MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Anti-G_{D2} 3F8 Monoclonal Antibody and GM-CSF for High-Risk Neuroblastoma

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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
ALT, alanine aminotransferase
ANC, absolute neutrophil count
ASCT, autologous stem-cell transplantation
AST, aspartate aminotransferase
BM, bone marrow
BUN, blood urea nitrogen
CBC, complete blood count
CT, computed tomography
CR, complete remission
CRDB, Clinical Research Database
DSM, Data and Safety Monitoring
DSMB, Data and Safety Monitoring Board
DSMC, Data and Safety Monitoring Committee
FDA, Food and Drug Administration
GM-CSF, granulocyte-macrophage colony-stimulating factor
HAMA, human anti-mouse antibody
ICH, International Conference of Harmonization
IND, investigational new drug
IRB/PB, Institutional Review Board/Privacy Board
iv, intravenous
MIBG, metaiodobenzylguanidine
MoAb, monoclonal antibody
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
PD, progressive disease
PET, positron emission tomography
PI, Principal Investigator
PFS, progression-free survival
PPR, Protocol Participant Registration
q, every
QA, quality assurance
RA, Research Authorization
RSA, research study assistant
SAE, serious adverse event
sc, subcutaneous
VGPR, very good partial remission

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Efficacy of anti-GD2 monoclonal antibody (MoAb) therapy is proven. This is a single-arm, open-label, open access study to provide the anti-GD2 murine IgG3 MoAb 3F8 combined with granulocyte-macrophage colony stimulating factor (GM-CSF) to patients with high-risk neuroblastoma (NB). This immunotherapy has shown efficacy against minimal residual disease (MRD) in such patients. 3F8 is not yet licensed by the Food and Drug Administration (FDA) and commercially available but is listed on the FDA site as orphan designated.[1a]

2.0 OBJECTIVES AND SCIENTIFIC AIMS

The objective of this open access protocol is to provide 3F8 to patients with high-risk NB. The treatment IND will collect additional usage, tolerability and safety data.

3.0 BACKGROUND AND RATIONALE

3.1 Disease background: NB is the most common extracranial solid tumor of childhood; 50-60% of patients present with an unresectable primary tumor and metastases in bone marrow (BM).[1] Intensive induction chemotherapy and aggressive surgery have improved remission rates in young patients[2-4]; results have been less impressive in adolescents and adults in whom NB is especially chemoresistant.[5,6] Realization of an effective strategy for eradicating MRD has remained a formidable challenge. Post-surgical use of local radiotherapy helps control MRD in the primary site.[7] Myeloablative therapy with autologous stem-cell transplantation (ASCT) has been the most common approach for eradicating MRD in distant sites. The vitamin A derivative 13-*cis*-retinoic acid (isotretinoin) helped to prolong relapse-free survival in a randomized study.[8] Immunotherapy using the anti-GD2 ch14.18 MoAb plus GM-CSF and interleukin-2 in alternating cycles improved outcome in a randomized study.[9] These results have made treatment with isotretinoin and anti-GD2 MoAb standard of care for high-risk NB.

3.2 Rationale for immunotherapy: Various strategies have undergone clinical testing to induce or augment immune-mediated attack against cancer. However, few clinical trials have used monoclonal antibody-targeted immunotherapy against solid tumors in children or adults, especially with antibody-dependent cellular cytotoxicity (ADCC) as the principal underlying immune cytotoxic mechanism. Also, an antineoplastic role for granulocytes has received scant attention.[10] A treatment program combining 3F8 and GM-CSF thus represents an attempt to help fill a gap in the emerging field of immunotherapy.

3.3 Rationale for 3F8: 3F8 is a murine IgG3 MoAb that is well suited for targeted immunotherapy. The reasons are several. First, the intensive chemotherapy required to produce the minimal disease state optimal for immunotherapy in NB patients results in prolonged severe lymphopenia.[11] This setting is unfavorable for active immunotherapy but allows passive immunotherapy since the patient will be unable to reject allogeneic, xenogeneic, or genetically-engineered antibodies. Second, 3F8 recognizes the ganglioside GD2.[12] This target antigen is expressed at high density on NB (but has restricted distribution in normal human tissues), is not modulated from the cell surface when bound by antibodies, and is genetically stable (unlike tumor-associated antigens such as immunoglobulin idiotypes on lymphoma cells).[13-15] Third, scintigraphy using ^{131}I-3F8 confirms that 3F8 localizes selectively to GD2(+) tumor deposits in patients.[16] The excellent targeting potential of 3F8 was evidenced by its high tumor to non-tumor ratio, the high percent injected dose per gram uptake, and the limited, if any, nonspecific liver and spleen

uptake.[16,17] Fourth, 3F8 mediates destruction in vitro of GD2(+) human solid tumor cells by human complement[18] and by human lymphocytes, cultured monocytes, and neutrophils.[19-21] Finally, the capacity of 3F8 to activate complement on NB cells (which lack decay accelerating factor[22]) raises the possibility, not only of complement-mediated lysis in patients, but also of the release of complement fragments that may elicit an inflammatory influx of granulocytes capable of lysing 3F8-labeled tumor cells. Furthermore, the deposition of complement fragments C3b and iC3b on NB cells may enhance ADCC because the receptor for iC3b – variously called Mac-1, CR3, CD11b/CD18, or $\alpha_M\beta_2$-integrin – is a key element in anti-GD2 MoAb-mediated tumor cell kill by neutrophils,[23,24] which are the most abundant circulating class of leukocyte.

3.4 Rationale for GM-CSF: GM-CSF has the potential for amplifying 3F8 antitumor activity in patients via effects on granulocytes and tissue-based macrophages. Reasons for combining GM-CSF with 3F8 include the following. First, granulocyte production is only transiently suppressed with chemotherapy and GM-CSF increases numbers of circulating neutrophils and eosinophils, does not affect complement levels, and is well tolerated compared to other cytokines such as interleukin-2.[25] Second, granulocytes from patients receiving chemotherapy and from normal volunteers are effective in mounting ADCC against NB cells via non-oxidative mechanisms, and GM-CSF enhances this cytotoxicity.[24,26-30] Third, eosinophilic infiltration of some cancers has favorable prognostic significance, and eosinophils exhibit potent antitumor activity in animal models.[31,32] Fourth, activated monocytes-macrophages efficiently phagocytose NB cells, and exposure in vitro or in vivo to GM-CSF primes monocytes-macrophages for greater antineoplastic cytotoxicity.[33-38] Fifth, GM-CSF enhances the proliferation, maturation, and function of antigen-presenting cells, including antigen processing and presentation by macrophages and dendritic cells[25,39,40] - effects that might promote induction, or antitumor activity, of an idiotypic network.[41-44] Finally, GM-CSF is not a growth factor for NB cells in vitro.[45]

3.5 GM-CSF-mediated activation of neutrophils: Neutrophils are the predominant class of circulating leukocytes; chemotherapy only transiently decreases their numbers and largely spares their cytotoxic capabilities. These features are advantageous for immune-based attack against cancer.[10] We and others have shown that Mac-1 (CD11b/CD18), FcRII, and FcRIII are required for optimal ADCC in a system using human neutrophils as effectors, human NB cells as targets, and clinically active anti-GD2 MoAbs as mediators.[23,24] Efficient tumor-cell kill occurs despite the large size of the targets (which precludes a role for phagocytosis) and their relative resistance to reactive oxygen species.[29] GM-CSF enhancement of ADCC in this system correlates with upregulation of Mac-1 and with increased exocytosis of azurophil (primary) granules which contain cathepsin G and defensins capable of lysing NB cells.[30] To elucidate the cytotoxic mechanisms of anti-GD2 MoAb-mediated ADCC, we used blocking MoAbs, neutrophils devoid of Mac-1 (from donors with leukocyte adhesion deficiency), and neutrophils that do not generate reactive oxygen species (from donors with chronic granulomatous disease),[23] while others used blocking MoAbs, electron microscopy, and neutrophils from healthy donors as well as from patients.[24] Activation of neutrophils by GM-CSF is correlated with improved anti-NB effects of treatment with anti-GD2 MoAb.[46]

3.6 Clinical experience with 3F8 plus GM-CSF: The optimal method of using GM-CSF with the aim of enhancing ADCC in patients with solid tumors has only recently been elucidated. In the past, we chose to administer GM-CSF by a 2-hr iv infusion (followed by a 1-hr interval before starting 3F8 infusion) because GM-CSF disappears rapidly (<2-3 hr) from the blood with that schedule.[47,48] We wished to avoid the prolonged (>12 hr) bioactive levels in blood associated with the

subcutaneous (sc) route; our concern was that high serum levels of GM-CSF might impede granulocyte trafficking into tissues.[49] However, for circulating tumor cells in BM or blood, this concern is dwarfed by the more potent antitumor effects from prolonged cytokine exposure following subcutaneous injection.[25]

The 03-077 study of 3F8+scGM-CSF immunotherapy plus isotretinoin enrolled patients in 1st complete/very good partial remission (CR/VGPR) (n=85), patients with primary refractory NB in BM (n=79), and patients in ≥2 CR/VGPR (n=101). Salient findings included:

1) Acute toxicities were manageable which allowed outpatient treatment. This finding stands in stark contrast with studies using other anti-GD2 MoAbs either alone or in combination with interleukin-2, where treatment is inpatient, often in an intensive care unit, with complications such as capillary leak syndrome.[9]

2) For patients in 1st CR/VGPR, prior myeloablative therapy + ASCT had no impact on 5-year progression-free survival (PFS) 62% or overall survival (OS) 81%.[50] This supports the welcome possibility of avoiding the risk of significant acute and long-term toxicities of myeloablative therapy.

3) For patients with primary refractory NB, CR rate in BM by histology was 87% and CR rate of metaiodobenzylguanidine (MIBG) scans was 38%. These results vividly document the anti-NB activity of anti-GD2 MoAb immunotherapy, i.e., the possibility of overcoming histologically- and/or radiologically-visible chemoresistant NB in BM using an anti-GD2 MoAb regimen that has manageable toxicity and is administered outpatient.

4) Patients treated in ≥2 CR/VGPR achieved 48-month PFS 33% and OS 53%, with no adverse impact if patients had received anti-GD2 MoAb and isotretinoin as part of initial (pre-relapse) therapy and with only 2/35 very long-term relapse-free survivors having received ASCT as part of salvage. These results are noteworthy, given that studies show <5% long-term OS after relapse[51-55] and anti-GD2 MoAb, isotretinoin, and ASCT are standard treatments for newly-diagnosed patients.[56] Indeed, the results support a change in mind-set: no longer should relapse of high-risk NB be considered "invariably lethal",[57] but rather a curative strategy should be applied.

5) Positive MRD after 2 cycles of 3F8+scGM-CSF was a significantly adverse independent prognostic factor for all groups of patients.[50,58,59] This finding should be taken into account when determining subsequent therapy in a given patient.

6) Correlative studies highlight the anti-neoplastic potency of myeloid effectors whereas lymphocytes are the effectors usually heralded in cancer immunotherapy.

7) GM-CSF by sc route is associated with better PFS and OS than by iv route,[60] as used in the predecessor study of 3F8+ivGM-CSF.[61]

3.7 Isotretinoin (13-*cis*-retinoic acid) was shown in a randomized national study to decrease the risk of relapse in patients treated in complete remission.[8] This agent has subsequently become standard of care for NB patients in complete remission. It will be used in this protocol after patients are evaluated for response (and for toxicity) to 3F8/GM-CSF.

3.8 Interpretations and implications The ease of administration of the treatments (a subcutaneous injection, which is given at home, and a 30-minute intravenous infusion in the

outpatient clinic), plus their transient acute side-effects, are compatible with widespread usage of this regimen beyond our hospital – a strong positive factor in furthering product development.

In addition to the outpatient treatment with anti-GD2 MoAb, the Memorial Sloan-Kettering Cancer Center (MSKCC) neuroblastoma treatment program remains unique in that ASCT is not included and the immunotherapy is available for patients in $\geq 2^{nd}$ CR/VGPR. Results to date, as summarized in section 3.6, are highly encouraging and may have implications world-wide.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This treatment uses 3F8/GM-CSF and isotretinoin for: ***Group 1*** patients are in 1^{st} CR/VGPR; ***Group 2*** patients are in a $\geq 2^{nd}$ CR/VGPR; and ***Group 3*** patients have primary refractory NB in BM. All patients will receive 3F8/GM-CSF through 24 months.

4.2 Intervention

Road Map/Schema for Group 1 (1^{st} CR/VGPR) and Group 2 ($\geq 2^{nd}$ CR/VGPR) patients: Cycle 1 3F8 (iv) + GM-CSF subcutaneous (sc) (1 wk)
2-4-wk interval
Cycle 2 3F8 (iv) + GM-CSF (sc) (1 wk)
2-4-wk interval* – oral isotretinoin x14 days
Cycle 3 3F8 (iv) + GM-CSF (sc) (1 wk)
2-4-wk interval – oral isotretinoin x14 days
Cycle 4 3F8 (iv) + GM-CSF (sc) (1 wk)
6-8-wk interval – oral isotretinoin x14 days on, 14 days off, 14 days on
Cycle 5 3F8 (iv) + GM-CSF (sc) (1 wk)
6-8-wk interval – oral isotretinoin x14 days on, 14 days off, 14 days on (6^{th} cycle)
Cycle 6 3F8 (iv) + GM-CSF (sc) (1 wk)
6-8-wk interval
Cycle 7 3F8 (iv) + GM-CSF (sc) (1 wk)

Continue with 6-8-wk intervals through 24 months from 1^{st} dose of 3F8.
* assessment of BM status by standard histology

Road Map/Schema for Group 3 patients (BM positive): The break between end of a cycle of 3F8/GM-CSF and start of next cycle is approximately 2-to-4-weeks through 4 cycles after achievement of CR in BM; subsequent breaks are ~6-8 weeks. Please see roadmap below for a patient achieving CR in BM after cycle 1.

Cycle 1 3F8 (iv) + GM-CSF (sc) (1 wk)
2-4-wk interval* – BM negative
Cycle 2 3F8 (iv) + GM-CSF (sc) (1 wk)
2-4-wk interval* – oral isotretinoin x14 days

Cycle 3 3F8 (iv) + GM-CSF (sc) (1 wk)
2-4-wk interval – oral isotretinoin x14 days
Cycle 4 3F8 (iv) + GM-CSF (sc) (1 wk)
2-4-wk interval – oral isotretinoin x14 days
Cycle 5 3F8 (iv) + GM-CSF (sc) (1 wk)
6-8-wk interval – oral isotretinoin x14 days
Cycle 6 3F8 (iv) + GM-CSF (sc) (1 wk)
6-8-wk interval – oral isotretinoin x14 days on, 14 days off, 14 days on (6^{th} cycle)
Cycle 7 3F8 (iv) + GM-CSF (sc) (1 wk)
6-8-wk interval

Continue with 6-8-wk intervals through 24 months from 1^{st} dose of 3F8.
* assessment of BM response by standard histology

The treatment schedule may require minor adjustment as clinically indicated or due to circumstance (e.g., due to Pediatric Day Hospital closure for holidays or due to inclement weather). Patients can complete the missed day of 3F8 the following week, so they receive a full 5-day cycle. GM-CSF will be continued through the 3F8 make-up day at the 500 mcg/m^2 dose.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 3F8 monoclonal antibody (IND number of BB-IND-8449)

m3F8 is an investigational new drug developed at MSKCC and manufactured by a qualified CMO in compliance with GMP guidance appropriate for phase I clinical trials. The final product specifications, analytical test methods and storage conditions/stability are described in the CMC section of this IND. Briefly, m3F8 is purified by column chromatography followed by viral filtration and finale sterile filtration. The final product is tested to assure that it is free of aggregates, nucleic acid, murine viruses, bacteria, fungi, mycoplasma and pyrogens. m3F8 is also tested for antibody specificity. For iv administration, 3F8 should be diluted into 10 ml 5% human serum albumin and millipore (0.2 μm) filtered before use. Route of Administration: iv infusion.

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 0.5 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, 0004-0156-01-ISOTRETINOIN, ACCUTANE)

5.3.1 Source and Pharmacology: The exact mechanism of retinoic acid-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. Retinoic acid also appears to enhance normal hematopoietic differentiation by increasing the responsiveness of myeloid and erythroid progenitor cells to the action of myeloid colony stimulating activity and erythropoietin, respectively. Metabolism: Retinoic acid 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/m^2 isotretinoin will produce peak plasma levels of 1-2mM. The mean peaktime as 3.2 hours after 80mg orally, with a terminal $t_{½}$ of 10 to 20 hours. Administering 160 mg/m^2/day to children after ASCT has been shown to achieve 13-cis-retinoic acid 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: Take orally 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

- Diagnosis of NB as defined by international criteria,[62] i.e., histopathology (confirmed by the MSKCC Department of Pathology) or BM metastases plus high urine catecholamine levels.
- High-risk NB, as defined by risk-related treatment guidelines and the International NB Staging System, i.e., stage 4 with (any age) or without (>18 months) *MYCN*-amplification,[63] or *MYCN*-amplified NB other than stage 1.[64,65]
- Patients are in CR/VGPR or have primary refractory NB in BM – i.e., NB resistant to standard therapy, as evidenced by persistence of NB in BM by histology or MIBG scan, but all other findings in scans show VGPR.
- Children and adults are eligible.
- Signed informed consent indicating awareness of the scheduling and side effects, as well as testing requirements, of this program.

6.2 Subject Exclusion Criteria

- Existing severe major organ dysfunction, i.e., renal, cardiac, hepatic, neurologic, pulmonary, or gastrointestinal toxicity $\geq$ grade 3, except for grade 3 hematologic toxicity.
- Progressive disease (PD) (section 12).
- History of allergy to mouse proteins.
- Active life-threatening infection.
- Human anti-mouse antibody (HAMA) titer >1000 Elisa units/ml.
- Pregnant women
- Inability to comply with protocol requirements.

7.0 RECRUITMENT PLAN

Patients will be offered participation in this open access study by their attending physician in the Department of Pediatrics, MSKCC. No patient will be identified by chart review or direct advertising. The attending physician will be responsible for explaining the study, obtaining written informed consent, and registering the patient on study. 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.

8.0 PRETREATMENT EVALUATION

Pre-treatment evaluations should be completed within 30 days of start of treatment.

8.1 Complete history and physical examination.

8.2 Complete blood count (CBC), serum creatinine, blood urea nitrogen, serum aspartate aminotransferase, serum alanine aminotransferase, and serum total bilirubin

8.3 BM aspirates from bilateral anterior and bilateral posterior iliac crests, and biopsies from any two separate sites. The specimens are studied by standard histochemical methods for the presence of tumor cells.

8.4 Computed tomography (CT) or magnetic resonance imaging (MRI) of primary tumor site, plus other specific or suspected sites of tumor.

8.5 Scintigraphic studies (MIBG scan[66] and/or positron emission tomography [PET] scan.[67]).

8.6 Serum for analysis of HAMA, if applicable (i.e., for patients previously treated with murine or chimeric antibody). If the patient was previously treated with an antibody, HAMA results since last antibody treatment can be used.

8.7 Pregnancy test, if applicable

9.0 TREATMENT/INTERVENTION PLAN

9.1 Schedule: The total dosage of 3F8 per cycle is 100 mg/m^2, administered at 20 mg/m^2/day and infused iv over ~1.5 hr or less (0.5 hr is customary), with analgesics and antihistamines used as needed for expected side-effects. 3F8 is started after GM-CSF administration. GM-CSF is dosed at 250 mcg/m^2/day from day –5 to day +1 (Wednesday to Tuesday is customary), and is 500 mcg/m^2/day thereafter (i.e., on days +2 to +4; Wednesday to Friday), as in the predecessor protocol.[61] Patients come off study for PD or a life-threatening grade 4 toxicity from 3F8; otherwise, patients will continue treatment through 24 months. It is expected that patients will receive ~4-to-10 cycles. Patients may receive local radiation therapy.

For ***Group 1 and 2*** patients (enrolled respectively on study in 1st or $\geq$2nd CR/VGPR, i.e., with no evidence of disease), the break between end of a cycle and start of next cycle is approximately 2-to-4 weeks through 4 cycles; subsequent breaks are ~6-8 weeks. Isotretinoin is started after cycle 2 of 3F8/GM-CSF. It is only to be started after cycle 1 if HAMA develops and precludes timely administration of cycle 2. Road map/schema is in section 4.2.

For ***Group 3*** patients (enrolled on study for treatment of primary refractory disease), the break between end of a cycle of 3F8/GM-CSF and start of next cycle is approximately 2-

to-4-weeks through 4 cycles after achievement of CR in BM; subsequent breaks are ~6-8 weeks. Isotretinoin is started after cycle 2 of 3F8/GM-CSF. It is only to be started after cycle 1 if HAMA develops and precludes timely administration of cycle 2. Road map/schema is in section 4.2.

Patients who develop early HAMA, which precludes timely treatments with 3F8/GM-CSF, are eligible to receive low-dose maintenance regimens such as irinotecan alone,[68] temozolomide alone,[69] irinotecan-temozolomide,[70] or cyclophosphamide-topotecan.[71] These patients can also receive anti-HAMA agents such as rituximab and cyclophosphamide. They resume treatment with 3F8/GM-CSF if HAMA becomes negative.

9.2 3F8/GM-CSF treatment schedule (one cycle).

Days -5 to -1: GM-CSF 250 mcg/m^2/day, subcutaneously.

Days 0 and +1: GM-CSF 250 mcg/m^2/day, subcutaneously.
3F8 20 mg/m^2/day by iv infusion over ~1.5 hr or less (usually 0.5 hr).

Days +2 to +4: GM-CSF 500 mcg/m^2/day, subcutaneously.
3F8 20 mg/m^2/day by iv infusion over ~1.5 hr or less (usually 0.5 hr).

Note: The daily GM-CSF is not administered if the absolute neutrophil count (ANC) is >20,000/ µl. The last documented ANC count will be used to determine whether GM-CSF will be administered or not. If HAMA develops, cycles are deferred until HAMA titer decreases to <1000 Elisa units/ml. Emla cream (lidocaine 2.5% and prilocaine 2.5%) can be used to prevent pain from GM-CSF shots.

9.3 Isotretinoin is administered at 160 mg/m^2/d, divided into two doses, x14 days. 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 3F8. All patients receive a minimum of 2 cycles of 3F8/GM-CSF before starting isotretinoin (only to be started after cycle 1 if HAMA develops and precludes timely administration of cycle 2). All patients take a total of 6 cycles of isotretinoin, but those patients who have persistence of disease (but no progressive disease) can take this agent until BM and MIBG show CR. Dose reductions due to expected side effects of isotretinoin (e.g., headaches, dry skin, etc.) are allowed.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Note: If for any reason tests cannot be completed on day 0, they will be completed on day 1.

10.1 CBC on days 0 and +3 (Mondays and Thursdays). If ANC is >10,000/ µl, CBC is repeated the next day.

10.2 C3 and CH50 on day 0 before and approximately 3 hours after 3F8 in cycle 1

10.3 HAMA is checked before each cycle of 3F8 (Dr. Cheung, Research Lab).

10.4 Serum creatinine, blood urea nitrogen, serum aspartate aminotransferase, serum alanine aminotransferase, and serum total bilirubin on days 0 and +4 of each cycle.

10.5 BM studies (section 8.4) at end of cycle 2 and subsequently, BM studies 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 Day 0 (Monday) and 3 (Thursday) of each cycle. If ANC >10,000/µl, CBC is repeated the next day. CBC is not repeated after Day 4.	-
C3 & CH50	-	Before and approximately 3 hours after 3F8 on Day 0 (Monday) of cycle 1.	-
Liver and Renal function tests (ALT, AST, BUN and creatinine)	✓	On Day 0 (Monday) and 4 (Friday) of each cycle.	-
Pregnancy test, if applicable	✓	Prior to starting cycle 1 of isotretinoin	-
Blood for HAMA	✓, if applicable	Before each cycle	-
Bone marrow studies	✓	After cycle 2, 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	Approximately q 3 months for 2 years from first treatment or until patient is off study, whichever is earlier and thereafter as clinically indicated

		by virtue of metastases in distant lymph nodes: approximately every 6 months while on study	
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

11.1 Toxicities are graded by the Common Toxicity Criteria (Version4.0) developed by the National Cancer Institute (NCI) of the USA.

11.2 3F8: Reversible side-effects include pain, paresthesia, hypertension, hypotension, tachycardia, urticaria, fever, nausea, emesis, and rarely, diarrhea, serum sickness, hyponatremia, somnolence and posterior reversible encephalopathy syndrome. Other potential side-effects that may occur are bronchospasm, anaphylaxis, peripheral neuropathy, impaired accommodation of the eye, and poor reactivity of pupils to light.

11.3 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.

11.4 Isotretinoin: Dry skin, cheilitis, dry eyes, hypercalcemia, pseudotumor cerebri, headaches, hepatotoxicity, teratogenic effect on fetus, depression, suicidal ideation.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 Response duration is calculated from first day of treatment with 3F8.

12.2 All patients are considered a treatment failure under this protocol if PD develops.

12.3 Disease status is defined by the International Neuroblastoma Response Criteria,[62] supplemented by results of MIBG scans.

- Complete response/remission (CR): no evidence of disease.
- Very good partial response/remission (VGPR): >90% decrease in all disease parameters, including primary tumor, except bone scan unchanged or improved; bone marrow must be free of disease; MIBG scan negative in osteomedullary sites.
- Partial response/remission: >50% decrease in all disease parameters, except bone scan unchanged or improved; no more than 1 positive bone marrow site; MIBG scan improved in all lesions.
- Mixed response: >50% decrease in $\geq$1 but not all disease markers, MIBG scan improved in some but not all sites.
- Stable disease: <50% decrease in all tumor markers.
- Progressive disease (PD): 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 dose of 3F8 will have an adequate trial.

13.0 CRITERIA FOR REMOVAL FROM STUDY

13.1 All patients come off study if PD develops at any time after cycle 1 (see section 12.3).

13.2 All patients come off study if there is a life-threatening grade 4 toxicity clearly attributable to 3F8.

13.3 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 removed from the study 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 Case Report Form or on the Serious Event Report, as applicable.
- **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, for example, clinical need to administer a concomitant medication that is excluded by the protocol, in the absence of an adverse event.
- **Violation of the study protocol**, including failure to return for required treatments or assessments.

14.0 BIOSTATISTICS

This trial concerns the anti-NB activity of 3F8/GM-CSF. The response endpoints are relapse-free survival for patients treated in CR/VGPR (Groups 1 and 2) and CR of BM disease for patients with primary refractory NB (Group 3). Response will be assessed and compared to historical data, in the context of the large experience with the 03-077 predecessor study (section 3.7).

We will treat NB patients with 3F8/GM-CSF provided they meet eligibility criteria of this protocol. This protocol will remain open until such a time that 3F8 is licensed for broader use (i.e., as an FDA-approved drug). All enrolled patients who receive any 3F8 will be considered in the evaluation of safety/toxicity.

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 or not 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).

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA 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 RSA to monitor patient accruals and completeness of regsitration data. Routine data quailty 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 (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. 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" which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: http://inside2/clinresearch/Documents/MSKCC%20Data%20and%20Safety%20Monitoring%20Plans.pdf

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical 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 clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

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. Patients are responsible for the costs of physician visits and usual laboratory tests, hospitalizations, and outpatient care. They are not responsible for the cost of 3F8. 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.

17.1 Privacy

MSKCC'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 described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:

The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.1

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, dry skin from isotretinoin, urticaria from 3F8 or GM-CSF, myelosuppression from the combination of 3F8 and GM-CSF, pain and transient hypoxia from opioids, and breath-holding with or without transient oxygen requirement. Pre-existing conditions, e.g., hearing loss, abnormal liver function tests from total parenteral nutrition, 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.

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.

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20.0 APPENDICES

Appendix A: Research Patient Diaries