclear cell
PD, progressive disease
PET, positron emission tomography
PI, Principal Investigator
PFS, progression-free survival
PMN, polymorphonuclear cell
PO, orally
PPR, Protocol Participant Registration
q, every
qRT-PCR, quantitative reverse-transcription-polymerase chain reaction QA, quality assurance
RA, Research Authorization
SAE, serious adverse event
sc, subcutaneous
TPN, total parenteral nutrition UHR, ultra-high risk
VGPR, very good partial remission

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This phase II study of the anti-G_{D2} humanized IgG1 monoclonal antibody (mAb) hu3F8 combined with granulocyte-macrophage colony stimulating factor (GM-CSF) will assess relapse-free survival (RFS) of patients with high-risk neuroblastoma (HR-NB) who are in first complete/ver y good partial remission (CR/VGPR). The hu3F8 dosage is three-fold greater than the dosage of other antiG_{D2} mAbs in widespread use for HR-NB, including murine 3F8 (m3F8). We will apply real-time quantitative reverse-transcription-polymerase chain reaction (qRT-PCR) methodology for monitoring minimal residual disease (MRD) in bone marrow (BM); we will test the hypothesis that the MRD response in BM after 2 cycles of hu3F8/GM-CSF has prognostic importance. Isotretinoin will be started after patients are evaluated for response to hu3F8/GM-CSF.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objective

- Assess the impact of hu3F8/GM-CSF on RFS of HR-NB patients in first CR/VGPR.

Secondary Objective

- Apply real-time qRT-PCR targeting a MRD marker panel comprising of *CCND1*, *GD2 synthase*, *ISL1*, and *PHOX2B* to test the hypothesis that the MRD findings in BM after the second cycle of hu3F8+GM-CSF have significant correlation with outcome.

3.0 BACKGROUND AND RATIONALE

3.1 <u>Disease background:</u> NB is the most common extracranial solid tumor of childhood; 50-60% of patients present with an unresectable primary tumor and metastases in BM.[1,2] Intensive induct ion chemotherapy and aggressive surgery have improved remission rates in young patients;[3-5] results have been less impressive in adolescents and adults in whom NB is especially chemoresistant. [6 - 8] Eradicating MRD has remained a formidable challenge. Post-surgical use of local radiotherapy helps control MRD in the primary site.[9] Myeloablative therapy with autologous stem-cell transplantat ion (ASCT) and treatment with the vitamin A derivative isotretinoin (13-*cis*-retinoic acid) are commonly used to eradicate MRD in distant sites.[10] Attempts at treatment intensification have met with both acute and long term side effects, both of grave concern for young patients. There is a scarcity of promising new agents, and to date, few if any target/pathway-specific small molecules have shown major clinical benefit in patients with NB, although many promising leads continue to emerge.[11] With a cure rate of <30% at toxicity limits among stage 4 patients diagnosed at ≥18 months of age, there is substantial room for improvement.[12]

3.2 <u>Rationale for anti-GD2 antibody for HR-NB:</u> Several factors make NB well suited for mAbtargeted immunotherapy. First, mAbs mediate highly efficient antibody-dependent cell mediated cytotoxicity (ADCC) of NB in the presence of human leukocytes. Second, mAbs induce complementmediated cytotoxicity (CMC) of NB cells, which lack decay accelerating factor CD55[13] and homologous restriction factor CD59.[14] Complement deposition on NB cells enhances ADCC through activation of the iC3b receptor on neutrophils,[15,16] available even after dose-intensive or myeloablative chemotherapy plus stem cell transplantation, if colony stimulating factors are given. [17] Third, the use of intensive chemotherapy (standard of care for HR-NB) to achieve clinical remissio n

causes prolonged T-lymphopenia and immunosuppression,[18] such that patients are less likely to reject chimeric or humanized mAbs.[19] Fourth, unlike chemotherapy or radiotherapy, mAbs are not myelosuppressive or genotoxic, generally with few long-term toxicities. These are critical considerations for young children. Finally, mAb is effective against metastatic cancer in blood, BM, and bone, typically found in HR-NB.

GD2 an ideal target for mAb-based therapy in NB: G_{D2} is an adhesion molecule abundant on NB. It is also widely expressed among melanoma, small cell lung cancer, bone or soft tissue sarcoma, retinoblastoma and brain tumor.[20] It is rarely expressed in normal tissues except neurons, skin cells and pain fibers. This antigen is genetically stable, rarely lost and relatively inert on the cell membrane. Scintigraphy studies using radiolabeled mAb confirms excellent tumor targeting. [20] At least two anti-NB antibody families have been tested clinically, i.e., 3F8[21] and 14.18.[22] Chimeric ch14.18 consists of the variable region of murine mAb 14.18 and the constant regions of human IgG1κ.[23] It demonstrates ADCC and CMC of NB and melanoma cells in vitro.[24-27] Based on encouraging clinical responses in phase I studies, ch14.18 was tested in large phase II studies as consolidation therapy for stage 4 NB (German NB90 and NB97 studies). For the 166 patients >12 months at diagnosis, even though event-free survival (EFS) was similar in patients receiving ch14.18 compared to patients on maintenance chemotherapy, overall survival (OS) was improved, and the rate of BM relapse reduced in the ch14.18 arm.[28,29] The Children's Oncology Group (COG) reported a randomized phase III trial showing a significantly superior PFS and OS at 2 years with the use of ch14.18+GM-CSF+IL-2 in patients in remission post-ASCT (ClinicalTrials.gov NCT00026312).[30]

Efficacy of m3F8 plus a single cytokine GM-CSF against primary refractory NB: m3F8, a murine IgG3 mAb specific for G_{D2}, induces cell death, and mediates efficient ADCC and CMC against NB in vitro.[20] ADCC appears to play an important role also in vivo. Among 80 patients with ultra-high-risk NB, (i.e., stage 4 diagnosed at $\geq$18 months of age with chemoresistant BM disease despite dose-intensive induction plus aggressive salvage therapy), 80% achieved BM remission with m3F8/GM-CSF.[31] This response rate was much higher than when m3F8 was used without any cytokine. Patients who received subcutaneous (sc) GM-CSF (ClinicalTrials.gov NCT00072358) had significantly longer PFS and OS than those who received intravenous (iv) GM-CSF (ClinicalTrials.gov NCT00002560). Post-GM-CSF absolute neutrophil counts (ANCs) and activated adhesion molecule (CD11b) expression on granulocytes were significantly higher in the sc GM-CSF group.[32] Of note, murine IgG mAbs, including m3F8, have preferential binding to the FcγR2A-R131 over the FcγR2AH131 present on myeloid effectors. The association of *FCGR2A* polymorphism and patient outcome was consistent with an anti-tumor effect of myeloid-ADCC.[33]

Efficacy of m3F8 plus single cytokine GM-CSF and 13-*cis*-retinoic acid for high-risk patients in first remission (Table 1)[34]: Given the activity of m3F8 against chemoresistant marrow disease, the use of m3F8 was expanded to patients in first remission. 169 children diagnosed with stage 4 NB (1988-2008) were enrolled in consecutive anti-G_{D2} m3F8±GM-CSF±13-*cis*-retinoic acid (CRA) protocols after achieving first CR/VGPR. Regimen A (n=43 high-risk [HR] patients) used m3F8 alone; Regimen B (n=41 HR) used m3F8+ivGM-CSF+CRA, following ASCT; Regimen C (n=85) used m3F8+scGM-CSF+CRA, 46/85 following ASCT, and 28/85 requiring additional induct ion therapy and therefore labeled by the MSK investigators as ultra-high-risk (UHR). Marrow MRD was measured by qRT-PCR. Survival probability was calculated by Kaplan-Meier method, and prognostic variables were analyzed by multivariate Cox regression model. At 5 years from the start of immunotherapy, PFS improved from 44% for HR patients on Regimen A, to 56% on Regimen B, and 62% on Regimen C. OS was 49%, 61%, and 81%, respectively. PFS and OS of UHR patients were 36% and 75%, respectively. Relapse was mostly at isolated site. Independent adverse prognostic

factors included UHR (PFS) and Post–MRD (i.e., post-cycle-2 MRD) (PFS and OS), whereas the prognostic factors for improved outcome were missing killer immunoglobulin- like receptor (KIR) ligand (PFS and OS), human anti-mouse antibody (HAMA) response (OS), and Regimen C (OS).[34] In the predecessor trial (IRB 03-077) using m3F8 at standard dosage of 100 mg/m^2/cycle, combined with GM-CSF, plus isotretinoin, the 56 patients treated in 1st CR/VGPR after conventional therapy achieved 70% two-year RFS.

	Table 1: Patient Outcome, m3F8 as adjuvant*							
Regimen	**Treatment group**	**SCT**	**N**	**PFS**	**OS**	**N**	**PFS**	**OS**
A	m3F8 only	-	43	44%	49%			
B	m3F8+(iv)GM-CSF+ CRA	+	41	56%	61%			
C	m3F8+(sc)GM-CSF+ CRA	+	57	62%	81%	37*	69%	78%
		-				20	50%	84%
		+	28**	36%	75%	9*	44%	78%
		-				19	32%	74%

*SCT=autologous stem cell transplantation; CRA = 13-*cis*-retinoic acid; GM-CSF = granulocyte-macrophage colony stimulating factor; iv = intravenous; sc = subcutaneous; ** Ultra high-risk (UHR) patients received additional cyclophosphamide ± topotecan or irinotecan for refractory disease after standard induction therapy before m3F8 immunotherapy.

Prognostic variables following m3F8+GM-CSF[34]: Variables with univariate p<0.1 were included in the multivariable model (Table 2). Regimens A and B were combined into a single category, since there was no significant difference between their effectiveness in the multivariable models. Positive Post-MRD (a measure of lack of responsiveness to immunotherapy) was an independent adverse prognostic factor for PFS and OS, while UHR (a measure of refractoriness to induction) was independently prognostic for adverse PFS. In contrast, missing KIR ligand had a favorable influe nce on PFS and OS. For OS, having HAMA response, and the addition of sc GM-CSF in Regimen C versus no GM-CSF (Regimen A) or iv GM-CSF (Regimen B) had an independent positive impact. However, the definitive proof of efficacy contributed by Regimen C will require randomized comparisons. When survival after relapse was analyzed, time-to-relapse and Regimen C were independent predictors of OS in a multivariable model.[34]

Table 2: Multivariable Analysis				
Variables Tested	Progression-free Survival		Overall Survival	
	p value	Hazard Ratio (95% CI)	p value	Hazard Ratio (95% CI)
(UHR) Refractory to induction	0.02	2.2 (1.2, 4.4)	-	-
ASCT consolidation before immunotherapy	0.41	0.82 (0.51, 1.3)	-	-
Positive post-MRD	<0.001	6.52 (3.9, 10.8)	<0.001	7.9 (4.4,14.3)
Missing KIR ligand	0.05	0.62 (0.38, 1)	0.045	0.55 (0.31,0.99)
HAMA response	-	-	<0.001	0.36 (0.21,0.64)

Addition of (sc)GM-CSF + CRA (Regimen C)	0.58	0.85 (0.49, 1.6)	0.026	0.52 (0.29,0.92)

3.3 <u>Rationale for quantifying MRD in BM by qRT-PCR:</u> Curative strategies for stage 4 NB must encompass control of both the primary mass and distant disease. Dose-intensive chemotherapy has had a favorable impact on resectability of primary tumors and surgical removal of all visible tumor, followed by local radiotherapy, reduces the risk of relapse in the primary site to <10%.[9] Dose-intensive chemotherapy eliminated histologically evident BM involvement in 90% of high-risk cases. These encouraging results have shifted the focus of curative strategies to the problem of MRD – which is readily assessed in BM. Adjuvant treatments, such as immunotherapy with mAbs, may make gradual and not quantum changes in MRD, which by definition is beyond the sensitivity of standard histologic or radiologic techniques. The availability of more sensitive methods for measuring occult NB cells in sequential BM specimens can allow determination of the efficacy and optimal duration of adjuvant treatment. The absence of detectable tumor cells in BM can serve as a surrogate end point for the adjuvant treatment strategy, while quantitative data on BM disease will help detect relapse earlier and indicate a need for a change in treatment. We and others have reported a series of studies aimed at improving the utility of BM testing, focusing on immunological and molecular methods for identifying MRD.[35-37] The limit of detection for immunocytology is $1/10^5$ to $1/10^6$ cells; qRT-PCR has slightly superior sensitivity with a range of $1/10^5$ to $1/10^7$ cells. The results suggest that using a panel of sensitive markers, BM MRD is highly predictive of clinical outcome for m3F8+GMCSF immunotherapy in patients in first CR/VGPR,[34] those with primary refractory disease,[31] and patients in second remission.[38] A MRD marker panel of 4 genes has been adopted for all future studies in NB: cyclin D1 (*CCND1*), ISL LIM homeobox 1 (*ISL1*), paired-like homeobox 2b (*PHOX2B*), and GD2 synthase (*B4GALNT1*). Among patients treated with m3F8/GM-CSF in first CR/VGPR (Table 2), early MRD response was the strongest independent prognostic factor of both PFS and OS.[34]

3.4 <u>Rationale for hu3F8:</u> These favorable clinical outcomes with m3F8 would not be possible if m3F8 could not be given in multiple treatment cycles over months. Early (before the 4th cycle of m3F8) HAMA can delay treatment, prevent further cycles, or more importantly compromise the anti-tumor effects of mAb treatment. An analysis of a recent 5-year experience with m3F8/GM-CSF in patients in 1st CR/VGPR showed HAMA first becoming (+) in 2/66 (2%) patients post-cycle 1, in 7/61 (11%) patients post-cycle 2, in 5/54 (9%) patients post-cycle 3, and in 1/52 (1%) of patients post-cycle 4. Patients with CNS/leptomeningeal disease benefit from repeated intrathecal ^{131}I-m3F8 injections partly because HAMA cannot cross the blood-brain barrier.[39,40] Reducing or removing mouse epitopes should reduce HAMA response, but even chimeric mAbs can induce human anti-chimeric antibody (HACA) unless the patient is immunocompromised.[41] An obvious strategy was to humanize m3F8.

hu3F8 was made using standard recombinant methods, transduced into CHO-DG44 cells and selected for high IgG expression. It was purified by protein A affinity chromatography, anion exchange membrane, viral and bacterial filters. hu3F8 has advantages over 14.G2a and its chimeric form ch14.18 in affinity and antigen crossreactivity[42]: 1) 10-fold slower k_{off} which translates into 10-fold higher affinity; 2) since affinity for antigen is a key determinant of efficacy in mAb therapy, [43] only m3F8 demonstrated direct cytotoxic ity on tumor cells in vitro in the absence of luekocytes or serum complement; 3) a slow k_{off} allows longer retention of mAb on the tumor cell surfaces, translating into more efficient ADCC and CMC; and 4) since m3F8 cross-reacts with GD1b, although

at low levels (4%), it is applicable for another common ganglioside in NB. Early (before the 4th cycle of mAb) development of HAMA or HACA can delay treatment, prevent further cycles, or more importantly compromise the anti-tumor effects of mAb treatment. Our phase I study of hu3F8 suggests that development of human anti-humanized antibody (HAHA) is much less common resulting in fewer treatment delays. Both blood mononuclear cell (PBMC)-ADCC and neutrophil (PMN)-ADCC of hu3F8 were superior (10 to >100 fold based on EC50, Table 3) to that of m3F8 in vitro, while CMC was slightly less intense. This superiority was consistently observed in ADCC assays, irrespective of whether donor leukocytes or NK92 cells transfected with human CD16 or CD32 were used as killers.

Cross-reactivity with other gangliosides was similar to that of m3F8.

Table 3: Potency of anti-GD2 antibodies in vitro

Antibody	*ADCC-PBMC*	*ADCC-PMN*	*CMC*
14.G2a	0.03	1	0.46
ch14.18	11	3	0.16
hu3F8	217	19	0.40
m3F8	1	1	1

Clinical significance of ADCC: This large improvement in ADCC is most desirable given recent evidence for its role in the anti-tumor effects of mAbs in patients. Among lymphoma patients treated with rituximab, both high affinity FcR2A and FcR3A were shown to have better response and survival advantage.[44] While the high affinity receptor FcR3A translated into <10 improvement in ADCC in vitro,[45] overall response and time to progression improved by 200%.[44] When metastatic breast cancer was treated with Herceptin, patients with high affinity FcR3A had better overall response
(83% versus 35%, p=0.03), and longer PFS (p=0.005).[46] For metastatic colorectal cancer treated with Cetuximab, patients with low affinity FcR2A and FcR3A had comparable hazard ratios as patients with mutated *KRAS*.[47] With m3F8, patients with the high affinity FcR3A receptor for m3F8 on myeloid cells were shown to have better survival.[33]

Clinical significance of CMC: CMC is highly effective against human NB[48] because NB has low expression of CD55[13] and CD59.[14] Anti-G_{D2} mAbs mediate efficient CMC, and m3F8 seems particularly efficient. Yet studies of rituximab have suggested a negative role of complement activation in down regulating ADCC.[49] In clinical studies, higher activity of complement component C1qA was associated with less favorable response to rituximab therapy.[50] For anti-G_{D2} mAbs, complement activation was thought to be largely responsible for the pain side effects; hence the Fc-CH2 domain mutated version (hu14.18K322A).[51] Given these considerations over-drive of CMC is probably not desirable. It is reassuring that hu3F8 had less but still substantial CMC compared to m3F8.

3.5 Current status of anti-GD2 immunotherapy for HR-NB: There are currently two anti-G_{D2} antibodies in clinical use. As discussed above, we have used m3F8 in phase II studies with demonstrated clinical efficacy.[31,34,38] Although toxicities are tolerable, pain and allergic reactions are clinically significant in the short term and development of HAMA often precludes repeated administration. The COG randomized phase III trial showing the efficacy of anti-G_{D2} immunotherapy

combined ch14.18 with two cytokines, GM-CSF and IL-2.[30] However, IL-2 is associated with significant toxicity and, like other chimeric mAbs, ch14.18 can induce a HACA response.[52] When m3F8 was combined with a single cytokine GM-CSF (Tables 1 and 2), the toxicity profile was substantially less compared to ch14.18+GM-CSF+Il-2.[34] In addition, the 2-year PFS and OS were 79% superior to ch14.18+GM-CSF+IL-2. We therefore studied the third generation anti-G_{D2} mAb hu3F8 combined with GM-CSF.

3.6 Rationale for GM-CSF: GM-CSF can amplify m3F8 anti-NB activity in patients via effects on granulocytes and macrophages.[32] Other reasons for using GM-CSF with hu3F8 include: 1) granulocyte production is only transiently suppressed by chemotherapy, and GM-CSF increases neutrophil and eosinophil production; 2) GM-CSF is well tolerated compared to other cytokines such as IL-2;[30,53] and 3) GM-CSF can activate monocytes-macrophages to phagocytose NB cells, and enhances the proliferation, maturation, and function of antigen-presenting cells that promote host antitumor response. Myeloid activation markers on granulocytes (CD11a, CD63, CD87, CD11b and its activation epitope CBRM1/5) were increased following GM-CSF therapy in HR-NB patients. For CBRM1/5, the increase was in both frequency and mean fluorescence intensity. CBRM1/5 activatio n was significantly higher after scGMCSF than after ivGMCSF. CBRM1/5 activation was strongly correlated with patient outcome in a multivariable model.[32] The favorable results of m3F8+GM-CSF in HR-NB (Table 1) provide a compelling rationale for combining hu3F8 with GM-CSF.

3.7 Phase I trial of hu3F8+GM-CSF: Dose-limiting toxicity (DLT) of hu3F8+GM-CSF was defined as ≥grade 3 toxicities occurring through 21 days from the 1st dose of hu3F8 in cycle 1. DLT occurred in only four of the 57 study patients treated through dose level 15 (3.3 mg/kg/dose): a grade 4 anaphylactic reaction with the 1st dose in two patients (including in one who had a similar reaction with m3F8), transient renal impairment in one patient, and transient hypertension in one patient. The latter was the only DLT among the 9 patients who received multiple cycles at dose levels 14 and 15, and would not be a reason for coming off a phase II trial. Otherwise, dose-escalation proceeded uneventfully. The only other grade 4 toxicities were an anaphylactic reaction with the 1st dose of 6th cycle in one patient and angioedema after the 3rd dose of 2nd cycle in one patient; these patients were the only study subjects to come off protocol because of toxicity other than DLT. Common side effects were as expected with anti-G_{D2} mAbs, namely, grade 1-2 pain, urticaria, and cough. Treatment was outpatient. Pharmacokinetic studies showed a rise in serum levels through dose level 13 (2.8 mg/kg/dose); the next dose level (14) was chosen for a phase II trial based on absence of MTD and the aim to assure optimal serum levels, given inter-patient variability. HAHA developed as follows : postcycle 1 in four patients (one not previously treated with anti-G_{D2} mAb, one previously HAMA-positive to m3F8, and two previously treated with both the chimeric anti-G_{D2} mAb ch14.18 and m3F8) and after cycle 2 in seven patients (one not previously treated with anti-G_{D2} mAb, two previous ly treated with m3F8, and four previously treated with ch14.18).

3.8 Isotretinoin (13-*cis*-retinoic acid) was shown in a randomized national study to decrease the risk of relapse in patients treated in complete remission post-ASCT.[8] This agent subsequently became standard of care for NB patients in complete remission. It will be used in this protocol after patients are evaluated for MRD response to hu3F8/GM-CSF.

3.9 Significance and implications: Various strategies have been applied to induce or augment immune- mediated attack against cancer. However, few clinical trials have successfully used mAbtargeted immunotherapy against solid tumors in children or adults, especially with ADCC as the key underlying immune mechanism. Building on the favorable results of m3F8, hu3F8 has several potential advantages over m3F8 and ch14.18, including: 1) low immunogenicity allowing repeat treatments over years, 2) greatly improved ADCC potency, 3) longer serum half-life reducing the necessity of daily injections, and 4) substantial reduction of pain side effects, anaphylactic or anaphylactoid reactions as well as immune complex diseases.

This phase II trial exploits recent findings that promise to optimize the anti-NB activity of antiG_{D2} antibody-based immunotherapy. Furthermore, qRT-PCR analysis of MRD response in post-cycle 2 and 5 BM, which has proven to be informative in past studies, will be applied to monitor anti-NB activity of the immunotherapy, with an emphasis on early recognition of treatment efficacy or responsivene ss. Risk-related molecular guidelines can be rapidly incorporated into future treatment programs. With NB, where randomized trials are difficult to carry out because of the small number of patients, such quantitative analyses of future adjuvant strategies have great utility for rapidly identifying both the best agent and its optimal use in a curative treatment program.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

In this phase II single arm trial, patients with HR-NB in first CR or VGPR undergo consolidation by using hu3F8/GM-CSF x5 cycles and isotretinoin x6 cycles. Isotretinoin starts after cycle 2 of hu3F8/GM-CSF, allowing assessment of MRD response to hu3F8/GM-CSF. RFS and MRD responses will be compared to those in the predecessor trials which used m3F8/GM-CSF (IRB# 03077).

4.2 Intervention

Road Map/Schema: Allowing flexibility for holidays, family travel, bed availability, etc., the break between end of a cycle of hu3F8/GM-CSF and start of next cycle is 2-to-4-weeks through 4 cycles, then the interval is up to 6-8 weeks.

Cycle 1 hu3F8 (iv) + GM-CSF (sc) 2-4-wk interval

Cycle 2 hu3F8 (iv) + GM-CSF (sc) 2-4-wk interval* – oral isotretinoin x14 days

Cycle 3 hu3F8 (iv) + GM-CSF (sc) 2-4-wk interval – oral isotretinoin x14 days

Cycle 4 hu3F8 (iv) + GM-CSF (sc) 6-8-wk interval – oral isotretinoin x14 days on, x14 days off, x14 days on

Cycle 5 hu3F8 (iv) + GM-CSF (sc) - oral isotretinoin x14 days, x14 days off, x14 days on

* **assessment of BM status by qRT-PCR and by standard histology**

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 HUMANIZED MONOCLONAL ANTIBODY hu3F8-IgG1 (IND-112594)

Hu3F8-IgG1 is a humanized antibody of the IgG1 subclass. Hu3F8 is manufactured under GMP conditions by a contracted manufacturer. The final product is manufactured, formulated and tested for identity, purity, potency, sterility, endotoxin and stability as described in the CMC section of IND 112594. The purified antibody hu3F8 is stored at 2-8° C in glass vials, at 4mg/ml. Stability of a punctured naxitamab vial is 6 hours after initial puncture at controlled room temperature (15 – 25° C), provided aseptic conditions are kept. Stability of prepared dose of naxitamab for administration is ≤ 12 hours at controlled room temperature (15 – 25° C), or ≤ 24 hours if refrigerated (2 – 8° C). For IV use, naxitamab should be administered in a diluted 5% human serum albumin and normal saline solution, in the volumes mentioned on Table 1 of the Investigational Medicinal Product Manual.

5.2 Sargramostim (YEAST-DERIVED HUMAN RECOMBINANT GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR; GM-CSF)

5.2.1 ***Source and Pharmacology:*** Yeast derived recombinant human GM-CSF.

5.2.2 ***Supplier:*** LEUKINE (sargramostim) is distributed by Genzyme Corporation, Cambridge, MA and manufactured by Bayer HealthCare Pharmaceuticals, Seattle, WA.

5.2.3 ***Formulation and Stability:*** Sargramostim is available as a sterile, white, preservative free, 250 mcg single use vial of lyophilized powder or a 500 mcg/1 mL injectable solution.

The 250 mcg vials require aseptic reconstitution with 1.0 mL Sterile Water for Injection, USP (without preservative). During reconstitution, the Sterile Water for Injection, USP should be directed at the side of the vial and the contents gently swirled to avoid foaming during dissolution. Avoid excessive or vigorous agitation; do not shake. The reconstituted sargramostim solutions are clear, colorless, isotonic with a pH of 7.4 ± 0.3, and contain 500 mcg/mL of sargramostim. The single-use 250 mcg vials contain no antibacterial preservative, and therefore should be administered as soon as possible, and within 6 hours following reconstitution. They are intended for single use only and should not be re-entered or reused. Do not save any unused portion for later administration. Do not use beyond the expiration date printed on the vial.

Vials containing 500 mcg/1 mL of sargramostim are already in solution and are multiple dose vials. For subcutaneous administration, further dilution is not required. Liquid sargramostim may be stored for up to 20 days at 2-8°C once the vial has been entered. Discard any remaining solution after 20 days.

Store sargramostim powder, or reconstituted solutions, under refrigeration at 2-8°C (36-46°F); do not freeze or shake.

Aseptic technique should he employed in the preparation of all sargramostim solutions. To assure correct concentration following reconstitution, care should be exercised to eliminate any air bubbles from the needle hub of the syringe used to prepare the diluent. Parenteral products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

5.2.4 ***Route of Administration:*** Subcutaneous

5.3 13-*cis*-Retinoic Acid (NDC #0004-0155-01,0004-0169, 0004-0156-01ISOTRETINOIN, ACCUTANE)

5.3.1 **Source and Pharmacology:** The exact mechanism of retinoic acid (RA)-induced maturation of tumor cells is not known. In neuroblastoma cell lines it has been shown to down regulate MYCN RNA and protein expression, and such down regulation correlates with the ability of isotretinoin to induce tumor cell growth arrest. Recent studies using gene transfection directly implicate down regulation of MYCN expression by isotretinoin as a key event in achieving sustained arrest of neuroblastoma cell proliferation. RA also appears to enhance normal hematopoietic differentiation by increasing the responsiveness of myeloid and erythroid progenitor cells to the action of myeloid colony stimulat ing activity and erythropoietin,. respectively. Metabolism: RA is 99.9% bound in plasma (almost entirely to albumin) and has a half-life of 10-20 hours. The major metabolite is 4-oxoisotretinoin, and excretion is in the urine and feces. A single oral dose of 100mg/m2 isotretinoin will produce peak plasma levels of 1-2mM. The mean peak time as 3.2 hours after 80mg orally, with a terminal $t_{½}$ of 10 to 20 hours. Administering 160 mg/m2/day to children after autologous bone marrow transplantation has been shown to achieve 13-cis-RA levels of 5 to 7 micromular.

5.3.2 **Formulation and Stability:** Isotretinoin, which is the 13-cis isomer of retinoic acid, will be used. This is a yellow-orange crystalline powder with a molecular weight of 300.44. Isotretinoin is sensitive to light and oxygen, and so it should not be removed from the capsule for longer than one hour prior to administration to the patient and it should be kept in subdued light as much as possible.

5.3.3 **Guidelines for Administration:** PO with fat-containing food or milk to enhance absorption. Administration of the entire capsule is to be encouraged and small children can be trained to swallow them using similar sized candy.

5.3.4 **Supplier:** Isotretinoin is available commercially under the trade name ACCUTANE (Roche Laboratories) in 10mg, 20mg, and 40mg soft gelatin capsules. See package insert for further information.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

6.1.1 Diagnosis of NB as defined by a) histopathology (confirmed by the MSK Department of Pathology), or b) BM metastases or MIBG-avid lesion(s) plus high urine catecholamine levels.

6.1.2 Patients must have high-risk NB (*MYCN*-amplified stage 2/3/4/4S of any age and *MYCN*-nonamplified stage 4 in patients greater than 18 months of age).

6.1.3 Patients must be in first CR/VGPR (as defined below in Section 12.3).

6.1.4 Patients must have a negative human anti-hu3F8 antibody (HAHA) titer.

6.2 Subject Exclusion Criteria

6.2.1 Existing major organ dysfunction, i.e., renal., cardiac, hepatic, neurologic, pulmonary, or gastrointestinal toxicity $\geq$ grade 3, with the exception of hearing loss and hematolo gic toxicity.

6.2.2 Active life-threatening infection.

6.2.3 Inability to comply with protocol requirements

7.0 RECRUITMENT PLAN

Patients will be offered participation in this study by their attending physician in the Department of Pediatrics, Memorial Sloan Kettering Cancer Center. No patient will be identified for recruitment by chart review or direct advertising. The attending physician will be responsible for explaining the study and obtaining written informed consent. Patients will mainly be children and adolescents because of the nature of NB (90% of patients are <6 years old at diagnosis). Patients of both sexes and all ethnic backgrounds are eligible for this study.

Some potential research participants/ research participant's legally authorized representatives (LAR) will discuss participation in this trial with a consenting professional while on site but for various valid reasons will leave without signing consents. As some of these patients might have far apart scheduled visits but would like to participate, consents, along with a cover letter (Appendix B), may be delivered by mail or facsimile to/from the potential research participant /LAR for review and signature.

Upon receipt of the consent documents, the consenting professional will conduct the consent discussion by telephone when the research participant or the LAR can read the document as it is discussed. All other applicable conditions for documentation of the informed consent will be met and the 1 signed copy will be returned to the consenting professional for signature and date. If a participant has not responded within about one week, a consenting professional may attempt to contact the participant. The consenting professional will follow the Reminder Phone Script (Appendix C) and try contacting the participant no more than three times.

As this consent process includes participants mailing back a signed consent document, the participant will be requested to mail the signed consent document as soon as possible. Patients will be provided with an overnight shipping label and envelope in which to return the consents to MSK. Once received, the consenting professional will sign and date the copy of the consent form and a completely signed copy will be mailed back to the participant for his/her records.

8.0 PRETREATMENT EVALUATION

Pretreatment evaluations (Table 4) should be completed within 30 days of start of treatment. Extent of disease evaluations can be completed within 45 days prior to treatment provided that the patient has not received any treatment after the tests were completed.

8.1 Complete history and physical examination.

8.2 Complete blood count, serum creatinine, blood urea nitrogen, serum aspartate aminotransferase, serum alanine aminotransferase, serum lactate dehydrogenase, serum alkaline phosphatase, serum total bilirubin, albumin, sodium, potassium, chloride and bicarbonate.

8.3 BM aspirates and biopsies from bilateral iliac crests for standard histochemical studies, and pooled aspirates for qRT-PCR.

8.4 Imaging by computed tomography (CT) and/or magnetic resonance imaging (MRI) of primary tumor site plus other specific or suspected sites of tumor, and metaiodobenzylguanid ine (MIBG) scan [54] and/or positron emission tomography (PET).[55]

8.5 Pregnancy test, if applicable

8.6 Serum for analysis of human anti-hu3F8 antibody (HAHA) if applicable (i.e., for patients previously treated with m3F8, hu3F8, ch14.18, or hu14.18), anti- idiotypic antibody, and anti-anti- idiotypic antibody. For phase I patients only, sera will be archived for baseline cytokine studies if needed. If the patient was previously treated with an antibody, any HAHA results since last antibody treatment can be used.

9.0 TREATMENT/INTERVENTION PLAN

9.1 Schedule: Hu3F8 is given iv, at a dose of 3.0 mg/kg/day. To modulate pain from hu3F8, patients can be premedicated with analgesics (e.g., hydromorphone) and/or antihistamines (e.g., diphenhydramine or hydroxyzine). Hu3F8 is started after GM-CSF. GM-CSF is dosed at 250 mcg/m2/day from day –4 to day 0 (e.g., Wednesday to Sunday), and is 500 mcg/m2/day on days +1 to 5 (e.g., Monday to Friday), as in the phase I trial (IRB #12-230). Patients may also receive treatment on a modified schedule of 3 doses of intravenous hu3F8 over a maximum of 10 days, as needed (as in the phase II arm of IRB #12-230). Patients will come off study treatment if they have life threatening toxicity and if progressive disease occurs at any time during the study and will be followed for 30-days.

Patients receive 5 cycles. Allowing flexibility for holidays, family travel, bed availability, etc., the break from the end of a cycle to start of next cycle is ~2-to-4 weeks through cycle 4, then up to 6-to-8 weeks until cycle 5. Patients who develop HAHA will have their treatments with hu3F8/GM-CSF delayed until their HAHA turns negative; the next cycle must be started no later than 24 months from the first cycle. As in the predecessor study (IRB #13-260) with HAMA, patients who develop HAHA which precludes timely treatments (as determined by Principal Investigator taking into account titer of HAHA and clinical factors specific to a given patient) with hu3F8/GM-CSF are eligible to receive low-dose maintenance regimens such as irinotecan alone,[56] temozolomide alone,[57] irinotecantemozolomide,[58] or cyclophosphamide-topotecan.[59] They can also receive anti-HAHA agents such as rituximab and cyclophosphamide. They resume treatment with hu3F8+GM-CSF if HAHA becomes negative.

9.2 hu3F8/GM-CSF treatment schedule (one cycle)

Days -4 to 0: GM-CSF 250 mcg/m2/day, subcutaneously. Days 1 to 5: GM-CSF 500 mcg/m2/day, subcutaneously.

Day 1: hu3F8 infused iv over ~30 to 90 minutes.

Day 3: hu3F8 infused iv over ~30 to 90 minutes.

Day 5: hu3F8 infused iv over ~30 to 90 minutes.

Note: GM-CSF is not administered if ANC>20,000/ul by last documented CBC.

The treatment and evaluation schedule may require minor adjustment as clinically indicated or due to circumstance (e.g., holidays or inclement weather). Patients can complete the missed day(s) of hu3F8 the following week, so they receive 3 doses of hu3F8. With modified schedules of hu3F8, GMCSF will be administered at 250 mcg/m2/day x5 days before the first dose of hu3F8, as with the standard schedule, but then GM-CSF at 500 mcg/m2/day will be administered as follows: on day of the first dose of hu3F8, on the day before and on the day of the 2nd dose of hu3F8, and on the day before and on the day of the 3rd dose of hu3F8. This usage will assure that there is the same total dosage of GM-CSF for every variation of the hu3F8 schedule.

9.3 Isotretinoin is administered at 160 mg/m2/d, divided into two doses, x14 days. If a dose is missed, it can be made up at the end of the cycle. This treatment can be repeated after a minimum rest period of 14 days, for a total of 6 cycles. It is not taken on the same days as hu3F8. Isotretinoin is started after 2 cycles of hu3F8/GM-CSF unless HAHA develops and precludes timely administration of cycle 2 of hu3F8/GM-CSF. Dose reductions due to expected side effects of isotretinoin (e.g., headaches, dry skin, etc.) are allowed, with dosing based on best clinical judgment depending on symptoms, number of cycles, and other clinical factors and considerations.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Note: If for any reason tests cannot be completed on the day of the first dose, they will be completed on or before the day of the second dose.

10.1 CBC on days of the first and the second doses of hu3F8.

10.2 C3 and CH50 on day of the first dose of hu3F8, before and ~3 hours after hu3F8 in cycle 1.

10.3 HAHA is checked before each cycle of hu3F8 (Dr. Cheung, Research Lab). At the discretion of the PI, serum can also be used for HAMA, but is not required, and HAHA +/-HAMA can be checked approximately every 3 months for 2 years then every 6 months up to 5 years from the first hu3F8 dose.

10.4 Serum creatinine, blood urea nitrogen, serum aspartate aminotransferase, serum alanine aminotransferase, and serum total bilirubin on days of the first and the third hu3F8 doses of each cycle.

10.5 BM histological and molecular assessment (section 8.3) at end of cycle 2 and before a cycle of isotretinoin. Subsequently, BM studies (histology only) are repeated in conjunction with MIBG or PET scan (section 10.7) through 2 years while on study in patients with history of BM or cortical bone involvement, but are repeated ~every 6 months in other patients (i.e., patients who are stage 2 or 3, or were stage 4 by virtue of metastases in distant lymph nodes).

10.6 CT and/or MRI of primary site approximately every 3 months through 2 years while on study.

10.7 MIBG and/or PET scan approximately every 3 months through 2 years while on study.

10.8 Pregnancy screen (females of child-bearing age) before starting isotretinoin.

Table 4: Evaluations on Protocol

Tests	Pre-treatment	During Treatment	During Followup
Complete history and physical	✓	-	-
Complete blood count	✓	On days of the first and second doses of each cycle.	-
C3 & CH50	-	Before and ~3 hours after 3F8 on day of the first dose of cycle 1.	-
Liver and Renal function tests (ALT, AST, BUN, creatinine, and total Bilirubin)	✓	On days of the first and third doses of each cycle.	-
Pregnancy test, if applicable	✓	Prior to starting cycle 1 of isotretinoin	-
Blood for HAHA	✓, if applicable	Before each cycle	-
Bone marrow studies	✓	After cycle 2 (before the cycle of isotretinoin), and subsequently with MIBG or PET through 2 years for patients with history of BM or cortical bone involvement. For patients who are stage 2 or 3, or stage 4 by virtue of metastases in distant lymph nodes: approximately every 6 months while on study	Approximately q 3 months for 2 years from first treatment or until patient is off study, whichever is earlier and thereafter as clinically indicated
CT and/or MRI	✓, of primary tumor site, plus other specific or suspected sites of tumor	~Q3 months of primary site	Approximately q 3 months for 2 years from first treatment or until patient is off study, whichever is earlier and thereafter as clinically indicated

MIBG and/or PET scan	✓	~Q3 months	Approximately q 3 months for 2 years from first treatment or until patient is off study, whichever is earlier and thereafter as clinically indicated

11.0 TOXICITIES/SIDE EFFECTS

Toxicities are graded by the Common Toxicity Criteria (Version 4.0) developed by the National Cancer Institute (NCI) of the USA. All toxicities during cycle 1 will be recorded.

After cycle 1, only toxicities ≥ grade 3 will be recorded.

- Hu3F8 is expected to have the following side-effects: pain, paresthesia, hypertension, hypotension, tachycardia, urticaria, fever, nausea, emesis, and rarely, diarrhea, serum sickness, hyponatremia, somnolence and posterior reversible encephalopathy syndrome (PRES). Other potential side-effects that may occur are bronchospasm, anaphylaxis, peripheral neuropathy, impaired accommodation of the eye, poor reactivity of pupils to light and elevated transaminases.
- GM-CSF: Common side-effects include bone pain, flushing, local reaction at site of injection, leukopenia shortly after injection, and decrease in platelet count. Rare side - effects (predominantly in adults) include allergic reactions, weight gain, pleural or pericardial effusion, pericardial embolism, thrombosis, and difficulty breathing after first injection.
- Isotretinoin: Dry skin, cheilitis, dry eyes, hypercalcemia, pseudotumor cerebri, hepatotoxicity, teratogenic effect on fetus, depression, suicidal ideation.
- Toxicities will only be captured up to 30 days after the last treatment or until the patient begins a new treatment, whichever is earlier.

11.1 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant starts investigational treatment/intervention. SAE reporting is required for 30-days after the participant's last investigational treatment/intervention. Any event that occur after the 30-day period that is unexpected and at least possibly related to protocol treatment must be reported.

Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.

The report should contain the following information:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment(s)
- If the AE was expected
- Detailed text that includes the following ○ An explanation of how the AE was handled ○ A description of the participant's condition
  - ○ Indication if the participant remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

11.2 External SAE Reporting

Any additional SAE reporting information required by the funder or drug supplier should be included in this section. (remove if, not applicable)

For IND/IDE protocols:
The SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the IND Office

11.3 Definition of SAE

Serious Adverse Events (SAEs) are any adverse events occurring at any dose while on treatment and within 30 days of the last dose of Hu3F8 that result in any of the following outcomes:

SAEs are defined as grade 4 toxicities other than fatigue, weight loss or gain, anorexia, nausea, anxiety, constipation, urinary retention from opioid analgesics, somnolence , hallucinations, disorientation, confusion, agitation, anxiety from antihistamine and opioid premedications, hypomagnesemia, fever, rash, urticaria from hu3F8 or GM-CSF,

myelosuppression from the combination of hu3F8 and GM-CSF, pain and transient hypoxia from opioids, and breath-holding with or without transient oxygen requirement. Preexisting conditions, e.g., hearing loss, abnormal liver function tests from total parenteral nutritio n, and alopecia, are not counted as SAEs. Hospitalizations that arise from complications of chemotherapy are considered part of standard care and therefore will not be reported.

For Hospitalization only – Any medical event equivalent to CTCAE (Version 4.0) Grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported, regardless of designation as expected or unexpected and attribution.

The following events are expected:

a) Due to Hu3F8: pain, paresthesia, hypertension, hypotension, tachycardia, urticaria, fever, nausea, emesis, and rarely, diarrhea, serum sickness, hyponatremia, somnolence, and posterior reversible encephalopathy syndrome (PRES). Other potential side-effects that may occur are bronchospasm, anaphylaxis, peripheral neuropathy, impaired accommodation of the eye, poor reactivity of pupils to light and elevated transaminases.

b) Due to GM-CSF: Common side-effects include bone pain, flushing, local reaction at site of injection, leukopenia shortly after injection, and decrease in platelet count. Rare side effects (predominantly in adults) include allergic reactions, weight gain, pleural or pericardia l effusion, pericardial embolism, thrombosis, and difficulty breathing after first injection.

c) Due to antihistaminics: somnolence, hallucinations, disorientation, confusion, agitatio n, anxiety.

d) Due to prior therapy: alopecia, hearing loss, fatigue, weight loss or weight gain, anorexia, myelosuppression, hypomagnesemia; hyperbilirubinemia or elevated LFTs from TPN.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 RFS is calculated from first day of treatment with hu3F8.

12.2 Patients are considered a response failure under this protocol if progressive disease is evident before two years. Deaths from toxicity attributable to protocol treatment will be counted as events. Patients who withdraw or are lost to follow-up before two years will be considered as progressions.

12.3 Disease response for NB will use the International NB Response Criteria[60]: ▪

Complete response/remission (CR): no evidence of disease.

- Very good partial response/remission (VGPR): >90% decrease in all disease parameters, except bone scan unchanged or improved; BM must be free of disease.
- Partial response/remission: >50% decrease in all disease parameters, except bone scan unchanged or improved; $\leq$1 positive BM site.
- Mixed response: >50% decrease in $\geq$1 but not all disease markers.
- Stable disease: <50% decrease in all tumor markers.
- Progressive disease: new lesion, or >25 % increase in any disease marker.

12.4 Adequacy of trial: All patients who fulfill the eligibility requirements and receive a first cycle of hu3F8 will have an adequate trial and are considered evaluable for protocol.

13.0 CRITERIA FOR REMOVAL FROM STUDY

13.1 Patients come off study if there is progressive disease at any time and are followed for 30 days from last treatment or until they start a new treatment, whichever is earlier, before being removed from study. Best clinical judgment will be used to determine whether a patient must undergo tests between cycles to make sure disease is not progressing. However, if a patient develops recurrent disease that can be completely eliminated surgically or eliminated by other means, that patient may remain on study at the discretion of the Principal Investigator as long as the patient does not receive systemic therapy and the recurrence is promptly eliminated. Such patients will be evaluable for the primary endpoint if progressive disease occurs before two years.

13.2 Patients come off study if they have a life-threatening toxicity clearly attributable to the hu3F8.

13.3 Patients remain on follow up for 5 years for survivor data only from first dose of hu3F8, unless one of the following criteria is met: death, lost to follow up, or withdrawal of consent. 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 removed from the study due to adverse events will be treated and followed according to established, acceptable medical practice. All pertinent informat ion concerning the outcome of such treatment will be entered in the MSKCC institutional Clinica l Research Database (CRBD). Patients will be followed until resolution or stabilization of the adverse event.

- **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 withdrawa l 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 withdrawn from the study if more than one scheduled dose is missed.

14.0 BIOSTATISTICS

The objective of this phase II trial is to assess the relapse-free survival (RFS) of HR-NB patients in first CR/VGPR treated with hu3F8/GM-CSF and isotretinoin. The hu3F8 dosing is three-fold greater than the standard dosage of other anti-G_{D2} mAbs.

14.1 Primary Endpoint

The primary endpoint is two-year RFS.

14.2 Sample Size

We will use a Simon's two-stage design. The type I error rate is set as 0.1 and the type II error rate as 0.1.

In the predecessor trial (IRB 03-077) using murine 3F8 at standard dosage of 100 mg/m2/cycle, combined with GM-CSF, plus isotretinoin, the 56 patients treated in CR/VGPR after conventional therapy achieved 70% two-year RFS. We will consider a two-year relapse-free survival (RFS) rate 0.70 as not promising and 0.85 as promising with the new treatment. Twenty patients will be accrued initially, and if there are less than 15 two-year RFS survivors, further accrual to the protocol will be stopped and the new treatment will be declared ineffective in this protocol. If there are at least 15 two-year RFS survivors, then an additional 39 patients will be accrued for a total of 59. If there are at least 46 two-year RFS survivors out of 59 patients, the protocol will be considered to have a positive result and the new treatment will be considered worthy of further testing in this patient population. The probability of stopping the study earlier is 58% under the null hypothesis.

14.3 Accrual Rate

We expect to accrue 10-15 patients per year. This trial can be completed within 4-6 years. The accrual will continue while the first 20 patients are evaluated for the first stage of the study. The twostage design is used to guard against many early recurrences.

14.4 Secondary Endpoint

The secondary endpoint of MRD findings is determined by using BM collected after cycle 2 of hu3F8/GM-CSF. Quantitation by qRT-PCR of a four marker panel will assess the association of MRD status with RFS and overall survival from the time of MRD measurement. MRD negativity is defined as absence in BM of all four markers. Statistical analysis is based on Kaplan-Meier method and log-rank test.

15.0 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 whether 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. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.2 Randomization

Not applicable.

16.0 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 by the CRC 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 on an ongoing basis.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring Plan utilized for this study must align with the MSK DSM Plan, where applicable.

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan Kettering were approved by the National Cancer Institute in August 2018. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials."

There are several different mechanisms by which clinical studies are monitored for data, safety and quality. At a departmental/PI level there exists procedures for quality control by the research team(s). Institutional processes in place for quality assurance include protocol monitoring, compliance and data verification audits, staff education on clinical research QA and two institutional committees that are responsible for monitoring the activities of our clinica l trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinica l trials, report to the Deputy Physician-in-Chief, Clinical Research.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required.

The MSK DSMB monitors phase III trials and the DSMC monitors non-phase III trials. The DSMB/C have oversight over the following trials:

- MSK Investigator Initiated Trials (IITs; MSK as sponsor)
- External studies where MSK is the data coordinating center
- Low risk studies identified as requiring DSMB/C review

The DSMC will initiate review following the enrollment of the first participant/or by the end of the year one if no accruals and will continue for the study lifecycle until there are no participants under active therapy and the protocol has closed to accrual. The DSMB will initiate review once the protocol is open to accrual.

17.0 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 which could be induced by the infusion of hu3F8.
- **Costs**: Patients are responsible for the costs of physician visits, routine laboratory and radiographic studies, bone marrow aspirates and biopsies, hospitalizations, drug administration, outpatient care, and GM-CSF. Patients will not be charged for Hu3F8 or for research blood tests. 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.

18.0 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.

19.0 REFERENCES

1. Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. Lancet 2007;369:2106-20.
2. Mueller S, Matthay KK. Neuroblastoma: biology and staging. Current oncology reports 2009;11:431-8.
3. Kushner BH, Kramer K, LaQuaglia MP, Modak S, Yataghene K, Cheung NK. Reduction from seven to five cycles of intensive induction chemotherapy in children with high- r isk neuroblastoma. J Clin Oncol 2004;22:4888-92.
4. La Quaglia MP, Kushner BH, Su W, et al. The impact of gross total resection on local control and survival in high-risk neuroblastoma. J Pediatr Surg 2004;39:412-7; discussion -7. 5. Park JR, Scott JR, Stewart CF, et al. Pilot induction regimen incorporating
pharmacokinetically guided topotecan for treatment of newly diagnosed high-risk neuroblastoma: a Children's Oncology Group study. J Clin Oncol 2011;29:4351-7.
6. Franks LM, Bollen A, Seeger RC, Stram DO, Matthay KK. Neuroblastoma in adults and adolescents: an indolent course with poor survival. Cancer 1997;79:2028-35.
7. Kushner BH, Kramer K, LaQuaglia MP, Modak S, Cheung NK. Neuroblastoma in adolescents and adults: the Memorial Sloan-Kettering experience. Med Pediatr Oncol 2003;41:50815.
8. Maris JM. Recent advances in neuroblastoma. N Engl J Med 2010;362:2202-11.
9. Kushner BH, Wolden S, LaQuaglia MP, et al. Hyperfractionated low-dose radiotherapy for high-risk neuroblastoma after intensive chemotherapy and surgery. J Clin Oncol 2001;19:2821 - 8.
10. Grupp SA, Asgharzadeh S, Yanik GA. Neuroblastoma: issues in transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2012;18:S92-100.
11. Deyell RJ, Attiyeh EF. Advances in the understanding of constitutional and somatic genomic alterations in neuroblastoma. Cancer genetics 2011;204:113-21.
12. Pearson AD, Pinkerton CR, Lewis IJ, Imeson J, Ellershaw C, Machin D. High-dose rapid and standard induction chemotherapy for patients aged over 1 year with stage 4 neuroblastoma: a randomised trial. Lancet Oncol 2008;9:247-56.
13. Cheung NK, Walter EI, Smith-Mensah WH, Ratnoff WD, Tykocinski ML, Medof ME. Decay-accelerating factor protects human tumor cells from complement- mediated cytotoxicity in vitro . The Journal of clinical investigation 1988;81:1122-8.
14. Chen S, Caragine T, Cheung NK, Tomlinson S. CD59 expressed on a tumor cell surface modulates decay-accelerating factor expression and enhances tumor growth in a rat model of human neuroblastoma. Cancer Res 2000;60:3013-8.
15. Kushner BH, Cheung NK. Absolute requirement of CD11/CD18 adhesion molecules, FcRII and the phosphatidylinositol- linked FcRIII for monoclonal antibody-mediated neutrophil antihuman tumor cytotoxicity. Blood 1992;79:1484-90.
16. Metelitsa LS, Gillies SD, Super M, Shimada H, Reynolds CP, Seeger RC.
Antidisialoganglioside/granulocyte macrophage-colony-stimulating factor fusion protein facilitates neutrophil antibody-dependent cellular cytotoxicity and depends on FcgammaRII (CD32) and Mac-1

(CD11b/CD18) for enhanced effector cell adhesion and azurophil granule exocytosis. Blood 2002;99:4166-73.

17. Mackall CL. T-cell immunodeficiency following cytotoxic antineoplastic therapy: a review. Stem Cells 2000;18:10-8.
18. Mackall CL, Stein D, Fleisher TA, et al. Prolonged CD4 depletion after sequential autologous peripheral blood progenitor cell infusions in children and young adults. Blood 2000;96:754-62.
19. Kushner BH, Cheung IY, Kramer K, Modak S, Cheung NK. High-dose cyclophospha mide inhibition of humoral immune response to murine monoclonal antibody 3F8 in neuroblasto ma patients: broad implications for immunotherapy. Pediatr Blood Cancer 2007;48:430-4.
20. Modak S, Cheung NK. Disialoganglioside directed immunotherapy of neuroblastoma. Cancer Invest 2007;25:67-77.
21. Cheung NK, Saarinen UM, Neely JE, Landmeier B, Donovan D, Coccia PF. Monoclonal antibodies to a glycolipid antigen on human neuroblastoma cells. Cancer Res 1985;45:2642-9.
22. Mujoo K, Kipps TJ, Yang HM, et al. Functional properties and effect on growth suppression of human neuroblastoma tumors by isotype switch variants of monoclonal antiganglioside GD2 antibody 14.18. Cancer Res 1989;49:2857-61.
23. Gillies SD, Lo KM, Wesolowski J. High-level expression of chimeric antibodies using adapted cDNA variable region cassettes. J Immunol Methods 1989;125:191-202.
24. Barker E, Mueller BM, Handgretinger R, Herter M, Yu AL, Reisfeld RA. Effect of a chimeric anti-ganglioside GD2 antibody on cell-mediated lysis of human neuroblastoma cells. Cancer Res 1991;51:144-9.
25. Barker E, Reisfeld RA. A mechanism for neutrophil- mediated lysis of human neuroblasto ma cells. Cancer Res 1993;53:362-7.
26. Mueller BM, Reisfeld RA, Gillies SD. Serum half-life and tumor localization of a chimeric antibody deleted of the CH2 domain and directed against the disialoganglioside GD2. Proc Natl Acad Sci USA 1990;87:5702-5.
27. Mueller BM, Romerdahl CA, Gillies SD, Reisfeld RA. Enhancement of antibody-depende nt cytotoxicity with a chimeric anti-GD2 antibody. J Immunol 1990;144:1382-6.
28. Simon T, Hero B, Faldum A, et al. Consolidation treatment with chimeric anti-GD2-antibod y ch14.18 in children older than 1 year with metastatic neuroblastoma. J Clin Oncol 2004;22:3549-57. 29. Simon T, Hero B, Faldum A, et al. Long term outcome of high- risk neuroblastoma patients after immunotherapy with antibody ch14.18 or oral metronomic chemotherapy. BMC cancer 2011;11:21.
30. Yu AL, Gilman AL, Ozkaynak MF, et al. Anti-GD2 antibody with GM-CSF, interleukin- 2, and isotretinoin for neuroblastoma. N Engl J Med 2010;363:1324-34.
31. Cheung NK, Cheung IY, Kramer K, et al. Key role for myeloid cells: phase II results of antiG(D2) antibody 3F8 plus granulocyte- macrophage colony-stimulating factor for chemoresista nt osteomedullary neuroblastoma. International journal of cancer 2014;135:2199-205.
32. Cheung IY, Hsu K, Cheung NK. Activation of Peripheral-Blood Granulocytes Is Strongly Correlated With Patient Outcome After Immunotherapy With Anti-GD2 Monoclonal Antibody and Granulocyte-Macrophage Colony-Stimulating Factor. J Clin Oncol 2012;30:426-32.
33. Cheung NK, Sowers R, Vickers AJ, Cheung IY, Kushner BH, Gorlick R. FCGR2A polymorphism is correlated with clinical outcome after immunotherapy of neuroblastoma with antiGD2 antibody and granulocyte macrophage colony-stimulating factor. J Clin Oncol 2006;24:288590.

34. Cheung NK, Cheung IY, Kushner BH, et al. Murine anti-GD2 monoclonal antibody 3F8 combined with granulocyte-macrophage colony-stimulating factor and 13-cis-retinoic acid in highrisk patients with stage 4 neuroblastoma in first remission. J Clin Oncol 2012;30:3264-70.
35. Cheung IY, Lo Piccolo MS, Kushner BH, Cheung NK. Early molecular response of marrow disease to biologic therapy is highly prognostic in neuroblastoma. J Clin Oncol 2003;21:3853-8.
36. Kreissman SG, Seeger RC, Matthay KK, et al. Purged versus non-purged peripheral blood stem-cell transplantation for high-risk neuroblastoma (COG A3973): a randomised phase 3 trial. Lancet Oncol 2013;14:999-1008.
37. Cheung NK, Ostrovnaya I, Kuk D, Cheung IY. Bone marrow minimal residual disease was an early response marker and a consistent independent predictor of survival after anti- GD2 immunotherapy. J Clin Oncol 2015;33:755-63.
38. Kushner BH, Ostrovnaya I, Cheung IY, et al. Prolonged progression-free survival after consolidating second or later remissions of neuroblastoma with Anti-G immunotherapy and isotretinoin: a prospective Phase II study. Oncoimmunology 2015;4:e1016704.
39. Kramer K, Humm JL, Souweidane MM, et al. Phase I study of targeted radioimmunotherap y for leptomeningeal cancers using intra-Ommaya 131-I-3F8. J Clin Oncol 2007;25:5465-70.
40. Kramer K, Kushner BH, Modak S, et al. Compartmental intrathecal radioimmunother apy: results for treatment for metastatic CNS neuroblastoma. Journal of neuro-oncology 2010;97:409-18.
41. Albert D, Dunham J, Khan S, et al. Variability in the biological response to anti-CD20 B cell depletion in systemic lupus erythaematosus. Ann Rheum Dis 2008;67:1724-31.
42. Cheung NK, Guo H, Hu J, Tassev DV, Cheung IY. Humanizing murine IgG3 anti-GD2 antibody m3F8 substantially improves antibody-dependent cell-mediated cytotoxicity while retaining targeting in vivo. Oncoimmunology 2012;1:477-86.
43. Tassev DV, Cheung NK. Monoclonal antibody therapies for solid tumors. Expert opinion on biological therapy 2009;9:341-53.
44. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphis ms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 2003;21:3940-7.
45. Niwa R, Hatanaka S, Shoji-Hosaka E, et al. Enhancement of the antibody-dependent cellular cytotoxicity of low-fucose IgG1 Is independent of FcgammaRIIIa functional polymorphism. Clin Cancer Res 2004;10:6248-55.
46. Musolino A, Naldi N, Bortesi B, et al. Immunoglobulin G fragment C receptor polymorphis ms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neupositive metastatic breast cancer. J Clin Oncol 2008;26:1789-96.
47. Bibeau F, Lopez-Crapez E, Di Fiore F, et al. Impact of Fc{gamma}RIIa-Fc{gamma}RI IIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorect al cancer treated with cetuximab plus irinotecan. J Clin Oncol 2009;27:1122-9.
48. Saarinen UM, Coccia PF, Gerson SL, Pelley R, Cheung NK. Eradication of neuroblasto ma cells in vitro by monoclonal antibody and human complement: method for purging autologous bone marrow. Cancer Res 1985;45:5969-75.
49. Wang SY, Veeramani S, Racila E, et al. Depletion of the C3 component of compleme nt enhances the ability of rituximab-coated target cells to activate human NK cells and improves the efficacy of monoclonal antibody therapy in an in vivo model. Blood 2009;114:5322-30.
50. Racila E, Link BK, Weng WK, et al. A polymorphism in the complement component C1qA correlates with prolonged response following rituximab therapy of follicular lymphoma. Clin Cancer Res 2008;14:6697-703.

51. Navid F, Sondel PM, Barfield R, et al. Phase I trial of a novel anti-GD2 monoclonal antibody, Hu14.18K322A, designed to decrease toxicity in children with refractory or recurrent neuroblasto ma. J Clin Oncol 2014;32:1445-52.
52. Ozkaynak MF, Sondel PM, Krailo MD, et al. Phase I study of chimeric human/mur ine antiganglioside G(D2) monoclonal antibody (ch14.18) with granulocyte-macrophage colony-stimulating factor in children with neuroblastoma immediately after hematopoietic stem- cell transplantation: a Children's Cancer Group Study. J Clin Oncol 2000;18:4077-85.
53. Petrella T, Quirt I, Verma S, et al. Single-agent interleukin-2 in the treatment of metastatic melanoma: a systematic review. Cancer treatment reviews 2007;33:484-96.
54. Kushner BH, Yeh SD, Kramer K, Larson SM, Cheung NK. Impact of metaiodobenzylguanidine scintigraphy on assessing response of high-risk neuroblastoma to doseintensive induction chemotherapy. J Clin Oncol 2003;21:1082-6.
55. Kushner BH, Yeung HW, Larson SM, Kramer K, Cheung NK. Extending positron emissio n tomography scan utility to high-risk neuroblastoma: fluorine-18 fluorodeoxyglucose positron emission tomography as sole imaging modality in follow-up of patients. J Clin Oncol 2001;19:3397405.
56. Kushner BH, Kramer K, Modak S, Cheung NK. Five-day courses of irinotecan as palliat ive therapy for patients with neuroblastoma. Cancer 2005;103:858-62.
57. Rubie H, Chisholm J, Defachelles AS, et al. Phase II study of temozolomide in relapsed or refractory high-risk neuroblastoma: a joint Societe Francaise des Cancers de l'Enfant and United Kingdom Children Cancer Study Group-New Agents Group Study. J Clin Oncol 2006;24:5259 - 64.
58. Kushner BH, Kramer K, Modak S, Cheung NK. Irinotecan plus temozolomide for relapsed or refractory neuroblastoma. J Clin Oncol 2006;24:5271-6.
59. Saylors RL, 3rd, Stine KC, Sullivan J, et al. Cyclophosphamide plus topotecan in children with recurrent or refractory solid tumors: a Pediatric Oncology Group phase II study. J Clin Oncol 2001;19:3463-9.
60. Brodeur GM, Pritchard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol 1993;11:1466-77.

20.0 APPENDICES

Appendix A: Research Patient Diaries

Appendix B: Participant Mailing Letter

Appendix C: Reminder Phone Script