Phase II study of anti-GD2 3F8 antibody and GM-CSF for high-risk neuroblasto ma

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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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This phase II trial of the anti-GD2 murine IgG3 monoclonal antibody 3F8 combined with granulocyte-macrophage colony stimulating factor (GM-CSF) will assess response of minimal

residual disease (MRD) in patients with high-risk neuroblastoma (NB) and help establish the optimal way to use GM-CSF. The study will focus on two groups of patients: ***Group 1*** patients have primary refractory NB in bone marrow (i.e., incomplete response to standard treatment), and ***Group 2*** patients have no evidence of NB by standard studies but are at high risk for relapse. We will apply real-time quantitative RT-PCR methodology targeting GD2 synthase mRNA for monitoring MRD; we will test the hypothesis that early BM response has prognostic import. The method of using GM-CSF to achieve optimal antitumor biologic effect is not known but will be studied by comparing anti-NB effects in patients with the subcutaneous (sc) usage of GM-CSF in this protocol versus results achieved with the intravenous (iv) usage of GM-CSF in the prior study. We hypothesize that the sc route will augment overall immune function with consequent improved anti-NB activity. Changing to the simple sc usage, as compared to the prior 2-hr iv method, will vastly simplify treatment, both for patients and for medical staff, and will reduce costs; the ultimate result will be promoting wider availability of this treatment.

2.1 OBJECTIVES AND SCIENTIFIC AIMS

- Assess the efficacy of subcutaneous GM-CSF in enhancing 3F8-mediated ablation of BM disease.
- Apply real-time quantitative RT-PCR to test the hypothesis that minimal residual disease content of BM after the first treatments with 3F8/GM-CSF has significant prognostic impact on relapse-free survival.
- Simplify treatment with consequent reduction in cost.

3.1 BACKGROUND AND RATIONALE

3.2 Disease background: Neuroblastoma (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 minimal residual disease (MRD) has remained a formidable challenge. Post-surgical use of local radiotherapy helps control MRD in the primary site.[7] Myeloablative therapy (with stem-cell support) has been the most common approach for eradicating MRD in distant sites. The long-term relapse-free survival rate in the most recent national study was only ~20%, although the vitamin A derivative 13-*cis*-retinoic acid (isotretinoin) helped to prolong relapse-free survival.[8] These results, plus the potentially severe toxicities of chemotherapy and radiotherapy, are compelling reasons for pursuing novel therapeutic approaches.

3.3 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[9-18] or adults,[19-22] especially with antibody-dependent cellular cytotoxicity (ADCC) as the principal underlying immune cytotoxic mechanism. Also, an antineoplastic role for granulocytes has

received scant attention.[23] A treatment program combining 3F8 and GM-CSF thus represents an attempt to help fill a gap in the emerging field of immunotherapy.

3.4 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. 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.[24] 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).[25-27] Third, scintigraphy using ^{131}I-3F8 confirms that 3F8 localizes selectively to GD2(+) tumor deposits in patients.[28] 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.[28,29] Fourth, 3F8 mediates destruction in vitro of GD2(+) human solid tumor cells by human complement[30] and by human lymphocytes, cultured monocytes, and neutrophils.[31-33] Finally, the capacity of 3F8 to activate complement on NB cells (which lack decay accelerating factor[34]) 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,[35,36] which are the most abundant circulating class of leukocyte.

3.5 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.[37] 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.[36,38-42] Third, eosinophilic infiltration of some cancers has favorable prognostic significance, and eosinophils exhibit potent antitumor activity in animal models.[43,44] 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.[33,39,45-50] Fifth, GM-CSF enhances the proliferation, maturation, and function of antigen-presenting cells, including antigen processing and presentation by macrophages and dendritic cells[37,51,52] - effects that might promote induction, or antitumor activity, of an idiotypic network.[53-56] Finally, GM-CSF is not a growth factor for NB cells in vitro.[57]

3.6 GM -CSF-me diate d activation of polymorphonucle ar ne utrophils (PM Ns): PMNs 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.[23] We and others have shown that Mac-1 (CD11b/CD18), FcRII, and FcRIII are required for optimal ADCC in a system using human PMNs as effectors, human NB cells as targets, and clinically active anti-GD2 MoAbs as mediators.[35,36] 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.[41] 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.[42] To elucidate the cytotoxic mechanisms of anti-GD2 MoAb-mediated ADCC, we used blocking MoAbs, PMNs devoid of Mac-1 (from donors with leukocyte adhesion deficiency), and PMNs that do not generate reactive oxygen species (from donors with chronic granulomatous disease),[35] while others used blocking MoAbs, electron microscopy, and PMNs from healthy donors as well as from patients.[36]

3.7 Rationale for quantitating BM disease by real-time RT-PCR: 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%.[4,7] Dose-intensive chemotherapy eliminates histologically evident BM involvement in 90% of high-risk cases.[3] 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 monoclonal antibodies, may make gradual and not quantum changes in MRD, which by definition is beyond the sensitivity of conventional histologic[58] or radiographic techniques. The availability of more sensitive methods for identifying and quantitating occult tumor cells in sequential BM specimens can allow determination of the efficacy and optimal duration of adjuvant treatment. Thus, 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[59-65] and others[66-70] have identified MRD in BM by immunological and molecular methods. The limit of detection for immunocytology is $1/10^5$ to $1/10^6$ cells; RT-PCR has slightly superior sensitivity with a range of $1/10^5$ to $1/10^7$ cells. A drawback to immunocytology is the need for freshly collected samples and the technique's labor intensive aspect (counting cells under a microscope). In contrast, molecular monitoring of tumor cells by RT-PCR uses cryo-preserved mononuclear cells and experiments can be repeated multiple times and for multiple markers as they are being developed. Pitfalls in the use of molecular-based assays of MRD include false positivity due to the presence of the "tumor-specific" markers in normal cells, and the process of illegitimate transcription (i.e., the transcription of any gene in any cell type).

Development of a real-time quantitative RT-PCR assay broadens the potential for monitoring MRD. The advantages of this technique include a wide linear dynamic range and superior sensitivity and accuracy, which allows good intra- and inter-assay reproducibility, plus high throughput capacity, speed, and elimination of lengthy post-PCR handling steps, which prevents potential carryover contamination. Real-time quantitation of GD2 synthase mRNA provides information on expression of the key enzyme in the biosynthesis of GD2.[63,64]

3.8 Results using real-time quantitative RT-PCR of GD2 synthase mRNA: Most recently, we have used this method to assess molecular response in BM from patients with high-

risk NB treated with 3F8 plus GM-CSF by 2-hr iv infusion; we now intend to apply this method for comparing anti-NB activity of the subcutaneous versus iv route of GM-CSF usage. In our retrospective study, the patients fell into four groups before treatment with 3F8 and iv GM-CSF: 33 patients were in complete/very good partial remission, 32 had primary refractory disease (incomplete response to induction), 11 had secondary refractory disease (incomplete response to treatment for relapse), and 16 had progressive disease. Molecular detection of tumor cells was evident in pretreatment BMs of all four groups (32%, 59%, 64%, and 75%, respectively) with mean GD2 synthase transcript levels of 7.5, 22.3, 54.7, and 436.3, respectively (Table 1).

Table 1. NB cell detection in BM before 3F8/GMCSF treatment in 4 groups of patients

		% positivity by		GD2 synthase
	N	Histology	QRT-PCR*	Transcript Level (mean±SE)
First CR/VGPR	33	0%	32%	7.5 ± 2.9
Primary Refractory	32	68%	59%	22.3 ± 5.6
Secondary Refractory	11	82%	64%	54.7 ± 34.2
Progressive Disease	16	73%	75%	436.3 ± 232.4

*GD2 synthase transcript < 5 was defined as negative.

In these 4 groups, there was molecular evidence of BM response in 43%, 67%, 44%, and 0% of patients, respectively (Table 2).

Table 2. Response rates by molecular testing and by histology in 4 groups of patients

	GD2 synthase mRNA		Histology
	Response Rate	Negative Transcript*	Response Rate
First CR/VGPR	43% (6/14)	19	(not applicable)
Primary Refractory	67% (14/21)	11	60% (15/25)
Secondary Refractory	44% (4/9)	2	60% (6/10)
Progressive Disease	0% (0/10)	1	0% (0/10)

*Pre- and post-treatment BMs were (-).

The response was consistent over time, that is, disease status after one or two cycles of 3F8/GM-CSF correlated with disease status at a later time point during treatment. Transcript levels in pretreatment BMs of responders (28.2±6.8) were lower than those of non-responders (139.0±77.7) (Table 3).

Table 3. GD2 synthase transcript levels* in BM before and after 3F8/GMCSF

		Pre-treatment sample	Post-treatment sample
	N	mean ± SE	mean ± SE
Responders	24	28.2 ± 6.8	0.5 ± 0.2
Non-responders	30	139.0 ± 77.7	312.4 ± 95.9
Negative transcript	33	0.9 ± 0.3	1.0 ± 0.3

*GD2 synthase transcript < 5 was defined as negative.

Excluding the progressive disease group, progression-free survival (PFS) was statistically different among non-responders, responders, and patients with persistently negative transcript (p=0.02) (Figure 1). Among patients with histologically negative BMs, molecular responders had a significantly lower risk of disease progression (p=0.02) (Figure 2). The results suggest that GD2 synthase might serve as a sensitive marker of MRD in BM and that its level might reflect clinical disease status as well as correlate with histologic response to immunotherapy.

3.9 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 is not known. 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

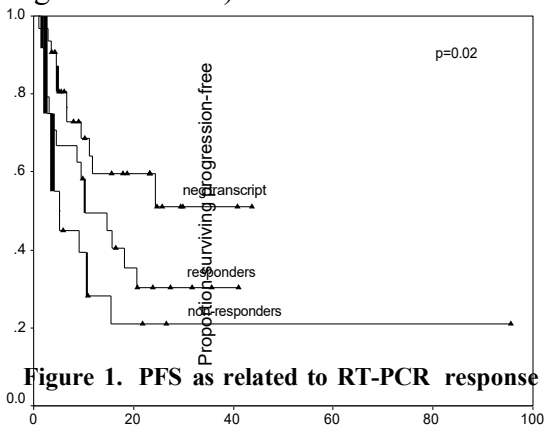


Figure 1. PFS as related to RT-PCR response

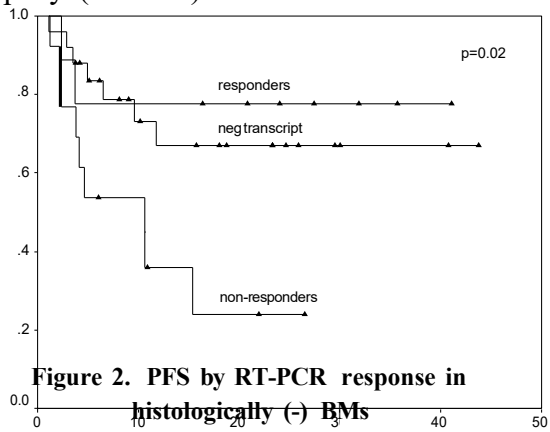
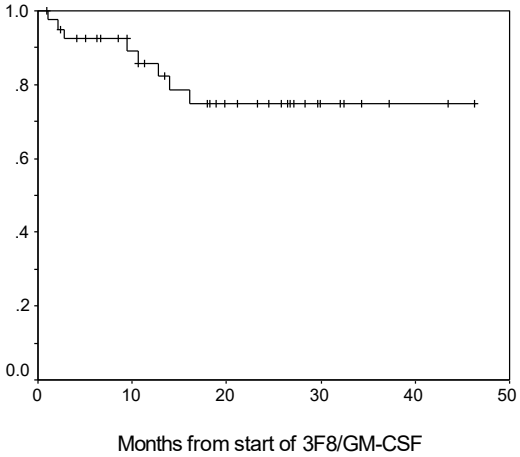


Figure 2. PFS by RT-PCR response in histologically (-) BMs

schedule.[71,72] We wished to avoid the prolonged (>12 hr) bioactive levels in blood associated with the sc route; our concern was that high serum levels of GM-CSF might impede granulocyte trafficking into tissues.[73] 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.[37]

Major findings included: 1) treatment for progressive disease: no major responses in 16/16 patients; 2) treatment for primary refractory disease: CR (by histology) in BM in 21 (75%) of 28 patients, including 5/5 adolescents/adults, and with CR evident after 1-3 cycles; and 3) treatment in first complete/very good partial remission post-transplant (n=41): 72.6% (±8.0%) relapse-free survival at 18 months from first dose of 3F8 (Figure 3), with many patients receiving 10 or more cycles and with use of 13-cis-retinoic acid after cycle 2.[6,18,74]

Figure 3. Patients treated in CR/VGPR



3.10 HAMA response/idiotypic network: In a study of 34 patients with MRD treated with 3F8 alone, patients with a self-limited (transient) HAMA response had significantly better PFS than patients who had persistently high

HAMA titers or no HAMA formation.[15] This association of a transient low level HAMA/anti-idiotype (Ab2) antibody response with better PFS was a seemingly paradoxic finding since HAMA/Ab2 blocks 3F8 binding to tumor cells. The unexpected observation regarding HAMA/Ab2 and survival led to the hypothesis of a host idiotypic network induced by 3F8 (Ab1) and responsible for long-term tumor control. The idiotypic network was first postulated by Jerne[75] for immune regulation and later demonstrated in human disease models, including cancer patients.[76-79] Anti-idiotypic antibodies (Ab2) in the HAMA pool may mimic antigen GD2 and stimulate an Ab3 antibody response. We previously reported the association of anti-anti-idiotype (Ab3) with prolonged survival among patients who underwent myeloablative therapy with autologous stem-cell rescue.[53] Other investigators have postulated the development of an idiotypic network in patients treated with 17-1A antibodies, where both Ab3 antibodies as well as anti-idiotypic and anti-anti-idiotypic T cells have been demonstrated.[54,55]

In our study of 34 patients, consistent with the emergence of an idiotypic network, Ab3 (anti-anti-idiotype) and Ab3' (anti-G_{D2}) response at 6-14 months after treatment with 3F8 alone correlated with improved PFS, and higher Ab3 titers showed a stronger correlation with improved outcome, while non-idiotype antibody responses (anti-mouse or anti-tumor nuclear antigen) had no apparent impact on outcome.[56] Since GD2 is an auto-antigen, induced Ab3' response represented a breakdown in self-tolerance. We hypothesized that significant rises in Ab3 or Ab3' would not be possible unless suppressor pathways were removed and naive T or B cells were allowed to repopulate. Following intensive chemotherapy that eliminates a large part of the lymphoid system, exposure to tumor-selective Ab1s (3F8) might induce unique Ab2s that could bias the recovering repertoire towards the GD2 antigen network. The potential for biasing the immune system towards specific antigens has been well documented in murine models[80] and human disease states.[81,82] If true, one would expect such an idiotypic network to be most successful following intensive immunosuppressive therapy, and not easily induced in immunocompetent patients. In fact, we found that myeloablative therapy suppressed HAMA and Ab2 but was followed by the appearance of Ab3 which in turn correlated with prolonged survival.[53,56] The prerequisite of strongly immunosuppressive therapy may explain why in most clinical studies of MoAbs, such an idiotypic network is not commonly observed. However, when found in patients with colorectal or ovarian carcinoma treated with specific MoAbs, the idiotypic network gave patients superior survival advantage.[54,55,76,77,83-87]

3.11 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.

3.12 Interpretations and implications: This phase II trial explores the utility of RT-PCR for monitoring anti-NB activity of immunotherapy, with an emphasis on early recognition of treatment efficacy. The studies focus on BM; findings may not reflect total body tumor burden. Metastatic NB is, however, a systemic disease, and, as with leukemia,[88] BM status appears to approximate the overall disease state. If the RT-PCR method proves to be sensitive, it will provide a useful tool for measuring NB-cell content in BM as a patient goes through treatment. The ability to identify patients not likely to benefit from further use of a given adjuvant therapy will allow a more timely switch to a possibly better treatment. We hypothesize that quantifying GD2 synthase transcript levels will help stratify patients by tumor load, define

tumor thresholds that correlate with favorable clinical outcome, and provide prognostic classification for patients receiving immunotherapy. Risk-related molecular guidelines can be rapidly incorporated into future treatment programs.

Our RT-PCR method provides a quantitative measure, such that the percentage of patients who achieve CR in BM, and the degree of response with 3F8/GM-CSF, can be calculated and compared to other adjuvant treatments. With NB, where randomized trials are difficult to carry out because of the small number of affected patients, such quantitative analyses acquire even greater utility for rapidly identifying both the best agent and its optimal use in a curative treatment program.

The findings regarding RT-PCR utility in the adjuvant setting, granulocyte activation, and idiotypic network will have general implications for antibody-based therapeutic strategies against solid tumors in humans. Also, 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.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a phase II, open-label, single arm study to assess the activity of 3F8/GM-CSF against neuroblastoma and to investigate whether subcutaneous (sc) usage of GM-CSF is as good or better than short-term (2-hr) intravenous (iv) usage for optimizing anti-neoplastic activity. ***Group 1*** patients have refractory bone marrow disease, and ***Group 2*** patients have no evidence of disease by standard studies, but are at high risk for relapse. Clinical results will be compared to those observed in the predecessor trial in which only iv GM-CSF was used with 3F8. Real-time quantitative RT-PCR[63-65] will be used to assess minimal residual disease in bone marrow. All patients will receive a minimum of 4 cycles of treatment and will continue treatment through 24 months.

4.3 Intervention

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

Cycle 1 3F8 (iv) + GM-CSF (subcutaneous [sc]) (1 wk)
3-wk interval* – BM negative
Cycle 2 3F8 (iv) + GM-CSF (sc) (1 wk)
3-wk interval* – if BM negative -> 1st cycle of oral isotretinoin x14 days
Cycle 3 3F8 (iv) + GM-CSF (sc) (1 wk)
3-wk interval – oral isotretinoin x14 days
Cycle 4 3F8 (iv) + GM-CSF (sc) (1 wk)
3-wk interval – oral isotretinoin x14 days
Cycle 5 3F8 (iv) + GM-CSF (sc) (1 wk)
8-wk interval – oral isotretinoin x14 days
Cycle 6 3F8 (iv) + GM-CSF (sc) (1 wk)
8-wk interval – oral isotretinoin x14 days on, x14 days off, x14 days on (6th/last cycle)
Cycle 7 3F8 (iv) + GM-CSF (sc) (1 wk)
8-wk interval
Continue with 8-wk intervals through 24 months from 1st dose of 3F8.

* **assessment of BM response by RT-PCR and by standard histology**

Road Map/Schema for Group 2 patients (no evidence of disease):

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

8-wk interval – oral isotretinoin x14 days on, x14 days off, x14 days on
Cycle 5 3F8 (iv) + GM-CSF (sc) (1 wk)
8-wk interval – oral isotretinoin x14 days on, x14 days off, x14 days on (6th/last cycle)
Cycle 6 3F8 (iv) + GM-CSF (sc) (1 wk)
8-wk interval
Cycle 7 3F8 (iv) + GM-CSF (sc) (1 wk)
Continue with 8-wk intervals through 24 months from 1st dose of 3F8.

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

TABLE 4. PRETREATMENT EVALUATION AND DURING THE STUDY

WHAT?	WHEN?
Complete history and physical examination.	• Pretreatment evaluation
Complete blood count	1. Pretreatment Evaluation 2. On Monday (day 0) and Thursday (day +3). If ANC is >10,000/ul, CBC is repeated on next day.
Liver and kidney function Blood Tests	1. Pretreatment 2. On day 0 (Monday) and +4 (Friday) of each cycle
C3 and CH50	• On days 0 and +4 (Monday and Friday) of each cycle.
HAMA (Red Top Tube) (Dr. Cheung, Research Lab)	• ~Every 1-2 months while on study.
Bone marrow studies (including 10 ml heparinized for immunocytology and/or RT-PCR, Dr. Cheung, Research Lab)	1. Pretreatment 2. At end of cycles 2 & 4 3. After cycle 6 of Accutane 4. Subsequently, • BM studies are repeated with MIBG or PET through 2 years for patients with history of BM or cortical bone involvement, • But are repeated ~every 6 months in other patients (e.g., patients who were stage 4 by virtue of metastases in distant lymph nodes) while on study.
CT or MRI	1. Pretreatment: of primary tumor site, plus other specific or suspected sites of tumor. 2. Followup: of primary site ~every 3 months through 1 year.
Scintigraphic studies (^{99m}Tc-MDP-bone scan, MIBG scan,[92] and/or PET scan.[93]).	• Pretreatment
^{99m}Tc-MDP-bone scan	~Every 3 months while on study until normal
MIBG or PET scan	~Every 3 months through 2 years
Echocardiogram	Pretreatment
Urine catecholamines levels	1. Pretreatment 2. ~Every 3 months through 2 years.
Pregnancy screen (females of child-bearing age)	Before starting isotretinoin.

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

5.1 THERAPEUTIC/DIAGNOSTIC AGENTS

5.2 3F8 monoclonal antibody (IND number of BB-IND-8449)

5.2.1 Source and Pharmacology: Monoclonal antibody 3F8 is purified by column chromatography. The final product is tested to assure that it is free of nucleic acid, murine viruses, bacteria, fungi, mycoplasma and pyrogens. Plasma concentrations achieved after a 5 mg/m2 and 20 mg/m2 IV dose were 1.1 +/- 0.6 and 3.7 +/- 1.4 ug/mL, respectively. Supplier: 3F8 is manufactured as an investigational agent.

5.2.2 Formulation and Stability: The purified antibody is frozen at –80°C, at 2 mg/mL 0.1 M citrate-phosphate pH 4.2 buffer in glass vials. At –80°C it is stable for at least 3 years. For IV administration, 3F8 should be diluted into 10 ml 5% human serum albumin and millipore (0.2 um) filtered before use. Route of Administration: IV infusion.

5.3 Yeast-derived human recombinant granulocyte-macrophage colony stimulating factor (GM-CSF; LEUKINE (sargramostim)) is produced by Berlex Laboratories, Seattle, WA.

5.3.1 LEUKINE is a sterile, white, preservative-free, lyophilized powder suitable for IV infusion upon reconstitution of 250 mcg vials; these vials should be reconstituted aseptically with 1.0 ml Sterile Water for Injection, USP (without preservative). The reconstituted LEUKINE solutions are clear, colorless, isotonic with a pH of 7.4 ± 0.3, and contain 250 mcg/ml of Sargramostim. The single-use vial should not be re-entered or reused. Do not save any unused portion for later administration. Vials containing 500 mcg of LEUKINE are already in solution and are multiple dose vials.

5.3.2 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 rigorous agitation; do not shake.

5.3.3 Dilution for IV infusion should be performed in 0.9% Sodium Chloride Injection. USP. If the final concentration of LEUKINE is below 10 mcg/ml, Albumin (Human), add 1 mg Albumin (Human) per 1 ml 0.9% Sodium Chloride Injection, USP (e.g. use 1 ml 5% Albumin [Human] in 50 ml 0.9% Sodium Chloride Injection, USP).

5.3.4 An in-line membrane filter is not to be used for IV infusion of LEUKINE.

5.3.5 LEUKINE contains no antibacterial preservative and therefore should be administered as soon as possible, and within 6 hours following reconstitution and/or dilution for IV infusion. Store LEUKINE powder, reconstituted solutions, or diluted solutions under refrigeration at 2-8°C (36-46°F); do not freeze or shake. LEUKINE vials are intended for single

use only; discard any unused solution after 6 hours. Do not use beyond the expiration date printed on the vial.

5.3.6 In the absence of compatibility and stability information, no other medication should be added to infusion solutions containing LEUKINE. Use only 0.9% Sodium Chloride Injection, USP to prepare IV infusion solutions.

5.3.7 Aseptic technique should he employed in the preparation of all LEUKINE 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.

5.3 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 (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 stimulating 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/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 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.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

Patients must have neuroblastoma. They can have evaluable (bone marrow [+] by

histology, abnormal MIBG or PET scan consistent with bone marrow involvement), but not progressive, disease (***Group 1***). Patients in complete/very good partial remission (CR/VGPR; no evaluable or measurable disease) yet at high risk for relapse are also eligible (***Group 2***).

6.2 Subject Inclusion Criteria

- Diagnosis of neuroblastoma as defined by international criteria,[91] i.e., histopathology (confirmed by the MSKCC Department of Pathology) or bone marrow metastases plus high urine catecholamine levels.

- Neuroblastoma, as defined by risk-related treatment guidelines and the International Neuroblastoma Staging System, i.e., stage 4 with (any age) or without (>18 months) MYCN-amplification,[93a] or MYCN-amplified neuroblastoma other than stage 1.[93b,93c]

- Group 1 patients have neuroblastoma (as defined above) resistant to standard therapy, as evidenced by incomplete response in bone marrow, but no MIBG-avid soft tissue tumor and no progressive disease.

- Group 2 patients have no evidence of disease, i.e., are in CR/VGPR from neuroblastoma (as defined above).

- Signed informed consent indicating awareness of the investigational nature of this program.

6.3 Subject Exclusion Criteria

- Existing severe major organ dysfunction, i.e., renal., cardiac, hepatic, neurologic, pulmonary, or gastrointestinal toxicity $\geq$ grade 3.

- Progressive disease or MIBG-avid soft tissue tumor.

- History of allergy to mouse proteins.

- Active life-threatening infection.

- Human anti-mouse antibody (HAMA) titer >1000 Elisa units/ml.

- Inability to comply with protocol requirements.

7.0 RECRUITMENT PLAN

In each of the past 2 yrs, 23 patients have been treated on the predecessor of this protocol. We anticipate an increase in this accrual rate, which will allow rapid realization of preliminary conclusions, with completion of the trial in ~3 yrs. Given the favorable prior efficacy-toxicity profile with 3F8/GM-CSF, we expect that all patients will complete the trial. 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 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 neuroblastoma (90% of patients are <6 years old at diagnosis). Patients of both sexes and all ethnic backgrounds are eligible for this study.

8.1 PRETREATMENT EVALUATION (TABLE 4)

8.2 Complete history and physical examination.

8.3 Complete blood count, serum creatinine, blood urea nitrogen, serum aspartate aminotransferase, serum alanine aminotransferase, and serum total bilirubin

8.4 Echocardiogram.

8.5 Urine catecholamine levels.

8.6 Bone marrow 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.
- Immunocytology using a panel of monoclonal antibodies that react with antigens associated with neuroblastoma (Dr. Cheung, Research Lab).

8.7 CT or MRI of primary tumor site, plus other specific or suspected sites of tumor.

8.8 Scintigraphic studies (^{99m}Tc-MDP-bone scan or MIBG scan[92] or PET scan.[93]).

9.1 TREATMENT/INTERVENTION PLAN

9.2 Schedule: The total dosage of 3F8 per cycle is the same as in prior trials (100 mg/m^2), administered at 20 mg/m^2/day and infused 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 ~1 hr after completion of 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.[18,74] Patients come off study if progressive disease occurs or if there is life-threatening grade 4 toxicity from 3F8; otherwise, patients will receive a minimum of 4 cycles of treatment and will continue treatment through 24 months**.** It is expected that patients will receive ~10 cycles.

For ***Group 1*** 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 2-to-4-weeks through 4 cycles after achievement of CR in BM; subsequent breaks are ~8 weeks. In this group, isotretinoin is started after documentation of response to, and after ≥2 cycles of, 3F8/GM-CSF. Road map/schema is in section 4.2.

For ***Group 2*** patients (enrolled on study in CR/VGPR, i.e., with no evidence of disease), the break between end of a cycle and start of next cycle is 2-to-4 weeks through 4 cycles;

subsequent breaks are ~8 weeks. Isotretinoin is started after cycle 2 of 3F8/GM-CSF. Road map/schema is in section 4.2. Regarding patients in second or greater CR from relapse in the central nervous system, if they develop early HAMA which precludes timely completion of the minimum of 4 cycles of 3F8/GM-CSF, they are eligible to go off protocol, to be treated with low-dose maintenance regimens of irinotecan,[94] temozolomide,[95] or the two agents combined;[96] they can resume treatment with 3F8/GM-CSF if HAMA becomes negative.

Patients who develop early HAMA which precludes timely treatments with 3F8/GM-CSF (>4 week delay) are eligible to receive low-dose maintenance regimens such as irinotecan alone,[94] temozolomide alone,[95] irinotecan-temozolomide,[96] or cyclophosphamide-topotecan.[97] They can also receive anti-HAMA agents such as rituximab and cyclophosphamide. They resume treatment with 3F8/GM-CSF if HAMA becomes negative.

9.3 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 ANC is >20,000/ul. The ANC count from the previous treatment day 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.

9.4 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).

10.1 EVALUATION DURING TREATMENT/INTERVENTION (TABLE 4)

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

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

10.3 C3 and CH50 on days 0 and +4 (Monday and Friday) of each cycle.

10.4 HAMA is approximately every 1-2 months while on study (Dr. Cheung, Research Lab).

10.5 Liver and kidney function blood tests on days 0 and +4 of each cycle.

10.6 BM studies (section 8.6) at end of cycles 2 and 4 and after the 6th cycle of Accutane. Subsequently, BM studies are repeated in conjunction with MIBG or PET scan (section 10.8) through 2 years in patients with history of BM or cortical bone involvement, but are repeated ~every 6 months in other patients (e.g., patients who were stage 4 by virtue of metastases in distant lymph nodes).

10.7 CT or MRI of primary site approximately every 3 months through 1 year.

10.8 ^{99m}Tc-MDP-bone scan approximately every 3 months while on study until normal.

10.9 MIBG or PET scan approximately every 3 months through 2 years.[92,93]

10.10 Urine catecholamine levels approximately every 3 months through 2 years.

10.11 Pregnancy screen (females of child-bearing age) before starting *cis*-retinoic acid.

11.1 TOXICITIES/SIDE EFFECTS

11.2 Toxicities are graded by the Common Toxicity Criteria (Version 2.0) developed by the National Cancer Institute (NCI) of the USA.

11.3 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 (PRES). Other potential side-effects that may occur are bronchospasm, anaphylaxis, peripheral neuropathy, impaired accommodation of the eye, and poor reactivity of pupils to light.

11.4 GM-CSF: Common side-effects include bone pain, flushing, local reaction at site of injection, leukopenia shortly after injection, and decrease in platelet count.[98] Rare side-effects (predominantly in adults) include allergic reactions, weight gain, pleural or pericardial effusion, pericardial embolism, thrombosis, and difficulty breathing after first injection.

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

12.1 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

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

12.3 ***Group 1*** patients (i.e., patients treated for primary refractory disease) are considered a treatment failure under this protocol if progressive disease develops.

12.4 ***Group 2*** patients (i.e., patients in CR/VGPR) are considered a treatment failure under this protocol if progressive disease develops.

12.5 Disease status is defined by the International Neuroblastoma Response Criteria,[91] supplemented by RT-PCR findings in bone marrow (section 8.6)

- 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; bone marrow must be free of disease.
- Partial response/remission: >50% decrease in all disease parameters, except bone scan unchanged or improved; no more than 1 positive bone marrow site.
- Mixed response: >50% decrease in ≥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.

12.6 Adequacy of trial: All patients who fulfill the eligibility requirements and receive a first dose of 3F8 will have an adequate trial.

13.1 CRITERIA FOR REMOVAL FROM STUDY

13.2 ***Group 1*** patients come off study if progressive disease develops at any time after cycle 1. (see section 12.2).

13.3 ***Group 2*** patients come off study if progressive disease develops at any time after cycle 1 (see section 12.3).

13.4 Life-threatening grade 4 toxicity clearly attributable to 3F8.

13.5 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. Patients who fail to return for treatments will be withdrawn from the study if more than 4 scheduled doses are missed

13.6 Patients will not be removed from study in order to receive radiotherapy.

14.0 BIOSTATISTICS

Clinical results: This phase II trial assesses the anti-NB activity of 3F8 plus sc GM-CSF. The response endpoints are CR of BM disease for patients with primary refractory NB (Group 1), and two-year relapse-free survival for patients treated in CR/VGPR (Group 2). We expect to accrue 15 patients per year in each group. This trial can be completed within 3 years.

Group 1: 3F8 plus iv GM-CSF (IRB protocol 94-018) yielded an overall two-year relapse-free Kaplan-Meier survival probability of 13% in 31 patients with primary refractory disease. With the new treatment using 3F8 plus subcutaneous GM-CSF, we will consider a two-year relapse-free survival probability of 0.13 as not promising and 0.25 as promising. For a one stage design with type I error 0.05 and 0.85 power, 70 patients will be required. If 12 or more patients are relapse-free at two years, the treatment will be defined as promising, and an additional 15 patients (total: 85) will be enrolled with the goal of strengthening the findings.

Group 2: 3F8 plus iv GM-CSF (IRB protocol 94-018) yielded a two-year relapse-free Kaplan-Meier survival probability of 55% for patients treated in first CR/VGPR. With the new treatment, we consider a two-year relapse-free survival probability of 0.55 as not promising and 1.70 as promising. For a one stage design with type I error 0.05 and 0.85 power, 75 patients will be required. If 49 or more patients are relapse free at two years, the treatment will be defined as promising. Two separate cohorts will be accrued: 75 patients in first CR/VGPR after transplant and 75 patients in first CR/VGPR after conventional chemotherapy + 13-cis-retinoic acid and no transplant. Up to 85 patients in each cohort will be accrued with the aim of strengthening the findings.

Patients treated in $\geq 2^{nd}$ remission, long-term relapse-free survival is <5%, including 0/7 treated with 3F8 plus iv GM-CSF (all 7 relapsed within 15 months). Therefore, we consider a two-year relapse-free survival probability of 15% as promising and 1% as not promising. Patients will be divided into two groups, those with and without prior antibody therapy:

* For those without prior antibody treatment or other immunotherapy, Simon's two-stage optimal design will be used with a power of 90% and an alpha of 5%. For a total of 33

subjects, 18 will be accrued during stage 1 and 15 during stage 2. The trial stops early if none of the first 18 patients survive for two years without relapse. Otherwise, the trial continues until 33 patients are accrued. Treatment will be considered promising if two or more patients experience two-year relapse-free survival and accrual can continue up to 50 patients with the goal of strengthening the findings

* Patients with prior antibody treatment or immunotherapy are less common and so only a trial with lower power is feasible. We will accrue 19 patients, declaring treatment as promising if two or more experience two-year relapse-free survival. This design has a power of 80% and an alpha of 5%, a null hypothesis of 1% and an alternate hypothesis of 15%. Only a one-stage design is feasible with these characteristics as an observed zero response rate would consistent with a true response rate of 15% for an interim analysis conducted at all possible stopping points up to the 18th patient. If the findings are favorable, accrual can increase to 35, with the aim of strengthening the conclusions.

* Patients who went off protocol to receive therapy for relapse in the central nervous system, or to receive additional chemotherapy because of HAMA (section 9.1), are not included in the statistical analyses for these groups.

Utility of RT-PCR: The association between histologic examination and real time RT-PCR regarding BM status can be assessed by the Wilcoxon statistic. The clinical significance (relapse-free survival) of histologic results and real time RT-PCR findings in both groups can be assessed by log-rank test or Cox regression model or time dependent Cox regression model.

15.1 SUBJECT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

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.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the written consent/verbal script and a completed Eligibility Checklist must be faxed to PPR.

16.1 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.

16.2 Quality Assurance

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

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.3 Data and Safety Monitoring

Institutional monitoring plan for phase I and phase II trials: Memorial Sloan-Kettering Cancer Center (MSKCC) has established standard procedures for data safety monitoring of clinical research (see appendix). For Phase II trials, these procedures include consideration of accrual rates, toxicity, adherence to dose-escalation schedules, adverse event notification and data recording. Therapeutic responses are logged on a central database and approximately 50% are reviewed by an independent committee. An annual report of the trial's progress is sent to the IRB. All of the procedures for Phase II studies outlined in MSKCC's policy are applicable to the current trial and will be followed by the investigators. The analysis of safety will include all patients who receive at least one dose of study medication. Adverse events, including all toxic effects of treatment, will be tabulated individually, and summarized by body system, according to dosage of study medication (single dose as well as cumulative doses of 3F8 and GM-CSF), and according to severity or toxicity grade. Laboratory data will be tabulated and summarized by descriptive statistics, as well as on the basis of MSKCC specified normal ranges.

The policies and procedures for data safety and monitoring of clinical research at Memorial Sloan-Kettering Cancer Center are detailed in Appendix (Data Safety and Monitoring of Clinical Research).

17.1 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. 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.

17.2 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.

17.3 Serious Adverse Event (SAE) Reporting

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

All SAEs must be entered into the CRDB SAE form page.

SAEs are defined as grade 4 toxicities other than fatigue, weight loss or weight gain, anorexia, nausea, anxiety, constipation, urinary retention from opioid analgesics, somnolence/hallucinations/ disorientation/confusion/ agitation/anxiety from anti-histamine and opioid premedications, hypomagnesemia, fever, rash, dry skin from accutane, urticaria from 3F8 or GMCSF, myelosuppression from the combination of 3F8 and GM-CSF and pain and transient hypoxia from opioids, breathholding, with or without transient oxygen requirement. Preexisting conditions e.g. hearing loss, hyperbilirubinemia or elevated LFTs from TPN, alopecia are not counted as SAEs.

18.1 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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