PROTOCOL COVER PAGE

Protocol Title:

A **S**afety **P**ilot Study of High **R**isk **I**nduction Chemotherapy for **N**euroblastoma without Prophylactic Administration of Myeloid **G**rowth Factors (SPRING)

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A **S**afety **P**ilot Study of High **R**isk **I**nduction Chemotherapy for **N**euroblastoma without Prophylactic Administration of Myeloid **G**rowth Factors (SPRING)

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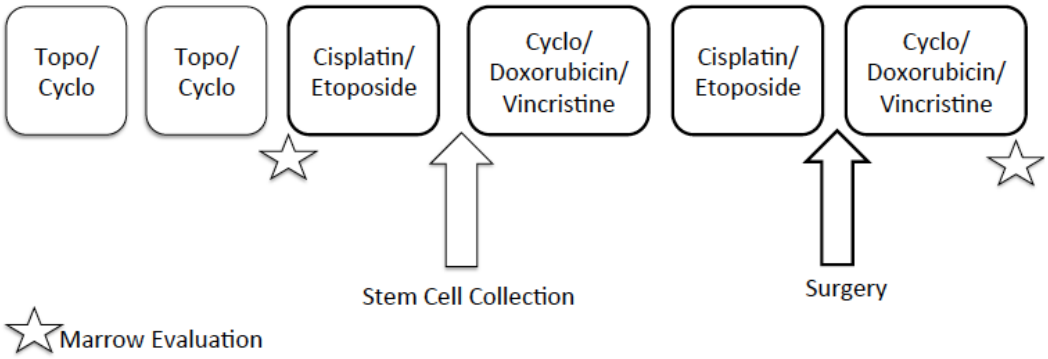
1 ABSTRACT:

Current induction chemotherapy for high risk neuroblastoma involves 6 cycles of chemotherapy followed by high dose chemotherapy with autologous stem cell rescue and then a combination of immunotherapy and differentiation therapy for maintenance. Standard practice for supportive care for these patients typically includes adjuvant G-CSF (Granulocyte Colony Stimulating Factor) to limit chemotherapy related neutropenia, based on the assumption that this reduces risk of infection. New data from preclinical models of neuroblastoma demonstrate that G-CSF promotes the maintenance of neuroblastoma cancer stem cells and may reduce the efficacy of chemotherapy by promoting neuroblastoma metastasis and drug resistance and use of G-CSF could reduce the overall efficacy of our current treatment protocols of high-risk neuroblastoma. Prior to performing randomized trials to test this hypothesis, we propose to determine the feasibility and safety of removing prophylactic G-CSF administration from our supportive care practice for these patients. Thus we propose the following non-inferiority protocol to test the safety and feasibility of this alternative supportive care approach to HR NB induction chemotherapy.

2 EXPERIMENTAL DESIGN SCHEMA:



3 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

3.1 Primary Aim:

To determine the safety and feasibility of administering induction chemotherapy for high risk neuroblastoma patients without routine G-CSF administration for supportive care.

3.2 Secondary Aims:

3.2.1 To determine the incidence and duration of delay in chemotherapy administration due prolonged neutrophil recovery in patients undergoing induction chemotherapy for high-risk neuroblastoma without prophylactic G-CSF

3.2.2 To determine the number of antibiotic days and hospital days due to fever and/or infection in patients undergoing induction chemotherapy for high-risk neuroblastoma without prophylactic G-CSF

3.2.3 To determine the number of platelet transfusions in patients undergoing induction chemotherapy for high-risk neuroblastoma without prophylactic G-CSF

3.2.4 To describe the response rate following induction chemotherapy without prophylactic G-CSF for patients with high-risk neuroblastoma

3.2.5 To describe caregiver burden in patients undergoing induction chemotherapy for high risk neuroblastoma

4 BACKGROUND

4.1 Introduction/Rationale for development:

Neuroblastoma is the most common extracranial solid tumor of childhood and accounts for 15% of pediatric cancer related mortality [1]. Only 50% of children with aggressive, metastatic tumors (i.e. high-risk disease) achieve long term survival despite aggressive treatment including multi-agent chemotherapy, surgery, radiation therapy and biologic agents [2]. The majority of deaths from neuroblastoma are due to relapsed disease. It is thought that most solid tumors, neuroblastoma included, are made up of heterogeneous populations of cells in various states of differentiation and with varying levels of tumorigenicity and chemosensitivity [2]. Though chemotherapy may eradicate the majority of these subpopulations, cancer stem cells (CSCs) are generally resistant to chemotherapy and their persistence may repopulate new tumors in metastatic sites after completion of chemotherapy, leading to relapse. CSCs have been described in the majority of aggressive malignancies including breast, colon, lung and brain tumors [3-5].

Recently, a novel CSC subpopulation in neuroblastoma has been characterized based on expression of CD114, the Granulocyte-Colony Stimulating Factor receptor (G-CSFR) [6]. Colony stimulating factors, including granulocyte-colony stimulating factor (G-CSF), are cytokines that are produced at low levels continuously and increase in response to several specific stimuli such as infection or reduction in the number of terminally differentiated hematopoietic cells. Recombinant human granulocyte colony-stimulating factor (r-metHuGCSF, filgrastim) is used extensively as an adjuvant to myelosuppressive chemotherapy

to decrease the duration of chemotherapy-induced myelosuppression. Over the past 10 years G-CSF was introduced into protocols for pediatric cancers based on data extrapolated from adults demonstrating a reduction in duration of severe neutropenia and reduction in risk of febrile neutropenia [7]. However, the risk versus benefit of routine adjuvant G-CSF has not been directly studied in neuroblastoma patients receiving our current regimen.

Recent data demonstrates that G-CSF acts as a CSC-specific growth factor via G-CSF receptor mediated activation of the STAT3 signaling pathway, a signaling mechanism critical in neural crest differentiation [8]. Exogenous G-CSF increases metastasis and tumor growth of neuroblastoma xenografts growing in immunodeficient mice (Figure 1), while STAT 3 inhibition has the opposite effect. Importantly, G-CSF also up regulates a number of STAT3 dependent anti-apoptotic and DNA repair mechanisms in the stem cell population. Additionally, orthotopic mouse xenograft models treated with chemotherapy followed by G-CSF had significantly larger tumors than those treated with chemotherapy alone, suggesting a pro-tumor effect of G-CSF (Figure 2). Together, these results raise the concern that routine administration of G-CSF after chemotherapy for neuroblastoma may be counter-productive and enhance drug resistance and persistence of metastatic disease. Thus, the role of G-CSF in promoting the tumorigenicity of neuroblastoma CSCs mandates a re-evaluation of the risks and benefits of the adjuvant use of G-CSF during chemotherapy for children with high risk neuroblastoma.

As a first step towards a definitive trial comparing the effects of G-CSF on survival, we must first assess the safety and feasibility of removing prophylactic G-CSF from chemotherapy. This protocol is a pilot study designed to test our hypothesis that the risk of severe infections during induction chemotherapy without prophylactic G-CSF will be equivalent to historical and published data. The results from this protocol will be used to inform a future randomized trial.

Figure 1. G-CSF signaling significantly promotes tumor growth *in vivo*:

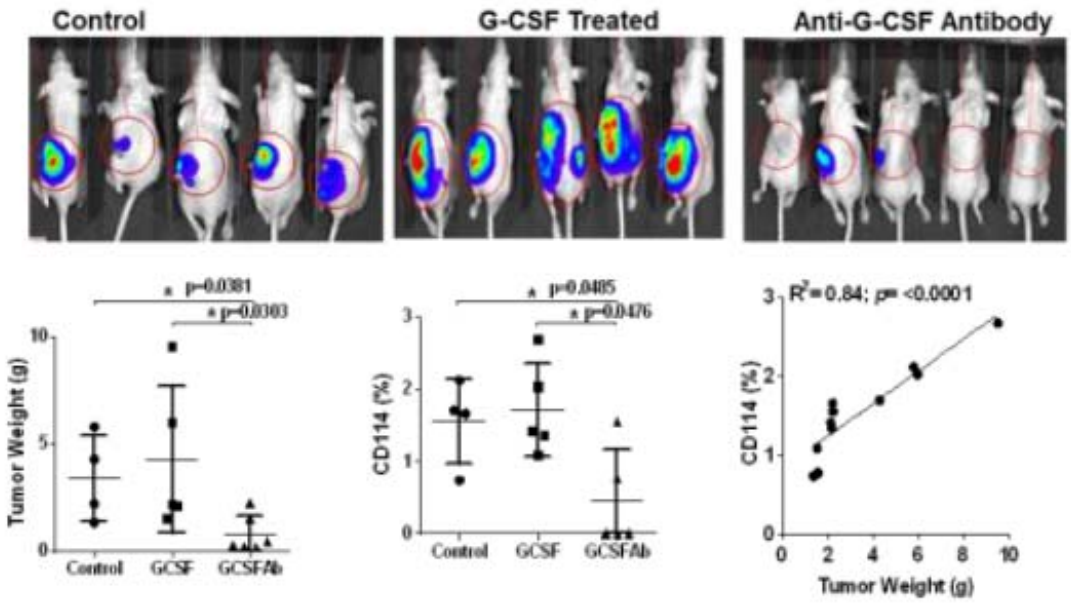


Figure 1. *In vivo* bioluminescence imaging of xenografts treated with G-CSF or neutralizing antibody to G-CSF demonstrated a marked effect of the exogenous cytokine promoting tumor growth. (From Agarwal et al 2015).

Figure 2. Chemotherapy with and without G-CSF administration in mice:

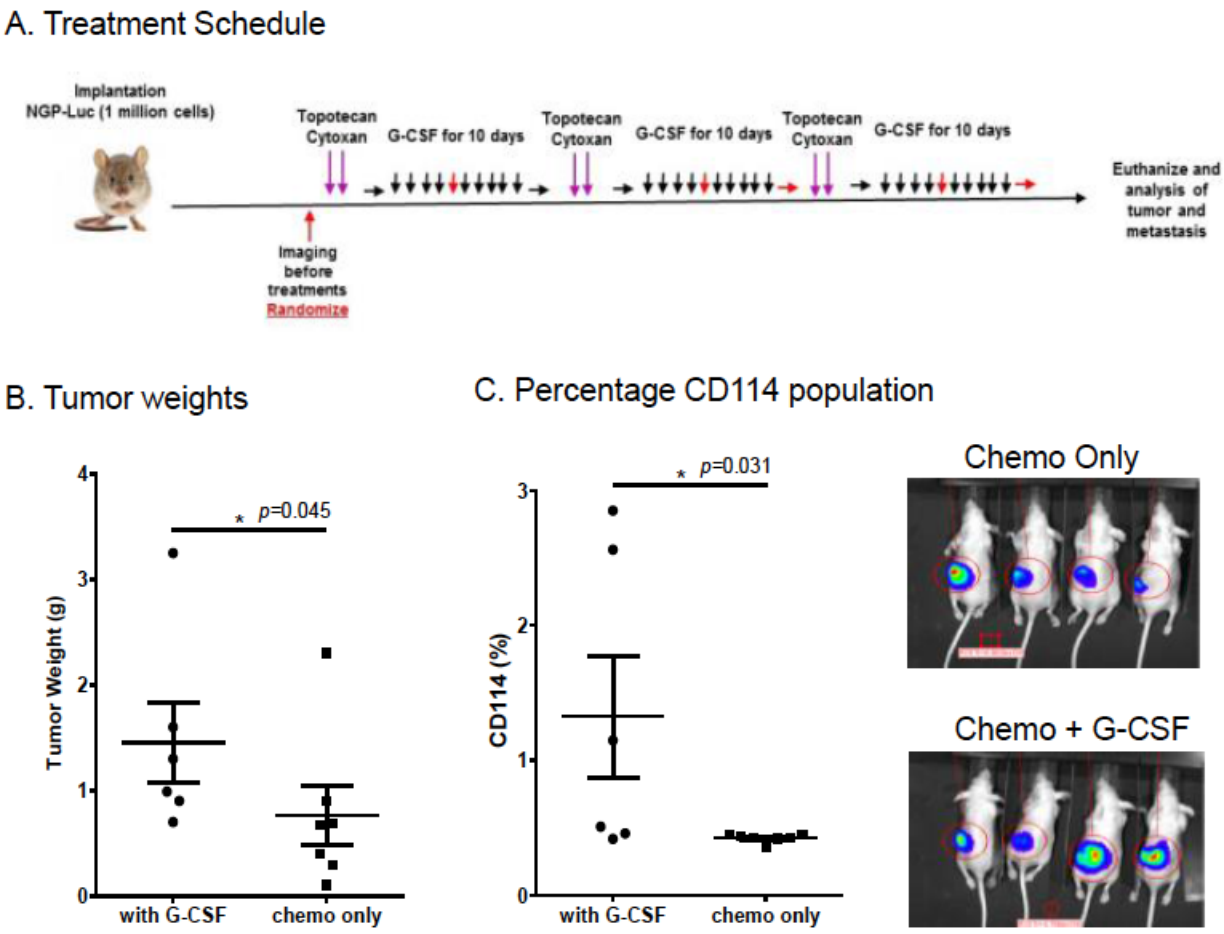


Figure 2. Preliminary data demonstrating G-CSF given after chemotherapy (topotecan and cyclophosphamide) reduces the efficacy of these drugs. A. Treatment schedule for chemotherapy regimen B. Tumor weights (N=6) and C. CD-114 positive neuroblastoma cells in cohorts of mice treated with chemotherapy with or without G-CSF. Far right: Representative imaging results of treatment cohorts after 3 cycles of therapy.

4.2 Induction Therapy

Standard chemotherapy for high-risk neuroblastoma begins with an initial phase of therapy aimed at inducing a remission of disease. Achievement of complete response following induction chemotherapy significantly impacts long-term survival [9]. Despite modest improvements in outcomes due to significant chemotherapy dose intensification, durable remission rates remain low, with 15-20% of patients progressing during induction therapy and another 40% progressing after an initial response to induction [10-12]. The current Children's Oncology Group induction chemotherapy regimen includes anthracyclines, alkylators, platinum compounds, and topoisomerase II inhibitors delivered every 21 days for 6 cycles [13]. Surgical resection of the primary tumor and bulky metastatic sits is usually needed to achieve a partial response by the end of induction therapy. Tumors frequently adhere to or invade local vital structures [14], resulting in significant complications including normal organ removal and hemorrhage [15, 16], particularly if resection is attempted before chemotherapy. Surgery after or near completion of induction therapy is an accepted practice and improves the likelihood of resection [17].

For the 80 to 95% of children with a good response to induction, treatment continues with consolidation and maintenance phases. These components of treatment are not part of this clinical trial, however current practice for these phases include radiation therapy to the primary tumor bed and residual metastatic sites, high-dose chemotherapy with autologous stem cell rescue, followed by six months of biologic and immunotherapy. Collection of autologous

stem cells occurs during induction therapy. Best practice at Texas Children's Cancer Center has been to perform this collection after induction chemotherapy course #3 via pharesis following stimulation of stem cell production and mobilization with G-CSF.

4.3 Prophylactic Use of Colony Stimulating Factors

The prophylactic use of myeloid colony stimulating factors (CSFs) has been described in two scenarios. Use of CSFs to decrease duration of myelosuppression after chemotherapy is referred to as primary prophylaxis, while an attempt to prevent febrile neutropenia or a delay in subsequent chemotherapy administration is called secondary prophylaxis. [18]. The American Society of Clinical Oncology (ASCO) has developed guidelines for the use of these medications based on review of the published literature, with the most recent update in 2015 [19]. Guidelines for use in adults recommend prophylactic use in patients in whom the expected incidence of febrile neutropenia is at least 20% and in patients who have already had an episode of febrile neutropenia. These guidelines have been applied to children without conclusive evidence supporting their use, and certainly without evidence specific to children with high-risk neuroblastoma.

While some studies have shown a reduction in rate of febrile neutropenia and length of hospitalization for febrile neutropenia, there has not been a demonstrated decrease in infection related mortality [20, 21]. A study of neuroblastoma patients undergoing the dose and time intensive chemotherapy protocol "Rapid COJEC" demonstrated similar findings with no decrease in rate of serious bacterial infections or mortality. This study demonstrated improvement

in compliance with chemotherapy schedule, however, the Rapid COJEC protocol delivers chemotherapy on an every 10 day schedule regardless of hematologic count criteria, provided the patient does not have an active infection [22]. This is not applicable to standard North American regimens which deliver chemotherapy in a less time intensive manner (10 days vs. 21-29 days between cycles) adhering to count recovery criteria. Furthermore, a study randomizing high risk neuroblastoma patients receiving dose and time intensive chemotherapy to either receive prophylactic G-CSF or not, found no impact of G-CSF on febrile episodes. This study demonstrated that patients receiving G-CSF had faster recovery of absolute neutrophil count, on average 3 days sooner to reach an absolute neutrophil count (ANC) above 500/μL. However, subjects who received G-CSF had slower platelet recovery for all chemotherapy following the third cycle. The authors concluded that while G-CSF use hastened neutrophil recovery, it did not impact dose intensity due to this delay in platelet recovery [23].

The most commonly used colony stimulating factor in pediatrics is granulocyte colony stimulating factor (G-CSF, filgrastim). This cytokine binds the G-CSF receptor (AKA CD114, also found on neuroblastoma stem cells) on neutrophil precursors to activate STAT3 dependent expansion and lineage differentiation to increase neutrophil counts in response to inflammatory stress. A commonly used formulation is peg-filgrastim, a pegylated form of G-CSF allowing for a single administration rather than daily injections. New evidence that G-CSF acts as a growth factor for neuroblastoma cancer stem cells (see section 2.1)

raises the concern that administration of this growth factor may be contributing to relapsed or refractory disease by stimulating the neuroblastoma cancer stem cells specifically. This concern warrants re-evaluation of the strategy of using colony stimulating factors for primary prophylaxis in neuroblastoma.

Granulocyte-macrophage colony stimulating factor (GM-CSF) is an alternative colony stimulating factor that acts on multiple cell lineages, including monocytes and neutrophils through binding its own surface receptor. It does not bind to the CD114 receptor found on neuroblastoma cancer stem cells, nor do neuroblastoma cells appear to have GM-CSF receptors. There have been few published trials of direct comparison of G-CSF with GM-CSF in children, however, these suggest little difference in overall efficacy, with GM-CSF demonstrating slightly longer time (1-2 days) to recover ANC compared with G-CSF, but no difference in incidence of antibiotic administration for febrile neutropenia or length of hospitalization [24]. These data suggest GM-CSF may be a reasonable alternative to maintain dose intensity for patients who manifest significant infectious complications or delays between chemotherapy cycles.

4.4 Infections in neuroblastoma patients during induction therapy

A review of 76 patients treated for high risk neuroblastoma at Texas Children's Hospital over the last ten years demonstrated that 58% of patients had one or more infections by the end of 5 cycles of induction chemotherapy (Table 1). Each cycle, approximately 19% of patients had an infection (Table 2). These patients were treated with 5 cycles of induction chemotherapy of similar intensity to the proposed regimen for this study. Patients were treated with prophylactic G-

CSF products (either filgrastim or peg-filgrastim) depending on the institutional standard of care at the time of treatment. Serious bacterial infections were defined as bacteremia with positive blood culture, signs/symptoms of sepsis with negative blood culture, pneumonia evident on chest radiograph with clinical symptoms, grade III urinary tract infection, grade III cellulitis and neutropenic enterocolitis. Review of Children's Oncology Group data from patients treated using the same regimen proposed in this study revealed a similar average rate of infections of 15% per cycle for all cycles (ANBL 02P1 Study Committee Progress Report, Section III. Toxicities). These data demonstrated the highest rate of infections after cycles 5 and 6, with an infection rate of 20% for these cycles.

# of Serious infections	# of patients (n=76) (%)
0	32 (42%)
1	25 (33%)
≥2	19 (25%)

Table 1. Analysis of rates of serious bacterial infections for HR neuroblastoma patients at TCH. Charts from patients undergoing HR neuroblastoma induction chemotherapy treated at Texas Children's Hospital from 2006-2015 were analyzed for serious bacterial infections. Number of infections per patient over 5 cycles is reported.

Cycle	Number with Serious bacterial Infection	% With infection
1	14/76	18.4%
2	13/75	17.3%
3	13/73	17.8%
4	17/71	23.9%
5	11/70	15.7%

Table 2. Analysis of rates of serious bacterial infections for HR neuroblastoma patients at TCH. Charts from patients undergoing HR neuroblastoma induction chemotherapy treated at Texas Children's Hospital from 2006-2015 were analyzed

for serious bacterial infections. Number of infections per cycle of chemotherapy is reported.

4.5 Caregiver Burden

The intensive treatment plan described above for high-risk neuroblastoma incurs a significant but poorly defined burden on the family. Having a child with cancer is associated with high financial costs [25] physical and psychological health of all members of the family and upheaval of normal family activities [26]. The frequency and duration of trips to the hospital and attending to medical needs of the child at home are two large contributors to family burden. Measuring how treatment affects patients and their families allows the family experience to be included in the overall assessment of the care. For example, a companion assessment of the leukemia clinical trials, P9904 and P9905, measured the family burden associated with inpatient versus outpatient methotrexate delivery. The pre-study assumption was that outpatient chemotherapy would be preferred, however they found that care was more burdensome and patient wellbeing was lower in the outpatient group [27]. This study examined care at one point in time. It is unclear how the time and effort changes over time.

The Care of My Child with Cancer (CMCC) instrument is a 28-item scale that measures the time and effort required to complete various tasks within the past week. The estimated time to complete the survey is 10 minutes. It is interpreted via physical and emotional subscales. Initial validation

demonstrated it has an internal consistency coefficient of 0.93 and a test-retest estimate of 0.9 with 3 to 7 days between administrations [28]. A second psychometric evaluation of the instrument was performed with 513 caregivers that supported its internal reliability [29]. This instrument is available in English and Spanish versions. This instrument was also used on ACCL01P3, the companion study to P9904 and P9905 described above. Completing this instrument in association with high-risk neuroblastoma induction therapy will allow description of the burden for this treatment.

5 ELIGIBILITY CRITERIA AND SUBJECT RECRUITMENT

5.1 Inclusion Criteria:

A. Age >12 months and <18 years old at diagnosis

B. Newly diagnosed neuroblastoma or ganglioneuroblastoma as verified by histology and/or demonstration of tumor cells in bone marrow with elevated urinary catecholamine metabolites

C. Must meet criteria for High Risk disease

1. Patients with INSS stage 4 disease are eligible with the following
    - *MYCN* amplification (greater than four-fold increase in *MYCN* signals as compared to reference signals), regardless of age or additional biologic features
    - Age >18 months (>547 days) regardless of biologic features
    - Age 12-18 months (365-547 days) with any of the following unfavorable biologic features (unfavorable pathology and/or DNA

index =1) or any biologic feature that is indeterminate/unsatisfactory/unknown

2. Patients with INSS stage 3 disease are eligible with the following
    - *MYCN* amplification, regardless of age or additional biologic features
    - Age >18 months (>547 days) with unfavorable pathology, regardless of *MYCN* status

3. Patients with INSS stage 2a/2b with *MYCN* amplification regardless of age or additional biologic features

4. Patients ≥365 days initially diagnosed with INSS stage 1 or 2 who progressed to a stage 4 without interval chemotherapy

D. Patients may have had no prior systemic therapy except
- Localized emergency radiation to sites of life threatening or functioning disease
- No more than 1 cycle of chemotherapy according to low or intermediate risk regimens prior to determination of MYCN amplification and histology, as long as the patient DID NOT receive G-CSF as part of that therapy.

E. Patients must have adequate hematopoietic function defined as
- Absolute neutrophil count (ANC) ≥ 750/μL,
- Platelet count ≥ 75,000/μL
- The above criteria do not have to be met if the patient has bone marrow involvement of tumor.

F. Patients must have adequate liver function defined as

- Direct bilirubin ≤ 1.5 mg/dL or total bilirubin ≤ 1.5 mg/dL
- AST and ALT ≤ 10 x upper limit of normal for age.

G. Patients must have adequate renal function as defined as:

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

Age	Maximum Serum Creatinine (mg/dL)	
	Male	Female
1 month to < 6 months	0.4	0.4
6 months to < 1 year	0.5	0.5
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

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

H. Patients must have adequate cardiac function as defined as:

- Shortening fraction of ≥ 27% by echocardiogram, or
- Ejection fraction of ≥ 50% by radionuclide angiogram

5.2 Exclusion Criteria

A. Patients who do not meet inclusion criteria

B. Patients who are pregnant or lactating.

C. Patients who have received G-CSF since the time of diagnosis of the current disease

5.3 Subject Recruitment

Subjects will be recruited from new patients with neuroblastoma presenting to Texas Children's Cancer Center and Rady Children's Hospital San Diego for treatment.

5.4 Informed Consent

Consent to participate in a research study will be obtained from the parent(s) or legal guardians prior to treatment. Assent will be obtained from the child, when age appropriate according to guidelines of Baylor College of Medicine and Texas Children's Hospital.

5.5 Patient registration and study enrollment

Patients may be enrolled on the study once all eligibility requirements for the study have been met. Eligibility checklist items will entered and uploaded into the electronic database eligibility section. The local investigator will review the eligibility checklist and electronically confirm accuracy. The coordinating center PI will then review the items and electronically confirm accuracy, at which point the subject will be considered registered and enrolled on study.

6 TREATMENT PLAN

This treatment plan describes induction chemotherapy only, including 6 cycles of chemotherapy, tumor resection, and stem cell collection. To ensure that safety data generated is directly comparable, the staging, disease evaluations and drug

administration will be followed according to our current practice and as close as possible to current national best practice. After two cycles of chemotherapy, disease reevaluation will take place including primary tumor imaging and bone marrow evaluation. According to our institutional standard of care, peripheral blood stem cells will be harvested for high dose chemotherapy/autologous stem cell rescue (HST/SCR) after cycle 3 of induction chemotherapy. The stem cell transplant itself, however, will take place after protocol therapy is completed. Surgical resection of the primary tumor will follow the fifth cycle of chemotherapy provided the tumor is deemed surgically resectable at that time. Consolidation therapy, including HDT/SCR and radiation therapy, and maintenance therapy will be conducted off-study according to the institutional standard.

6.1 Induction Chemotherapy

Induction chemotherapy will consist of six cycles of chemotherapy administered approximately 21 days apart. The first two cycles will be cyclophosphamide and topotecan. Cycles 3 and 5 will consist of cisplatin and etoposide, and cycles 4 and 6 will be cyclophosphamide, vincristine and doxorubicin. Disease re-evaluation will occur after cycle 2, and again after cycle 6. This chemotherapy regimen is consistent with the current national best practice according to the Children's Oncology Group.

6.1.1 Criteria to receive chemotherapy

Chemotherapy cycles (except cycle 1) may begin when the ANC is ≥750/μL and platelets ≥75,000/μL after post chemotherapy nadir. There are no hematologic criteria to begin cycle 1.

Patients who received one cycle of chemotherapy per low or intermediate risk neuroblastoma therapy prior to determination of *MYCN* amplification and histology will receive all 6 cycles of induction chemotherapy on this protocol.

6.2 Induction therapy administration

- For patients >12 kg, chemotherapy doses will be calculated by body surface area (BSA).
- For patients ≤12 kg, chemotherapy doses for all drugs EXCEPT topotecan will be dose per kg rather than per BSA.
- Topotecan dosing will be based on BSA regardless of age or weight

6.2.1 Induction therapy cycle 1 and 2

- Cyclophosphamide 400 mg/m^2/dose (or if ≤12 kg, 13.3 mg/kg) IV over 30-60 minutes, once daily for 5 doses on days 1-5.
- Topotecan 1.2 mg/m^2/dose IV over 30 minutes, once daily for 5 doses on days 1-5.

6.2.2 Induction therapy cycle 3 and 5

- Cisplatin 50 mg/m^2/dose (or if or if ≤12 kg, 1.66 mg/kg/dose) IV over 60 minutes, once daily on days 1-4. Suggest achieving urine specific gravity ≤ 1.010 prior to start of cisplatin.
- Etoposide 200 mg/m^2/dose (or if ≤12 kg, 6.67 mg/kg/dose) IV over at least 90 minutes, once daily for 3 doses on days 1-3.

6.2.3 Induction therapy cycle 4 and 6

- Cyclophosphamide 2100 mg/m^2/dose (or if or if ≤12 kg, 70 mg/kg/dose) IV over 6 hours, once daily for 2 doses on days 1-2.

- MESNA 420 mg/m^2/dose (or if or if ≤12 kg, 14 mg/kg/dose) IV immediately prior to each cyclophosphamide dose and again at 3, 6, 9 and 12 hours after the start of each cyclophosphamide infusion on days 1-2
- Doxorubicin 25 mg/m^2/dose (or if or if ≤12 kg, 0.83 mg/kg/dose) IV over 15 minutes once daily for 3 doses.
- Dexrazoxane 250 mg/m^2/dose (or if ≤12 kg, 8.3 mg/kg/dose) once daily x3 doses
- Vincristine
    - Patients <12 months of age: 0.017 mg/kg/dose once daily IV for 3 doses on days 1-3
    - Patients ≥ 12 months and ≤ 12 kg: 0.022 mg/kg/dose once daily IV for 3 doses on days 1-3
    - Patients ≥ 12 months and ≥ 12 kg: 0.67 mg/m^2 once daily IV for 3 doses on days 1-3

6.3 Stem Cell Collection

Patients will have peripheral blood stem cells harvested after the third cycle of chemotherapy, provided they are clinically stable enough to do so. G-CSF will be administered according to institutional standard procedures prior to stem cell collection. If adequate stem cell number is not able to be collected after cycle 3, re-collection will be attempted at a later time during induction chemotherapy.

6.4 Local Control- Surgery

6.4.1 Diagnostic Surgery

In all patients, the goal of the first surgical procedure is to obtain enough tumor tissue for a histologic diagnosis and well as MYCN determination, cytogenetics and other biologic studies. The surgeon should attempt to obtain at least $1cm^3$ of viable tumor tissue, if feasible, without putting the patient at undo risk. Complete excision should only be undertaken if doing so is unlikely to delay the start of chemotherapy or result in great morbidity.

6.4.2 Delayed Resection

Surgical resection of soft tissue disease should be planned for after induction cycle 5 (or later, if medically necessary). The goal of delayed resection is gross total resection of residual tumor in the primary site and any tumor in areas of regional dissemination (usually lymph nodes). All attempts should be made to preserve organs.

6.4.3 Prophylactic Growth factor prior to surgical resection

To prevent potential delays between chemotherapy administration and surgical resection, prophylactic rGM-CSF will be administered following the chemotherapy cycle immediately prior to surgical resection until ANC >750/μL. CBC will be checked twice weekly while receiving rGM-CSF.

6.5 Supportive Care

6.5.1 Myeloid Growth factor

No prophylactic G-CSF (filgrastim, peg-filgrastim) will be administered during this therapy with the exception of mobilization and harvesting of peripheral blood stem cells. Prophylactic GM-CSF (sargramostim) will be administered prior to

surgical resection and for patients who meet criteria for prophylactic GM-CSF administration for prior myelosuppression or prior infections (see sections 6.5.1 and 6.5.3).

If a subject is inadvertently given G-CSF (filgrastim or peg-filgrastim) rather than GM-CSF (sargramostim) for a cycle in which growth factor is indicated, this will be considered a protocol violation. These subjects will remain on protocol therapy.

If a subject is inadvertently given *one or two doses* of G-CSF (filgrastim) during a cycle where no growth factor was indicated, this will be considered a protocol deviation, and the cycle will still be considered evaluable. If the subject is given *more than two doses* of filgrastim in a single cycle or is given a dose of peg-filgrastim, that cycle will be considered inevaluable. If the subject is given more than two doses of filgrastim in two or more cycles or a dose of peg-filgrastim in two or more cycles, the subject will be removed from protocol therapy.

6.5.2 Hematopoietic Support

Patients should receive platelet and red cell transfusion as per institutional guidelines and optimal patient care.

Complete blood count (CBC) should be checked twice weekly for patients with Grade 4 thrombocytopenia (<25,000/μL) and Grade 4 neutropenia (<500/μL).

6.5.3 Nausea and vomiting

Patients should receive anti-emetics as per institutional guidelines and optimal patient care.

6.6 Dose modifications for toxicity during induction chemotherapy

Toxicities will be graded by CTCAE version 4 criteria. Neurologic toxicity will be graded using Balis Criteria.

6.6.1 Myelosuppression

Chemotherapy may begin when ANC ≥ 750/μL and platelet count is ≥ 75,000/μL (except to start cycle 1 if patient has bone marrow involvement). Delay the next cycle until both criteria are met.

Cycles should be approximately 21 days apart. Attempt to start all cycles on day 22 following the start of previous cycle of chemotherapy. Following any cycle, if the ANC <750/ μL and/or platelet count is <75,000/ μL, delay next cycle until recovery occurs or meets criteria to continue based on bone marrow tumor involvement. If patient recovery to ANC ≥ 750/μL and platelet count is ≥ 75,000/μL by day 29, proceed with next cycle at full dose.

Delay beyond day 22 due to Platelets <75,000/μL

- Recheck CBC in 1 week (day 29)

Delay beyond day 29 due to Platelets <75,000/μL

- If recovery occurs between day 30-43, reduce the doses of all drugs except vincristine by 25%
- If recovery occurs after day 43 of any cycle, reduce all doses of drugs by 50%, except for vincristine

Delay beyond day 22 due to ANC <750/μL

- If ANC ≥250/μL at day 22, continue to check CBC twice a week until ANC ≥750/μL, then begin chemotherapy at full dose. If ANC

remains <750/μL at day 29, see below for Delays beyond day 29 due to ANC<750/μL.

- If ANC <250/μL at day 22, begin GM-CSF daily 250 mcg/m^2 IV or SQ daily and check CBC twice weekly until ANC ≥750/μL, then begin chemotherapy at full dose.

Delay beyond day 29 due to ANC <750/μL

- If ANC <750/μL at day 29, begin GM-CSF 250 mcg/m^2 IV or SQ daily and check CBC twice weekly until ANC ≥750/ μL. For all future cycles of the chemotherapy that caused delay >29 days use prophylactic GM-CSF 250 mcg/m^2 IV or SQ daily. For example, if delay occurred after Topotecan/Cyclophosphamide, the next cycle of Topotecan/cyclophosphamide should include prophylactic GM-CSF
- If recovery (ANC < 750/ μL by day 29) occurs more than once AND the second delay occurs while the patient is receiving growth factors, reduce all subsequent chemotherapy by 25% (except vincristine).

6.6.2 Dosing of GM-CSF

- 250 mcg/m^2 IV or SQ daily until ANC >750 x1

6.6.3 Infections

Patients who experience the following infectious complications will be treated with prophylactic GM-CSF (250 mcg/m^2 IV or SQ daily until ANC ≥750/ μL x1) for ALL subsequent cycles of induction chemotherapy

- Any grade IV bacterial infection

- Any identified bacterial or presumed bacterial infection requiring IV antibiotics including
    - Bacteremia with positive blood culture
    - Signs/symptoms of sepsis with negative blood culture
    - Pneumonia evident on chest radiograph with clinical symptoms consistent with pneumonia
    - Grade III urinary tract infection
    - Grade III cellulitis
    - Neutropenic enterocolitis

Hematuria

For Cycles 1 and 2: If microscopic (> 2 abnormal urinalyses during a cycle of therapy with < Grade 2 hematuria) or gross hematuria occurs after Induction Cycle 1 cyclophosphamide, give MESNA with Induction Cycle 2 cyclophosphamide as follows: MESNA 80 mg/m^2 (or 2.67 mg/kg if < 12 kg) with cyclophosphamide infusion, then MESNA 80 mg/m^2 (or 2.67 mg/kg if < 12 kg) IV over 15 minutes at Hours 4 and 8 from start of cyclophosphamide infusion. If hematuria resolves prior to start of cycle 4 cyclophosphamide, administer cyclophosphamide and mesna in cycles 4 and 6 without modification.

For Cycles 4 and 6: If microscopic (> 2 abnormal urinalyses during a cycle of therapy with < Grade 2 hematuria) or gross hematuria occurs after Induction Cycle 4 cyclophosphamide, give mesna as a 24 hour continuous infusion with Induction Cycle 6 cyclophosphamide as follows: MESNA 2100 mg/m^2(or 70

mg/kg if < 12 kg) in required fluid over 24 hours starting with the start of the cyclophosphamide dose each day.

If Grade 3 or 4 hematuria occurs following a cycle of cyclophosphamide, do not give another cycle of cyclophosphamide, topotecan (CT) or cyclophosphamide, doxorubicin and vincristine (CDV) until hematuria resolves to Grade 2 or less. If patient is due to begin next cycle of cyclophosphamide containing chemotherapy prior to resolution of hematuria to $\leq$ Grade 2, substitute cisplatin and etoposide cycle. The intent of Induction is to give a total of 2 cycles each of CT, cisplatin/etoposide and CDV, therefore if substitution of cisplatin/ etoposide is made for CDV cycle or a CT cycle, make-up this missed cyclophosphamide-containing cycle later in therapy. If gross hematuria from cyclophosphamide recurs, delete cyclophosphamide from subsequent cycles.

6.6.4 Renal Toxicity

6.6.4.1 Cisplatin

No dose reductions in cisplatin will be made for a decrease in the baseline GFR or creatinine clearance as long as the value remains > 60 mL/min/1.73 m2. If the serum creatinine increases > 50% during a cycle of cisplatin-containing chemotherapy, omit the remainder of the cisplatin from that cycle. Repeat GFR prior to next scheduled cisplatin. If GFR or creatinine clearance is < 60 mL/min/1.73 m^2 prior to cisplatin/etoposide cycle substitute CDV cycle. The intent of Induction is to give a total of 2 cycles of CT, 2 cycles of cisplatin/etoposide, and 2 cycles of CDV, therefore if substitution of CDV cycle is made for cisplatin/etoposide cycle, give the cisplatin/etoposide cycle later in therapy. Omit further cycles of cisplatin therapy if GFR or creatinine clearance remains < 60 mL/min/1.73m^2.

Cyclophosphamide, Doxorubicin, Vincristine, Topotecan and Etoposide

No dose reductions in cyclophosphamide, doxorubicin, vincristine, topotecan or etoposide are necessary for decrease in creatinine clearance.

6.6.5 Cardiac Toxicity

6.6.5.1 For Symptomatic Congestive Heart Failure (CHF)

If at any time, the patient develops Grade 3 congestive heart failure or dysrhythmia or any Grade 4 cardiac toxicity not related to underlying infection or metabolic abnormality, omit doxorubicin from all subsequent cycles. If cardiac toxicity is resolved to < Grade 2 congestive heart failure or dysrhythmia, decrease the dose of cyclophosphamide to 50% for the next cycle containing cyclophosphamide. If this dose of cyclophosphamide is tolerated without > Grade 2 congestive heart failure or dysrhythmia, then decrease the dose of cyclophosphamide to 50% for the next cycle containing cyclophosphamide. If this dose of cyclophosphamide is tolerated without > Grade 2 congestive heart failure or dysrhythmia, then administer full dose of cyclophosphamide in subsequent cycles of chemotherapy.

6.5.5.2 For Dysrhythmia

If the patient develops Grade 2 cardiac dysrhythmia as defined in the Common Toxicity Criteria, repeat in one week. If Grade 2 toxicity resolves to Grade 0 or 1 toxicity, patient may continue on therapy without chemotherapy dose alterations.

If Grade 2 toxicity occurs prior to Cycle 4, substitute cisplatin/etoposide for CDV cycle until dysrhythmia resolves. Make a notation of chemotherapy substitution on data form. The intent of Induction is to give a total of 2 cycles of CDV and 2 cycles of cisplatin/etoposide, therefore if cisplatin/etoposide cycle substituted for

CDV, give CDV cycle later in therapy. If dysrhythmia symptoms occur prior to Cycle 6, proceed with cyclophosphamide, vincristine but omit doxorubicin.

6.5.5.3 Hypertension

Hypertension due to neuroblastoma will not be considered reason for removal from protocol therapy or alteration in chemotherapy doses.

6.5.6 Hepatotoxicity

If direct bilirubin is > 3 mg/dL prior to Cycle 4 chemotherapy, substitute cisplatin/etoposide for CDV cycle. If direct bilirubin is > 3 mg/dL prior to Cycle 6 chemotherapy, omit doxorubicin and vincristine. If direct bilirubin is > 1.5 but < 3 (Grade 3 toxicity) prior to Cycle 4 or 6 chemotherapy, reduce doxorubicin and vincristine dose by 50%.

6.5.7 Gastrointestinal Toxicity

6.5.7.1 Mucositis

If patient develops Grade 3 or 4 mucositis that resolves to < Grade 2 by Day 22-29 of next cycle, no dose adjustments will be made in chemotherapy and no prophylactic growth factor will be administered. If patient develops Grade 3 or 4 mucositis that is NOT attributable to infectious etiology AND recovery to < Grade 2 occurs between Day 30-43 for any cycle of Induction, administer GM-CSF for all subsequent cycles of chemotherapy. For the second occurrence of Grade 3 or 4 mucositis that resolves to < grade 2 by day 30-43 reduce the dose of doxorubicin or etoposide in the next 2 cycles of chemotherapy by 25%. If subsequent chemotherapy tolerated without recurrence of Grade 3 or 4 GI

toxicity then resume full doses of chemotherapy agents in all subsequent cycles of induction.

If patient develops Grade 3 or 4 mucositis that is NOT attributable to infectious etiology AND recovery to < Grade 2 occurs after Day 43 of any cycle, administer GM-CSF for all subsequent cycles of chemotherapy. For the second occurrence of Grade 3 or 4 mucositis that resolves to < grade 2 after day 43 of any cycle reduce dose of doxorubicin or etoposide in the next 2 cycles of chemotherapy by 50%. If subsequent chemotherapy tolerated without recurrence of Grade 3 or 4 GI toxicity then escalate dose by 25% in subsequent cycles of induction.

If patient develops mucositis that requires intubation for airway management or if patient develops grade 4 neutropenic enterocolitis or other grade 4 gastrointestinal toxicity hold subsequent chemotherapy until toxicity resolved to < Grade 2. If the toxicity resolves to < Grade 2 by Day 43, proceed with next 2 cycles of chemotherapy but administer prophylactic GM-CSF and reduce dose of doxorubicin or etoposide by 25%. If recovery to < Grade 2 occurs after Day 43 of any cycle, administer prophylactic GM-CSF and reduce dose of doxorubicin or etoposide in the next 2 cycles of chemotherapy by 50%. If subsequent chemotherapy tolerated without recurrence of Grade 3 or 4 GI toxicity then escalate dose by 25% in subsequent cycles of induction.

6.5.7.2 Diarrhea

If patient develops severe diarrhea (Grade 3 or 4) attributable to chemotherapy and not underlying infection (i.e. C. difficile), that resolves by Day 22-29 of cycle,

no dose adjustments will be made in chemotherapy. If recovery to < Grade 2 occurs between Day 30-43 for any cycle of Induction, reduce the dose of doxorubicin or etoposide in next cycle of chemotherapy by 25%. If subsequent chemotherapy tolerated without recurrence of Grade 3 or 4 GI toxicity then resume full doses of chemotherapy agents in all subsequent cycles of induction. If recovery to < Grade 2 occurs after Day 43 of any cycle, reduce dose of doxorubicin or etoposide in the next cycle of chemotherapy by 50%. If subsequent chemotherapy tolerated without recurrence of Grade 3 or 4 GI toxicity then escalate dose by 25% in subsequent cycles of induction.

6.5.8 Ototoxicity

For an inner ear/hearing toxicity ≥ Grade 3, decrease cisplatin dose by 50% for subsequent cycles. If loss extends below 2000 Hz, delete further cisplatin/etoposide cycles. If cisplatin is deleted, then complete total of 2 cycles of CDV, then proceed to consolidation therapy.

6.5.9 Neurologic Toxicity

If severe peripheral neuropathy (vocal cord paralysis, inability to walk or perform usual motor functions) or ileus develops from vincristine, vincristine therapy should be stopped or withheld until the ileus resolves or the peripheral neuropathy improves. Restart vincristine at 50% dose and escalate by 25% if tolerated with next course. If neuropathy recurs on escalating dose, return to previously tolerated dose once neuropathy improved.

6.5.10 Allergic Reactions

4.5.10.1 Etoposide

Etoposide allergic reactions may be managed with diphenhydramine 1mg/kg IV (maximum single dose 50 mg), ranitidine 1 mg/kg IV (maximum single dose 50 mg) and hydrocortisone 2 mg/kg IV (maximum single dose 100 mg) and by slowing the rate of the infusion. For those reactions which are unable to be controlled with medication and the slowing of the rate of etoposide infusion, etoposide phosphate may be substituted in the same dose and at the same rate. Pre-medication for etoposide phosphate is recommended.

6.5.10.2 Cisplatin and Carboplatin

Platinum allergic reactions may be managed with diphenhydramine 1mg/kg IV (maximum dose 50mg), ranitidine 1mg/kg IV (maximum single dose 50 mg) and Hydrocortisone 2 mg/kg IV (maximum single dose 100 mg).

6.7 Drug Shortages

In the event of a drug shortage of a medication that is not a G-CSF or GM-CSF product, the provider may use best clinical judgment regarding omission of the agent or substitution with a different agent. The medical and research records of study patients should reflect that the patient was informed of any delays and/or modifications in protocol therapy related to the shortage of the agent and the associated risks.

7 STUDIES TO BE OBTAINED WHILE ON PROTOCOL THERAPY

7.1 Required Observations

Observation	Pre-Treatment	Before each cycle	Weekly	After cycle 2	After cycle 6
CBC	x	x	x^		
Chem 10, AST, ALT, Bili	x	x			
GFR/Serum Cr	x			x*	
Audiogram/ABER (Suggested only)	x				x
EKG					x
ECHO	x				x
Tumor imaging with CT or MRI	x			x	x
MIBG (preferred) or bone scan or PET scan	X$			x	x
Bilateral BM Aspirate and biopsy	x			x#	x#
Urine HVA and VMA	x			x#	x#
Urine Pregnancy Test %	x				
Care of My Child with Cancer instrument		Before cycle 2 and 5&			

^Twice weekly while on growth factors
if positive/elevated at diagnosis
* if abnormal at diagnosis
% if female of childbearing potential
& Optional study
$ Within 2 weeks of enrollment if unable to obtain prior to diagnosis

8 EVALUATION CRITERIA

8.1 International Response Criteria [30]

- Complete response (CR): No evidence of primary tumor; no evidence of metastases (chest, abdomen, liver, bone, bone marrow, nodes, etc.), and HVA/VMA normal. MIBG scan must be negative to qualify for CR.

- Very good partial response (VGPR): Greater than 90% reduction in primary tumor; no metastatic tumor (as above except bone); no new bone lesions, all pre-existing lesions improved, HVA/VMA normal
- Partial Response (PR): 50-90% reduction of primary tumor; 50% or greater reduction in measurable sites of metastases; 0-1 bone marrow samples with tumor; number of positive bone sites decreased by 50%
- Mixed Response (MR): Greater than 50% reduction of any measurable lesion (primary or metastases) with, <50% reduction in any other site; no new lesions; <25% increase in any existing lesion (exclude bone marrow evaluation).
- No response (NR): No new lesions; <50% reduction by <25% increase in any existing lesion (exclude bone marrow evaluation).
- Progressive disease (PD): Any new lesion or increase of a measurable lesion by 25%; previous negative marrow positive for tumor. Any new site of disease documented by MIBG scan qualifies patient as having progressive disease.

9 DRUG INFORMATION

9.1 Sargramostim (Granulocyte macrophage colony stimulating factor, rhu GM-CSF, rGM-CSF, GM-CSF)

Source and pharmacology:

Sargramostim (recombinant human GM-CSF) is a glycoprotein produced in yeast (*S. cerevisiae*) by recombinant DNA technology. rGM-CSF is a hematopoietic growth factor which supports survival, clonal expansion, and differentiation of

hematopoietic progenitor cells. rGM-CSF induces partially committed progenitor cells to divide and differentiate in the granulocyte-macrophage pathways. rGM-CSF stimulates the production of monocytes, granulocytes, erythrocytes, and sometimes, megakaryocytes in the bone marrow. It also induces mature neutrophil and monocytes to increase phagocytosis, superoxide generation, ADCC, tumoricidal killing and cytokine production (IL-1 and tumor necrosis factor). Recombinant human GM-CSF is a glycoprotein of 127 amino acids characterized by three primary molecular masses of 15500, 16800, and 19500 daltons. The amino acid sequence differs from the natural sequence by a substitution of leucine at position 23 and the CHO moiety may be different from the native protein. After subcutaneous administration of sargramostim, peak levels were obtained in 1-4 hours and were detectable at therapeutic levels for 12-16 hours post injection. The elimination t½ ranges from 1.5-2.7 hours after SubQ or IV administration.

Toxicity

	Common 21-100 children out of every 100	**Occasional** 5-20 children out of 100	**Rare** <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Headache, malaise, fatigue, rash, pruritus, bone pain, myalgia, arthralgia, fever, chills	Abdominal Pain, weakness, anorexia, nausea, local injection reactions	Anaphylaxis, “first dose reaction” (hypoxia, dyspnea, hypotension, fever, tachycardia, diaphoresis, flushing, back pain), vomiting, diarrhea, phlebitis, SVT,

			pericardial effusion
Prompt: Within 2-3 weeks, prior to the next course		Weight gain	In high doses: capillary leak syndrome, pneumonitis, peripheral edema, elevation of creatinine, bilirubin and hepatic enzymes in patients with pre-existing renal or hepatic dysfunction
Delayed: Any time later during therapy		Thrombocytopenia	
Unknown frequency and Timing	Fetal and teratogenic toxicities: It is not known whether sargramostim can cause fetal harm or affect reproduction capacity when administered in pregnant women. It is unknown whether the drug is excreted in breast milk		

Formulation and Stability**:**

Sargramostim is available as a lyophilized sterile, white, preservative free powder with 250 mcg (1.4 million International Units) per vial. The sargramostim reconstituted lympholized vial contains 40 mg/mL mannitol, *USP*, as excipients. Do not freeze or shake.

Reconstitute lyophilized powder for injection with 1 mL SWFI or 1 mL Bacteriostatic Water for Injection. **Use SWFI without benzyl alcohol for neonates, infants, and children < 2 years of age or patients with hypersensitivity to benzyl alcohol**. During reconstitution, direct the diluent at the side of the vial and gently swirl the contents to avoid foaming during dissolution. Avoid excessive or vigorous agitation; do not shake. Reconstituted

solutions prepared with Bacteriostatic Water for Injection (0.9% benzyl alcohol) or the liquid preserved solution may be stored for up to 20 days following the first entry into the vial at 2°-8°C (36°-46°F). Discard reconstituted solution after 20 days have elapsed. Reconstituted solutions prepared with SWFI (without preservative) should be administered as soon as possible and within 6 hours following reconstitution.

Use sargramostim for subcutaneous injection without further dilution. Perform dilution for IV infusion in NS. If the final concentration is < 10 mcg/mL, add albumin (human) at a final concentration of 0.1% to the saline prior to addition of sargramostim to prevent adsorption to the components of the drug delivery system. For a final concentration of 0.1% albumin (human), add 1 mg albumin (human) per 1 mL NS. For example, for a final volume of 50 mL NS, add 50 mg (or 1 mL) of 5% albumin [human]. Intravenous dilutions are stable for up to 48 hours at room temperature or refrigerated but should be used within 6 hours due to microbiological concerns. Do not use an in-line membrane filter for IV infusion.
Supplier: Commercially available. See package insert for more detailed information.

10 OFF THERAPY AND OFF STUDY CRITERIA

10.1 Off Therapy Criteria

A. Tumor related

- Progressive or recurrent disease at any time such that provider feels it

is in patient's best interest to proceed to alternative chemotherapeutic agents not included in this protocol

B. Treatment related

- Unacceptable toxicity necessitating change in treatment plan.
- Greater than two doses of G-CSF (filgrastim) in two or more cycles or a dose of peg-filgrastim in two or more cycles administered in a cycle when prophylactic growth factor was not indicated

10.2 Off Study Criteria

- Death
- Patient/parent request to discontinue participation on the protocol
- Patient becomes pregnant

11 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

Subjects on study will have scheduled clinic visits and laboratory evaluations throughout the treatment period. During these visits, they will be closely monitored for clinical or laboratory evidence of toxicity associated with the administered chemotherapy. Adverse events related to the omission of hematopoietic growth factors will be collected. Each grade ≥3 adverse event will be collected for the following targeted adverse events: febrile neutropenia, bacterial infection, bacteremia, pneumonia, urinary tract infection, cellulitis, and neutropenic enterocolitis. Hematologic toxicities including neutropenia, anemia, and thrombocytopenia will be collected. Adverse events will be scored utilizing criteria listed in the Common Terminology Criteria for Adverse Events (CTCAE) version

4.0. For multiple occurrences of a toxicity in a single cycle, a separate grade will be listed for each occurrence. Further assessment, testing, and interventions for any adverse events will be at the discretion of the subject's primary clinician, unless specified in section 6.6 Dose Modifications for Toxicities: 6.6.1 Myelosuppression and 6.6.3 Infection.

An Unanticipated Problem Involving Risk to Subjects or Others (UPIRSO) is defined as incident, experience or outcome that is unexpected (in terms of the nature, severity or frequency) given (a) the description of the likely harms in the protocol, the consent form or the other materials submitted to the IRB and (b) the characteristics of the subject population; related to a subject's participation in the research; and suggests that the research places subjects or others at greater risk of harm - physical, psychological, economic or social harms - than was previously known or recognized. Study subjects should be advised to report any UPIRSOs they experience during the course of their participation in this study from time of consent through the end of the study.

All unanticipated problems involving risks to subjects or others (UPIRSOs) should be identified and reported locally per institutional standards. All UPIRSOs should be entered into the electronic database within 24 hours of study personnel becoming aware of the event.

The Principal Investigator will provide a Continuing Review Report to the Dan L. Duncan Data Review Committee (DRC) annually. The BCM IRB will be provided with the DRC’s determination at every renewal. In addition, all unexpected events

that increase the subject's risk of harm considered by the Principal Investigator to be possibly, likely, or definitely related to not receiving G-CSF or administration of GM-CSF or participation in this study will be reported to the IRB office per their policies. Expected adverse events will not be reported.

All safety data collected on study participants will be reviewed on a continuous basis for any evidence of a higher-than-expected incidence of adverse events. Since the study will not be blinded, it will be feasible to determine on an ongoing basis whether there is a higher incidence of adverse events or toxicities than expected. Any evidence for such a discrepancy will be brought to the attention of the DRC and then the IRB and the cumulative data will be reviewed to determine the suitability of continuing the study.

11.1 Follow-up

Adverse events (as defined in section 11) for all patients will be documented and reported to the Principal Investigator until the subject's ANC has recovered to greater than 1000 (units) (post-nadir) after cycle 6 chemotherapy or until the subject is removed from protocol therapy for progressive disease.

12 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

The study data will be captured through an electronic database. The Clinical Trials Management System (CTMS) is a secure web-based system. The CTMS is HIPAA compliant and 21 CFR Part 11 compliant to protect the integrity of trial data. The CTMS establishes individual user accounts to control access to site-specific patient data. All registered users will be assigned a unique user account to log in to the system. Access is restricted to the roles granted to the user by the

coordinating center. External sites will only be able to view data for patients enrolled at their center. Study personnel will periodically review study accrual, study procedures, and integrity and completeness of the data collected.

This study will undergo quality analysis per the Texas Children's Hospital monitoring policy. All patient eligibility will be reviewed by the study PI prior to enrollment.

13 FINANCIAL CONSIDERATIONS

The therapeutic components of this study will be paid for by the patient's usual methods of reimbursement. GM-CSF will be paid for by the patient's usual methods of reimbursement. The cost of treatment of infections will also be covered by the patient's usual methods of reimbursement whether or not the patient received prophylactic growth factor prior to developing the infection.

14 POTENTIAL RISKS AND BENE FITS

The potential risks of this therapy are substantial. The risk associated with the research question, not giving prophylactic G-CSF, is the potential that the patient will have increased risk of serious infections and/or delays in chemotherapy. There is a small possibility that substantial delays in therapy may lead to an increase in relapse or refractory disease. These will be mitigated with the addition of GM-CSF to reduce excessive delays when indicated.

Untreated, high-risk neuroblastoma is invariably fatal. The chemotherapy outlined in this protocol has been associated with improved survival in previous

trials; therefore there is a potential benefit to receiving the chemotherapy associated with this study. While G-CSF is considered a standard part of treatment for high-risk neuroblastoma, there is little data to support its need. It is possible the patient may benefit by not receiving a drug they do not need. If G-CSF increases neuroblastoma tumor cell growth, patients may benefit from not receiving this agent.

14.1 Risks of Chemotherapy Agents:

Possible Side Effects of Cisplatin (Table Version Date: April 20, 2015)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Cisplatin, more than 20 and up to 100 may have:
• Nausea, vomiting, • Infection, especially when white blood cell count is low • Anemia which may cause tiredness, or may require blood transfusions • Bruising, bleeding • Kidney damage which may cause swelling, may require dialysis • Hearing loss including ringing in ears

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Cisplatin, from 4 to 20 may have:
• Hair loss • Change in taste • Diarrhea • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Confusion • Difficulty with balance • Numbness and tingling of the arms and legs • Blurred vision or changes in ability to see colors (especially blue or yellow)

RARE, AND SERIOUS In 100 people receiving Cisplatin, 3 or fewer may have:
• Cancer of bone marrow caused by chemotherapy later in life • Seizure

Possible Side Effects of Cyclophosphamide
(Table Version Date: May 28, 2013)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Cyclophosphamide, more than 20 and up to 100 may have:
• Hair loss • Nausea, vomiting, loss of appetite • Sores in mouth • Infection, especially when white blood cell count is low • Absence of menstrual period which may decrease the ability to have children • Blood in urine

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Cyclophosphamide, from 4 to 20 may have:
• Damage to the bone marrow (irreversible) which may cause infection, bleeding, may require transfusions • Loss or absence of sperm which may lead to an inability to father children • Stuffy nose • Fluid around the heart

RARE, AND SERIOUS In 100 people receiving Cyclophosphamide, 3 or fewer may have:
• Severe skin rash with blisters and peeling which can involve mouth and other parts of the body • Damage to the heart or heart failure which may cause shortness of breath, swelling of ankles, cough or tiredness • A new cancer including cancer of bone marrow (leukemia) caused by chemotherapy • Swelling of the body including the brain which may cause dizziness, confusion • Scarring of the lungs

Possible Side Effects of Dexrazoxane (Table Version Date: October 24, 2013)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Dexrazoxane, more than 20 and up to 100 may have:
• Infection, especially when white blood cell count is low • Nausea • Fever • Bruising, bleeding

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Dexrazoxane, from 4 to 20 may have:
• Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Anemia which may cause tiredness, or may require transfusion • Pain at the site of injection • Vomiting

RARE, AND SERIOUS In 100 people receiving Dexrazoxane, 3 or fewer may have:
• Cancer of bone marrow caused by chemotherapy

Possible Side Effects of Doxorubicin (Table Version Date: October 24, 2013)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Doxorubicin, more than 20 and up to 100 may have:
• Hair loss • Vomiting • Red colored urine, saliva, or sweat

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Doxorubicin, from 4 to 20 may have:
• Heart failure or heart attack which may cause shortness of breath, swelling of ankles, cough or tiredness which may occur years after the dose • Swelling of the body which may cause shortness of breath • Swelling and redness at the site of the medication injection or area of previous radiation • Belly pain • Sores in the mouth, throat or stomach • Nausea, diarrhea • Hepatitis which may cause yellow eyes and skin • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Cancer of the bone marrow (leukemia) caused by chemotherapy • Damage to organs which may cause infection, bleeding, may require transfusions • Darkening of the nail beds or skin or hands and feet • Loss of nails

RARE, AND SERIOUS In 100 people receiving Doxorubicin, 3 or fewer may have:
• Infection, especially when white blood cell count is low • Bruising, bleeding • Severe blood infection

Possible Side Effects of Etoposide (Table Version Date: May 28, 2013)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Etoposide, more than 20 and up to 100 may have:
• Hair loss • Chills • Sores in mouth which may cause difficulty swallowing • Diarrhea, loss of appetite, nausea, vomiting • Infection, especially when white blood cell count is low • Anemia which may require transfusion • Bruising, bleeding • Tiredness • Fever

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Etoposide, from 4 to 20 may have:
• Heart failure or heart attack which may cause chest pain, shortness of breath, swelling of ankles, and tiredness • Severe skin rash with blisters and peeling which can involve inside of mouth and other parts of the body • Liver damage which may cause yellowing of eyes and skin, swelling

RARE, AND SERIOUS In 100 people receiving Etoposide, 3 or fewer may have:
• Cancer of bone marrow caused by chemotherapy • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat

Possible Side Effects of Filgrastim (Table Version Date: October 24, 2013)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Filgrastim, more than 20 and up to 100 may have:
• Nausea, vomiting • Pain in bone

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Filgrastim, from 4 to 20 may have:
• Anemia which may cause tiredness, or may require transfusion • Damage to the lungs which may cause shortness of breath • Internal bleeding which may cause coughing up blood • Swelling or tenderness of vessels

RARE, AND SERIOUS In 100 people receiving Filgrastim, 3 or fewer may have:
• Rupture of the spleen leading to bleeding in the belly

Possible Side Effects of Mesna (Table Version Date: May 28, 2013)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Mesna, more than 20 and up to 100 may have:
• Nausea, vomiting • Tiredness, headache • Pain in arms, legs • Unpleasant taste

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Mesna, from 4 to 20 may have:
• Low blood pressure which may cause feeling faint

RARE, AND SERIOUS In 100 people receiving Mesna, 3 or fewer may have:
• None

Possible Side Effects of Sargramostim (Table Version Date: October 24, 2013)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Sargramostim, more than 20 and up to 100 may have:
• Diarrhea, vomiting • Internal bleeding which may cause black tarry stool, or blood in vomit • Pain • Chills, fever, tiredness • Infection • Weight loss • Itching, rash

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Sargramostim, from 4 to 20 may have:
• Abnormal heartbeat • Bleeding of the eye which may cause blurred vision with a chance of blindness • Difficulty swallowing • Swelling of arms, legs • Bleeding in the brain which may cause headache, confusion • Worry • Kidney damage which may cause swelling, may require dialysis • Fluid in the organs which may cause low blood pressure, shortness of breath, swelling of ankles

RARE, AND SERIOUS In 100 people receiving Sargramostim, 3 or fewer may have:
• None

Possible Side Effects of Topotecan (Table Version Date: April 22, 2014)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Topotecan, more than 20 and up to 100 may have:
• Anemia which may require a blood transfusion • Constipation, diarrhea, nausea, vomiting • Fever • Pain • Bruising, bleeding • Infection, especially when white blood cell count is low • Tiredness • Shortness of breath • Hair loss

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Topotecan, from 4 to 20 may have:
• Sores in mouth which may cause difficulty swallowing • Headache • Cough • Scarring of the lungs • Rash

RARE, AND SERIOUS In 100 people receiving Topotecan, 3 or fewer may have:
• Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat

Possible Side Effects of Vincristine (Table Version Date: May 28, 2013)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Vincristine, more than 20 and up to 100 may have:
• Constipation • Hair loss • Pain or redness at the site of injection • Numbness and tingling of fingers or toes • Headache, jaw pain and/or muscle pain • Weakness and difficulty walking • Swelling of lower legs

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Vincristine, from 4 to 20 may have:
• Anemia which may cause tiredness, or may require transfusion • Drooping eyelids • Hoarseness

RARE, AND SERIOUS In 100 people receiving Vincristine, 3 or fewer may have:
• Seizure

14.2 Risks of stem cell collection

The risks of peripheral blood stem cell collection are the risks of apheresis, which are minimal. The specific risks to the individual patient will be explained in detail prior to the procedure by the apheresis team and consent for this procedure will be obtained separately at that time.

14.3 Risks of surgery

The risks of surgical resection are numerous including infection, hemorrhage and death. The specific risks to the individual subject are dependent on the extent and location of disease at time of resection. These risks will be explained in detail and consent for surgery will be obtained by the surgeon prior to surgery.

15 STATISTICAL CONSIDERATIONS

15.1 Statistical design

Sample size was calculated based on an estimation of infection rate for patients receiving prophylactic G-CSF of 58% (see section 4.4). This study will use Ahern's single stage design. A total of 13 subjects will be enrolled into this study. We will reject the null hypothesis if the proportion of patients who do NOT

develop an infection is ≤22% in favor of the alternative hypothesis that the proportion is ≥42% if at least 5 of 13 subjects complete six cycles of therapy without an infection. If the true proportion is 22%, then the probability of incorrectly rejecting the null hypothesis is 15% (type 1 error). If the true proportion is 42%, then the probability of failing to reject the null hypothesis is 25% (type II error).

15.2 Patient accrual and expected duration of trial

Texas Children's Hospital sees approximately 10-12 new high risk neuroblastoma patients per year. We plan to enroll 13 patients total. We anticipate this protocol will reach accrual goals in 2 to 2.5 years. The trial duration will be approximately 3 years.

An additional 13 caregivers will be enrolled to participate on the assessment of caregiver burden survey.

The expected duration of the trial for individual subjects is approximately 21 weeks.

15.3 Statistical analysis methods

15.3.1 Aim 1

The primary endpoint of this study is the incidence of the following infections in chemotherapy cycles NOT followed by hematopoietic growth factors:

- Any grade IV bacterial infection
- Any identified bacterial or presumed bacterial infection requiring IV antibiotics including

- Bacteremia with positive blood culture
- Signs/symptoms of sepsis with negative blood culture
- Pneumonia evident on chest radiograph with clinical symptoms consistent with pneumonia
- Grade III urinary tract infection
- Grade III cellulitis
- Neutropenic enterocolitis

This study will conclude that the proportion of patients who develop one of the above infections following cycles of chemotherapy when they did not receive hematopoietic growth factor is non-inferior to that of the standard regimen if 14 or fewer out of 21 total evaluable patients develop an infection. Otherwise, we will conclude that the new treatment regimen is not significantly non-inferior. The proportion of patients who develop an infection will be estimated with 95% confidence intervals using the methods of Koyama and Chen [31].

15.3.2 Secondary Aims:

The remainder of the aims will be descriptive in nature. We will use descriptive statistics to evaluate time between chemotherapy cycles, time to neutrophil recovery, antibiotic days, hospital days due to fever and/or infection and number of platelet transfusions. We will also describe response rates for patients enrolled in this study.

- Aim 2.1 To determine the incidence and duration of delay in chemotherapy administration due to prolonged neutrophil recovery in

patients undergoing induction chemotherapy for high-risk neuroblastoma without prophylactic G-CSF

We will capture start dates of each chemotherapy cycle reasons for delay cycles are >21 days apart. Assessment will be performed via descriptive analysis, calculating the proportion of each cycle delayed by cause of delay.

- Aim 2.2 To determine the number of antibiotic days and hospital days due to fever and/or infection for in patients undergoing induction chemotherapy for high-risk neuroblastoma without prophylactic G-CSF

  We will capture start and stop dates for antimicrobial agents (except agents for prevention of infection such as PJP prophylaxis agents and prophylactic anti-fungal agents). Admission and discharge dates for admissions for fever or documented or possible infection will be captured. Assessment of this aim will be performed via descriptive analytics summarizing the number of days of antibiotics and admitted to hospital for infection and compared by cycles with and without growth factors.

- Aim 2.3 To determine the number of platelet transfusions in in patients undergoing induction chemotherapy for high-risk neuroblastoma without prophylactic G-CSF

We will capture each platelet transfusion. Assessment of this aim will be performed via descriptive analytics summarizing the number platelet transfusions and compared by cycles with and without growth factors.

- Aim 2.4 To describe the response rate following induction chemotherapy without prophylactic G-CSF for patients with high-risk neuroblastoma

  Assessment of this aim will be performed via a descriptive analysis, calculating the number and proportion of patients in each response category and the proportion who achieve a CR, VGPR or PR after induction.

- Aim 2.5 To describe caregiver burden in patients undergoing induction chemotherapy for high risk neuroblastoma

  Assessment of this aim will be performed via a descriptive analysis, calculating the scores of the CMCC survey after cycle 1 and cycle 4. Summary statistics at each time point will be estimated. The individual scores and the change in scores for each patient will be compared to demographic factors and toxicities to identify potential predictors of increased caregiver burden for future study.

15.4 Gender and minority analysis

Given the low accrual of 13 patients, it is unlikely that a meaningful analysis of minorities is feasible. We shall report outcome and toxicity in a qualitative

manner by gender and race. No formal statistical comparisons will be applied to these subgroups.

16 ASSESSMENT OF FAMILY BURDEN

Participation in this aspect of this trial is voluntary.

Primary caregivers (one from each family) will complete the Care of My Child with Cancer instrument twice during induction: between day 21 and 29 of cycle 1 (before starting cycle 2) and between day 21 and 29 of cycle 4 (before starting cycle 5). The instrument will be completed in paper version, and kept with the research chart. It is not necessary that the same primary caregiver complete both surveys. Responses will be reviewed in real time by the research staff.

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