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RESEARCH CONSORTIUM

Protocol DFMO Phase II

NMTRC 003B

A Phase II Preventative Trial of DFMO (eflornithine HCl) as a single agent in Patients with High Risk Neuroblastoma in Remission.

NCT02395666

Last IRB Approval Board Action Date: 02/18/2020

Version 5.1 **February 18, 2015**

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Certificate of Action

Board Action Date: 02/18/2020	**Work Order Number:** 1-1248815-1
Sponsor: Giselle Sholler, M.D.	**Protocol Approval Expires:** 03/12/2021
Sponsor Protocol Number: NMTRC 003 **Amended Sponsor Protocol Number:** NMTRC 003B	**Continuing Review Frequency:** Annually
IRB Tracking Number: 20120325	**Panel:** 1 **Note: Panel 18 is a shared panel of CGIRB and WIRB. Panel 19 is a shared panel of Aspire and WIRB.**
Protocol Title: A Phase II Preventative Trial of DFMO (eflornithine HCl) as a Single Agent in Patients with High Risk Neuroblastoma in Remission.	

THE FOLLOWING ITEMS ARE APPROVED:
A Phase II Preventative Trial of DFMO (eflornithine HCl) as a Single Agent in Patients with High Risk Neuroblastoma in Remission.

Please note the following information about this review:

ALL IRB APPROVED INVESTIGATORS MUST COMPLY WITH THE FOLLOWING:

Consistent with AAHRPP's requirements in connection with its accreditation of IRBs, the individual and/or organization submitting shall promptly communicate or provide, and where necessary cause each investigator to promptly communicate or provide, the following information relevant to the protection of human subjects to the IRB in a timely manner:

- Upon request of the IRB, a copy of the written plan between sponsor or CRO and site that addresses whether expenses for medical care incurred by human subject research subjects who experience research related injury will be reimbursed, and if so, who is responsible in order to determine consistency with the language in the consent document.
- Any site monitoring report that directly and materially affects subject safety or their willingness to continue participation. Such reports will be provided to the IRB within 5 days.
- Reports from any data monitoring committee, data and safety monitoring board, or data and safety monitoring committee in accordance with the time frame specified in the research protocol.
- Any findings from a closed research when those findings materially affect the safety and medical care of past subjects. Findings will be reported for 2 years after the closure of the research.

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Thank you for using this WCG IRB to provide oversight for your research project.

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Genevieve Bergendahl, RN, NMTRC

This is to certify that the information contained herein is true and correct as reflected in the records of this IRB. WE CERTIFY THAT this IRB IS IN FULL COMPLIANCE WITH GOOD CLINICAL PRACTICES AS DEFINED UNDER THE U.S. FOOD AND DRUG ADMINISTRATION (FDA) REGULATIONS, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES (HHS) REGULATIONS, AND THE INTERNATIONAL CONFERENCE ON HARMONISATION (ICH) GUIDELINES.

Association for the Accreditation of Human Research Protection Programs, Inc. — Full Accreditation

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INVESTIGATOR SIGNATURE SHEET

I have read the attached protocol and agree that it contains all the necessary details for performing the study.

I will provide copies of the protocol and of the preclinical and clinical information (Investigator's Brochure) on the test article, which was furnished to me by the Neuroblastoma and Medulloblastoma Translational Research Consortium (NMTRC), to all members of the study team responsible to me who participate in the study. I will discuss this material with them to assure that they are fully informed regarding the test article and the conduct of the study.

Once the protocol has been approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), I will not modify this protocol without obtaining the prior approval of the NMTRC and of the IRB/IEC. I will submit the protocol modifications and/or any informed consent form (ICF) modifications to the NMTRC and the IRB/IEC, and approval will be obtained before any modifications are implemented.

I understand the protocol and will work according to it, the principles of Good Clinical Practice (GCP) [current International Conference of Harmonization (ICH) guidelines], and the Declaration of Helsinki (1964) including all amendments up to and including the Scotland revision (2000) and notes of clarification added in 2002 and 2004.

_____ _____

Investigator's Signature Date

_____

Investigator's Printed Name

_____

Investigational Site Name

TABLE OF CONTENTS

A Phase II Preventative Trial of DFMO (eflornithine HCl) as a Single Agent in Patients with High Risk Neuroblastoma in Remission.

PROTOCOL SYNOPSIS

PROTOCOL TITLE	A Phase II Preventative Trial of DFMO as a Single Agent in Patients with High Risk Neuroblastoma in Remission.
PROTOCOL NUMBER	NMTRC 003B
PHASE OF DEVELOPMENT	Phase II
OBJECTIVES	**Primary** • To evaluate the preventative activity of DFMO as a single agent in patients with neuroblastoma who are in remission based on: ◦ Event free survival (EFS) **Secondary** • To evaluate the preventative activity of DFMO as a single agent in patients with neuroblastoma who are in remission based on: ◦ Overall Survival (OS) • Correlation of urinary polyamine levels with progression of disease in neuroblastoma. • To determine the safety and tolerability of DFMO as a single agent in pediatric and young adult patients with high risk neuroblastoma that is in remission. • To evaluate the pharmacokinetics (PK) of new DFMO formulation in a minimum of 10 patients • Biological Correlates to minimally include: 1) ***Urine: polyamine levels*** and blood inflammatory markers, 2) Blood: microRNA analysis as predictor of DFMO effect, ODC SNP analysis in DNA isolated from nucleated cells, circulating tumor cell analysis, and explorative biomarker analysis 3) Bone Marrow: flow cytometry of minimal residual disease of tumor; explorative biomarker analysis
STUDY DESIGN	DFMO will be used in an open label, single agent, multicenter, study for patients with neuroblastoma that are in remission. **Cycle 1-27: DFMO** In this study subjects will receive twenty-seven (27) cycles of oral DFMO at a dose of 500 to 1000 mg/m^2 BID (per dosing chart in section 6.1.2) on each day of a 28 day cycle.

	Subjects will be evaluated in 2 strata: • Stratum 1: Subjects that are in remission at the end of upfront therapy defined as chemotherapy (5-7 cycles), surgery as indicated, consolidation therapy as indicated, radiation therapy as indicated, anti-GD2 antibody therapy with retinoic acid up to 6 cycles. • Stratum 2: Subjects that are in remission after any previous relapse or refractory therapy **Stratum 1**- A total of 127-140 subjects in Stratum 1 will be enrolled to ensure that there will be 127 evaluable subjects. **Stratum 2**- A total of 38 subjects in Stratum 2 will be enrolled to ensure that there will be 33 evaluable subjects.
ELIGIBILITY	**Inclusion Criteria** 1. Age: 0-21 years at the time of diagnosis. 2. Diagnosis: histological verification at either the time of original diagnosis or a previous relapse of high risk neuroblastoma. 3. Disease Status: Neuroblastoma that is in Remission 4. Greater than 30 days from completion of cytotoxic and antibody therapy and less than 120 days from previous therapy. 5. A negative serum or urine pregnancy test is required for female subjects of child bearing potential (onset of menses or ≥13 years of age). 6. Both male and female post-pubertal study subjects need to agree to use one of the more effective birth control methods during treatment and for six months after treatment is stopped. These methods include total abstinence (no sex), oral contraceptives ("the pill"), an intrauterine device (IUD), levonorgestrol implants (Norplant), or medroxyprogesterone acetate injections (Depo-provera shots). If one of these cannot be used, contraceptive foam with a condom is recommended. 7. ANC > 500/µl and platelet count >50,000/µl 8. Organ Function Requirements

Subjects must have adequate liver function as defined by AST and ALT <10x upper limit of normal
Serum bilirubin must be ≤ 2.0 mg/dl
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	05
1 to < 2 years	0.6	0.6
2 to < 6 year	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

9. Informed Consent: All subjects and/or legal guardians must sign informed written consent. Assent, when appropriate, will be obtained according to institutional guidelines

Exclusion Criteria

1. Lansky score < 60%
2. BSA (m^2) of <0.25
3. Investigational Drugs: Subjects who are currently receiving another investigational drug are excluded from participation.
4. Anti-cancer Agents: Subjects who are currently receiving other anticancer agents are not eligible. Subjects must have fully recovered from the effects of prior chemotherapy (hematological and bone marrow suppression effects).
5. Infection: Subjects who have an uncontrolled infection are not eligible until the infection is judged to be well controlled in the opinion of the investigator.
6. Subjects who, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study, or in whom compliance is likely to be suboptimal, should be excluded.

	Additional criteria: Subjects willing to participate in the correlative biologic studies will sign an additional consent form to provide bone marrow and blood for analysis.
ESTIMATED NUMBER OF SUBJECTS/GEOGRAPHIC REGIONS	A projected total of 160 evaluable subjects (Stratum 1-127 subjects, Stratum 2-33 subjects). Up to 25 sites in the United States will participate.
LENGTH OF STUDY	Accrual to this study is estimated to be at least 2 years, with follow up until all patients meet primary and secondary endpoints.
INVESTIGATIONAL PRODUCT DOSE/ROUTE/REGIMEN	DFMO is an oral tablet that will be administered daily on a 28-day cycle. Dose will be 500 to 1000mg/m2 given BID (per dosing chart in section 6.1.2)
STUDY ASSESSMENTS	Refer to Table of Assessments for timing of study procedures.
CRITERIA FOR EVALUATION	**Preventative Activity Measures: Primary objectives** • **Event Free Survival (EFS),** defined as period from the first day of administration of study drug to the first occurrence of relapse, progressive disease, secondary cancer, or death or, if none of these events occurred, until the last contact with the subject. **Safety Measures and Preventative Activity Measures: Secondary objectives** • **Correlation of urinary polyamine levels** with progression of disease in neuroblastoma**.** • **Overall Survival (OS)** will be defined as the first day of administration of study drug until death or will be censored at the last contact with the subject if death did not occur during the study. • **Safety analysis** will be conducted on all subjects who have received at least one dose of study drug, and will include the frequency of all adverse events and laboratory abnormalities as well as frequency of dose interruptions, dose reductions and treatment discontinuation.

STATISTICS/SAMPLE SIZE ESTIMATE	**Statistics**: All baseline subject characteristics will be summarized in a tabular format. Safety data will be described for all subjects receiving at least one dose of DFMO. Safety data will include values for hematology, serum chemistry, vital signs, and adverse events. The proportion of subjects experiencing adverse events, serious adverse events, and treatment delays will be summarized for each dosing cohort. **Sample Size:** **Stratum 1:** A 70% 2-year EFS rate was selected as the Phase I baseline rate. The baseline 70% EFS rate at 2-years was based upon published data by Yu et al. It is hypothesized that DFMO will increase the 2-year EFS rate to 80% which represents an approximately 10% increase in the duration of the EFS rate at 2-years. Assuming a directional 5% one population binomial test, a sample size of n = 127 patients will be required to achieve an 80% power to detect this 2-year difference in EFS (70% vs 80%). The comparison of the biomarker prevalence rates assumes that the Stratum 1 cohort will experience a relapse rate of 30% and a 70% non-relapse rate at up to two years of follow-up. It is also assumed that the corresponding prevalence of an elevated biomarker will be 60% for the relapse group and 30% in the non-relapse group, respectively. This hypothesized prevalence difference will be detectable with at least an 85% power with an overall sample size of N=127 patients (38 relapse and 89 non-relapse) using a Fisher's Exact Test and a non-directional Type I Error level of 5%. **Stratum 2:** Examination of EFS will require a sample size of n = 33 subjects in Stratum 2 to test whether treatment with DFMO can prolong the overall estimated EFS at two years to 30% from the 10% at two years estimate for the known historical data in this subject population. A one population binomial test with power 80% and a 5% two-tailed Type I error level will require n = 33 total patients The one population binomial test will reject the null hypothesis that the two year EFS rate = 10% in favor of the EFS = 30% if 7 or more of the n = 33 patients are observed to have at least a two year EFS.

	Pharmacokinetics: DFMO plasma concentration-time data will be determined for the first 10 or more subjects enrolled on the new DFMO formulation. The following PK parameters will be determined for the last dose of current formulation DFMO and after 2-5 days of new formulation DFMO: • Cmax • AUC (0 – 24 hr) • Plasma half-life (t1/2) • Plasma clearance (Cl) • Vd

Procedures and Assessments

	Pre	Cycles 1					Cycles 2-27					Follow Up
		Day 1	Day 8	Day 15	Day 21	End of cycle	Day 1	Day 8	Day 15	Day 21	End of Cycle	
Informed consent	X											
Medical & surgical history, demographics histologic evidence of malignancy	X											
Prior therapy for malignancy	X											
Physical examination	X	X		X			X					X
Vital signs	X	X		X			X					X
ECOG performance/Lansky play status	X						X					X
CBC and differential	X			X			X[f]					X
Serum chemistries (as defined in 7.0)	X			X			X[f]					X
CRP and ESR	X						X[f]					
Pregnancy test	X						X					
MIBG/CT/MRI Scan as needed	X										X[b]	X
Concomitant medications	X	X		X			X					X
BSA calculation	X						X					
Administration of DFMO		→	→	→	→	→	→	→	→	→	→	
Dispense (and collect) drug dosing diary		X					X					X
AE monitoring		X		X			X					X
PK blood samples (Minimum 10 subjects)							X[e]					
Urine for biological correlates	X	X		X			X					
Blood[a] for biological correlates		X		X			X[f]					
Blood for Circulating tumor cells[a]		X					X[g]					
Audiogram	X										X[d]	X[d]
Urine VMA/HVA	X						X					
Bone Marrow^	X										X[c]	X[c]

a *Voluntary – additional informed consent required for blood*
b End of cycle 3, 6, 9, 13, 20, and at off protocol therapy (end of Cycle 27)- or as clinically indicated.; see section 7.4 for specific details)
c If concern for progression of disease (and/or if hematologic toxicity grade 4)
d End of cycle 6, 12, and off protocol therapy (and as clinically indicated; see section 7.4 for specific details)
e PK's on last day of tablet formulation and Day 2-5 of starting new formulation per Sec 12.
f- Cycles 1-12, 14, 17, 21, 24, and off therapy (end of cycle 27)
g- Cycles 3, 6, 9, 12, 14, 17, 21, 24, and off therapy (end of cycle 27)
^Standard of Care Bone Marrow. (Separate consent required for additional research samples per section 12.0)

1.0 Protocol Concept

High risk Neuroblastoma (NB) remains a challenge in pediatric oncology, accounting for 15% of all pediatric cancer deaths. While most patients are able to attain remission, approximately 50% will relapse. Once relapsed, there is currently no curative treatment for these children, and for these children the 5-year survival rate is <10%. As such, new therapeutic approaches are needed to treat these children. Relapsed patients who are able to obtain a second remission are not eligible for relapse therapeutic trials since they have no evidence of disease and yet they are likely to relapse within 6 months-1year. Prevention of relapse is one such approach to improve outcome in these patients. This study will address this concept while using a well-tolerated targeted medication for children in remission.

These more aggressive forms of NB respond poorly to hormonal and chemotherapeutic approaches, and therefore, there is a great need for antineoplastic agents with novel mechanisms of action. The MYCN protein up-regulates *ornithine decarboxylase* (*ODC*), a gene encoding for the ODC enzyme that is pivotal in polyamine biosynthesis. High polyamine content and elevated ODC activities are commonly found in NB as well as many other tumors, and therefore, suppression of polyamines in cancer cells is an effective means to reduce tumor cell proliferation. Specific polyamine inhibitors such as DFMO have been evaluated in adult clinical cancer trials and shown to prevent formation of polyps and colon cancer. DFMO will be evaluated in this pediatric NB trial as a preventative single agent therapy.

2.0 Background and Preliminary Data

2.1 Previous Preclinical and Clinical Work

Neuroblastoma (NB) is a tumor of the autonomous nervous system originating from the adrenal medulla and autonomous ganglia in the chest and abdomen. After leukemia and brain tumors, NB is the third most frequent malignant tumor of childhood. The incidence in the United States is approximately one in 7,000 children (Ater, Gardner et al. 1998). Therapy for NB is very intense, especially in advanced stages of the disease with widespread metastases to liver, bone, lymph nodes, and bone marrow. Current therapy includes chemotherapy, radiation, and high dose chemotherapy with subsequent bone marrow transplantation followed by differentiating therapy. More recently, immunotherapy has been added using monoclonal antibodies to the GD_2 glycolipid antigen that is heavily expressed by NB cells (Bosslet, Mennel et al. 1989; Cheung, Kushner et al. 1998). Over the last 30 years, significant therapeutic progress has been made with an increase in the five-year relative survival rate from approximately 25% to 55%. However, almost 50% of patients are estimated to die of their tumor, and over the past decade improvement in the five-year survival rate of NB patients has been slow (Harras 1996). NB has a particularly poor prognosis in patients older than 2 years at diagnosis, advanced stage disease and/or disease characterized by *MYCN* gene amplification (Seeger, Brodeur et al. 1985; Brodeur 2003). These more aggressive forms of NB respond poorly to chemotherapeutic approaches, and therefore, there is great need for a better understanding of the cellular regulation of *MYCN*-amplified NB tumors in an effort to search for alternative molecular drug targets. Although a role for the MYCN oncoprotein has been established in NB pathogenesis, the mechanism by which MYCN contributes to both the development of this disease and its poor prognosis is still unclear. The

MYCN oncoprotein functions as a transcriptional regulator (Ben-Yosef, Yanuka et al. 1998) and thus may influence tumorigenesis and patient survival by regulating the expression of key genes involved in the NB malignant phenotype. MYCN regulates the expression of genes that encode ornithine decarboxylase (ODC), the multi-drug resistance-associated protein 1 (MRP1), and MDM2 (Slack, Chen et al. 2005). ODC is the rate-limiting enzyme in the production of polyamines (Marton and Pegg 1995). Although polyamines, and therefore ODC, are essential for normal cell proliferation, increased ODC activity can induce cellular transformation *in vitro* (Auvinen, Paasinen et al. 1992), and high ODC levels are associated with a variety of tumors, including those of the brain and prostate (Mohan, Challa et al. 1999; Ernestus, Rohn et al. 2001). Several studies have identified *ODC* as a target gene for the MYCN oncoprotein (Lutz, Stohr et al. 1996; Ben-Yosef, Yanuka et al. 1998; Lu, Pearson et al. 2003), and it is possible that ODC, and therefore polyamines, play a significant role in NB tumorigenesis.

Role of polyamines in cancer

The identification of novel inhibitors of enzymes involved in polyamine biosynthesis with antitumor activities has recently revived interest in polyamine homeostasis and in designing strategies of cancer chemotherapy (Mamont, Duchesne et al. 1978; Porter, Regenass et al. 1992; Seiler, Atanassov et al. 1998). Selective pharmacological interference with the synthesis of natural polyamines results in tumor cell growth inhibition under both in vitro and in vivo conditions (Mamont, Duchesne et al. 1978; McCann and Pegg 1992). Although the precise mechanism by which polyamines contribute to cell proliferation is not well known, it has been suggested that it may be a result of their ability to bind DNA and affect gene expression by bringing about structural changes in chromatin, thereby stimulating cell growth (Marton and Morris 1987). Furthermore, the dramatic increases in the activity of ODC in certain tumor cells have been linked to G_1-S transition (Fuller, Gerner et al. 1977; Kahana and Nathans 1984; Kaczmarek, Calabretta et al. 1987). As mentioned above, an apparent molecular basis for this derives from the fact that ODC is among those genes, which can be regulated by c-Myc and MYCN (Bello-Fernandez, Packham et al. 1993; Pena, Reddy et al. 1993; Wagner, Meyers et al. 1993; Lutz, Stohr et al. 1996; Lu, Pearson et al. 2003), both of which regulate entry into and exit from the cell cycle. Because cell growth is absolutely dependent on polyamines, interference with polyamine biosynthesis has long been considered a promising therapeutic approach against proliferative diseases, including various malignancies (Heby and Persson 1990; Auvinen, Paasinen et al. 1992; McCann and Pegg 1992). α-difluoromethylornithine (DFMO or eflornithine), a suicide substrate inhibitor of ODC (Metcalf, Bey et al. 1978; Poulin, Lu et al. 1992), has been the prototype tool for the study of therapeutic effectiveness of polyamine depletion in experimental tumors (McCann and Pegg 1992; Meyskens and Gerner 1999) (Fig. 1).

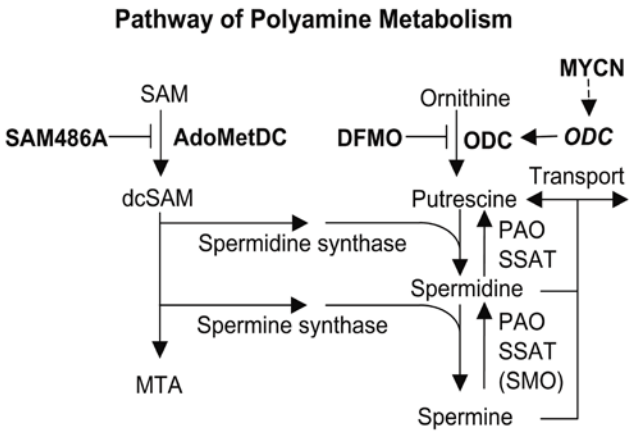

Figure 1. Pathway of polyamine metabolism showing two target enzymes of the polyamine inhibitors DFMO and SAM486A. By inhibiting ODC, DFMO depletes putrescine (Put) and spermidine (Spd) pools, whereas it only modestly affects spermine (Spm) pools. By inhibiting AdoMetDC, SAM486A depletes Spd and Spm, whereas it markedly increases Put. The inhibitor combination lowers all three polyamine pools until cells stop growing and the pools are no longer diluted by cell division. SSAT and PAO work in concert to acetylate and oxidize polyamines during retro-conversion. SMO converts Spm back to Spd without the need for an acetylation step. AdoMetDC, *S*-adenosylmethionine decarboxylase; MTA, methyl-thioadenosine; ODC, ornithine decarboxylase; PAO, polyamine oxidase; SAM, *S*-adenosylmethionine; SMO, spermine oxidase; SSAT, spermidine/spermine N^1-acetyltransferase

DFMO inhibits cell growth of many cancer cells including NB and induces cell differentiation (Chapman 1980; Melino, Farrace et al. 1988). These processes are accompanied by an apparent depletion of putrescine (Put) and spermidine (Spd) pools (Pegg 1988; Heby and Persson 1990; McCann and Pegg 1992). DFMO has also been shown to induce apoptosis and inhibit metastasis in a human gastric cancer model (Takahashi, Mai et al. 2000).

Role of polyamines in NB cell differentiation

The fluctuation in the levels of intracellular polyamines such as Put, Spd, and spermine (Spm) has been observed in association with cell differentiation (Heby 1981; Tabor and Tabor 1984; Pegg 1986), and inhibition of ODC by DFMO and reduction in polyamine pools stimulates various cancer cells including NB cells to differentiate (Chen, Nau et al. 1983; Melino, Farrace et al. 1988; Melino, Piacentini et al. 1991). DFMO treatment of NB cells can change the triangular NB morphology by inducing a different phenotype; one which resembles elongated fibroblast-like cells without typical neuritic processes. By comparison, treatment with retinoic acid (RA) induces neural differentiation of NB cells as indicated by the outgrowth of definite neurites (Melino, Farrace et al. 1988; Melino, Piacentini et al. 1991; Wainwright, Lasorella et al. 2001). The role of polyamines in cell differentiation has been studied for many years, and yet the precise role of polyamines at the cellular and molecular level is still not well understood and may play a key role in tumor regression.

Effect of polyamine inhibitor DFMO in a transgenic neuroblastoma animal model

Encouraging data by two groups (Hogarty, Norris et al. 2008; Rounbehler, Li et al. 2009) recently emerged and confirmed the effect of DFMO *in vivo* using the *TH-MYCN* NB mouse model, and DFMO in combination with cisplatin and cyclophosphamide increased the tumor-free survival of TH-MYCN homozygous mice (Fig. 2) (Hogarty, Norris et al. 2008). Additional studies have revealed that DFMO combined with SAM486A act synergistically and result in a significantly reduced tumor burden in TH-MYCN mice (56).

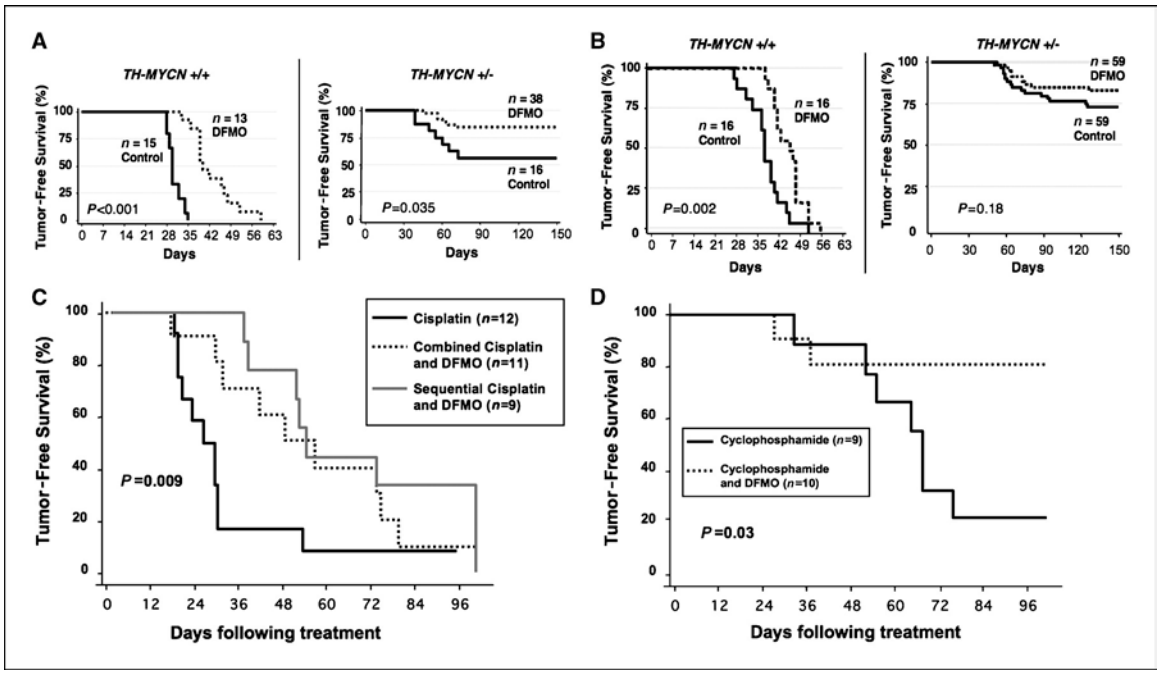

Figure 2. Extended tumor-free survival in neuroblastoma-prone mice treated with DFMO. **A,** tumor-free survival curves for homozygous (*TH-MYCN* +/+) or hemizygous (*TH-MYCN* +/-) mice stratified by DFMO therapy. DFMO-treated mice (dashed lines) received DFMO from birth onward (preemptive treatment trial). DFMO therapy was stopped at day 70 in tumor-free mice. **B,** delayed treatment trial: *TH-MYCN* homozygous and hemizygous mice were randomized to DFMO (dashed lines) or control (solid lines) following weaning at day 25. Tumor-free survival for *TH-MYCN* homozygous mice with advanced tumor from the time of treatment with (**C**) cisplatin alone (black line), cisplatin followed by DFMO (gray line), or cisplatin administered simultaneously with DFMO (dashed line) or (**D**) cyclophosphamide alone (solid line) or combined with DFMO (dashed line). P values using the method of Kaplan-Meier are shown (75).

Relevance of ODC in patients with neuroblastoma

Further evidence of the importance of ODC in NB tumorigenesis is available from our recent studies with human NB tumors. We analyzed the expression levels of ODC mRNA from 88 NB patients and found significant correlations between ODC expression and the overall survival probability. High levels of ODC were predictive of low survival probability and vice versa (Fig. 3A). Most surprisingly, ODC was also predictive in tumors without MYCN amplification (Fig. 3B), thus suggesting that ODC also plays a role in NB tumorigenesis independent of MYCN amplification (57). These findings were independently confirmed by two other groups (Hogarty, Norris et al. 2008; Rounbehler, Li et al. 2009).

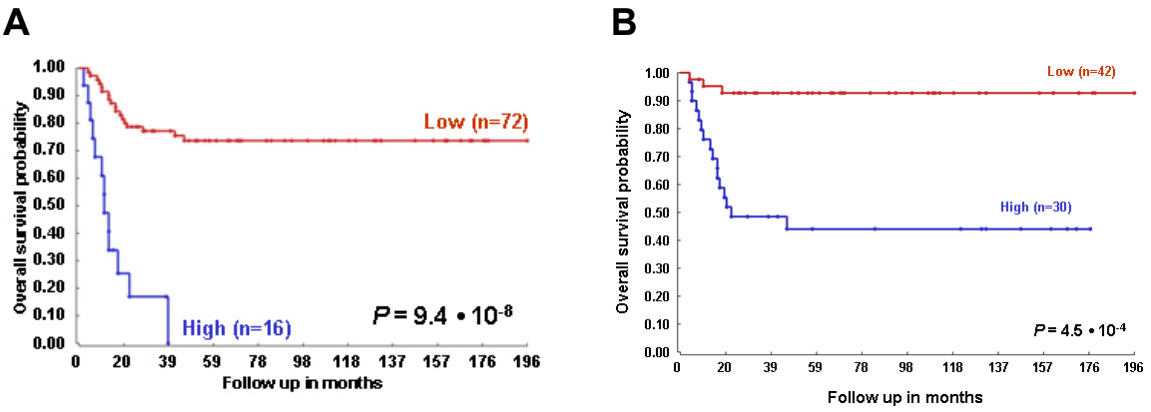

Figure 3. Correlation of ODC gene expression with NB patient survival prognosis. **A,** Kaplan-Meier graphs representing the survival prognosis of 88 NB patients based on high or low expression levels of ODC. Survival probability of NB patients (follow-up over 196 months) with low ODC expression is significantly higher than of patients with high ODC expression. **B,** Kaplan-Meier graphs representing the survival prognosis based on high or low expression levels of ODC stratified for patients without *MYCN* amplification. The survival probability of NB patients with low ODC expression is significantly higher than of patients with high ODC expression (80).

2.2 Clinical Work

Summary of Phase I Neuroblastoma Trial (NTC01059071)

A multi-center, investigator initiated dose escalation study that was started in 2010 and is still in progress. The following dose levels of DFMO (CPP-1X) are being investigated: 500 mg/m^2, 750 mg/m^2, 1000 mg/m^2 and 1500 mg/m^2. Cycles are 21 days, with CPP-1X given days 1-21 for all cycles and for cycles 2 and greater, etoposide is given on days 1-14 of each cycle. Patients must complete the first 2 cycles of treatment (cycle 1 CPP-1X alone, cycle 2 CPP-1X with 50 mg/m^2/dose etoposide) in order to be included in the dose limiting toxicity assessment. Patients who did not complete 2 cycles were replaced in the cohort. Patients are considered to have completed the study if they have received 5 cycles of treatment. They may continue treatment if there are no safety concerns, there is no disease progression, and/or there is an indication of clinical benefit.

Based on current data, 15 patients have received therapy, with 4 patients at 500 mg/m^2, 5 patients at 750 mg/m^2, 3 patients at 1000 mg/m^2, and 3 patients at 1500 mg/m^2. All patients were multiple relapsed/refractory, with 10 males and 5 females, mean age 9, range (5-15), and 60% White/Caucasian.

To date, there have been no confirmed DLT. To date, 8 patients have reported an adverse event while on study. The most prevalent adverse events were low hemoglobin, neutropenia and thrombocytopenia. The majority of the events were considered unrelated to treatment. One patient died during the follow-up period after going into hospice care once off study for progression after one cycle of therapy.

Six patients have completed at least 5 cycles of therapy. Time on study ranged from 20-645+ days, with 3 patients out 1 year or more.

Chemopreventive effects of DFMO and sulindac in sporadic colorectal adenomas

A recent randomized placebo-controlled double-blind trial was performed by Drs. Meyskens and Gerner to test whether the combination of a low dose of DFMO plus a low dose of sulindac reduces the recurrence of human colorectal adenomas (59). This study revealed that recurrent adenomatous polyps in patients can be markedly reduced by a combination of low oral doses of DFMO and sulindac (Table 1). Moreover, the study provided strong evidence that these two drugs are safe together with minimal side effects.

Table 1. Risk of adenomas; evidence of substantial effect in active arm

	Follow-up colonoscopy 2 to 39 mo after beginning treatment (n = 267)		Follow-up colonoscopy 33 to 36 mo after beginning treatment (n = 204)	
	Placebo (n = 129)	DFMO/sulindac (n = 138)	Placebo (n = 97)	DFMO/sulindac (n = 107)
Detection of any adenoma				
Cumulative incidence of adenomas detected at end of the treatment (%)	53 (41.1)	17 (12.3)	42 (43.3)	12 (11.2)
Risk ratio* (95% CI)		0.30 (0.18-0.49)		0.26 (0.15-0.46)
P		<0.001		<0.001
Detection of advanced adenomas†				
Cumulative incidence of advance adenomas detected at end of the treatment (%)	11 (8.5)	1 (0.7)	9 (9.3)	1 (0.9)
Risk ratio* (95% CI)		0.085 (0.011-0.65)		0.10 (0.013-0.78)
P		0.001		0.004
Detection of advanced adenomas with size ≥1 cm				
Cumulative incidence of advanced adenomas with size ≥1 cm detected at end of the treatment (%)	9 (7.0)	1 (0.7)	7 (7.2)	1 (0.9)
Risk ratio* (95% CI)		0.10 (0.013-0.81)		0.13 (0.016-1.03)
P		0.004		0.02
Detection of multiple adenomas (>1)				
Patients with >1 adenoma, incidence (%)	17 (13.2)	1 (0.7)	15 (15.5)	1 (0.9)
Risk ratio* (95% CI)		0.055 (0.0074-0.41)		0.060 (0.0081-0.45)
P		<0.001		<0.001
Sensitivity analysis imputing adenoma for patients without an end-point determination‡				
Cumulative incidence of adenomas detected at end of the treatment (%)	76/184 (41.3)	39/191 (20.4)		
Risk ratio* (95% CI)		0.49 (0.36-0.69)		
P		<0.001		

*Relative risk estimation by log-binomial regression. Likelihood ratio test P values are reported.
†Advanced adenomas in the placebo group included tubulovillous (3), intramucosal carcinoma (2), size ≥1 cm (6), and one serrated adenoma with high-grade dysplasia; the one advanced adenoma in the treatment group was an adenoma >1 cm.
‡Sensitivity analysis imputing adenoma for all patients without an end-of-study colonoscopy at the placebo rate of recurrence.

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Specific experience with DFMO and studies in patients with adenomatous polyps are described in four clinical chemoprevention studies that have been performed (59, 60, 61, 62)

Boyle JO et al in a pilot study established that DFMO could lower polyamine content in colorectal mucosa. They demonstrated that shed oral extracellular mucosal cells did not provide a reliable surrogate estimate of DFMO effects on the colon. Meyskens et al performed a dose de-escalation chemoprevention trial of 2 difluoromethylornithine in patients with colon polyps. The short-term (one month) Phase IIa study established that DFMO was both safe and effective in reducing colorectal mucosal polyamine contents when administered orally to patients as low as .25 gm/m^2 for 28 days. No ototoxicity was observed at doses up to twice this amount. They

demonstrated that both putrescine content and the ratio of spermidine to spermine and changes in these parameters as a function of DFMO treatment decreased as a function of donor age, which is an important consideration in the evaluation of DFMO effect. Meyksens then showed in an intermediate term (12 months) Phase IIb clinical chemoprevention trial demonstrated that polyamine levels in rectal mucosa can be continually suppressed by daily oral doses of DFMO that produced few or no side effects (62). Using a similar design, a smaller study has confirmed these results (63); this trial reported some reversible ototoxicity (at a dose of DFMO of 500 $mg/m^2/day$).

Usage of DFMO at high doses for therapeutic purposes indicated that hearing loss occurred and seemed to be related to the total dose; this toxicity was however reversible (64). The toxicity of low dose DFMO has been assessed in both a phase IIa and phase IIb trial and the evaluation of hearing changes was described in the phase IIb trial (65). They were unable to demonstrate an effect of DFMO on otoacoustic emissions at any dose of DFMO (0, -75, 200, 400 $mg/m^2/day$) administered. They detected a subtle decrease in pure tone threshold that was dose-related. However, even at the highest dose the decrease was less than 4 db. Since 1-2 db decreases per year of hearing in normal humans in this age range have now been shown (66,67), results should not raise undue concern since: (1) the effects of DFMO on hearing loss are known to be rapidly reversible after discontinuation of the drug and (2) 10-20 db losses need to occur before the effect is clinically evident. Nevertheless, all participants will receive a baseline audiogram, will be check throughout the study and if clinically indicated.

Summary

These studies suggest that ODC/polyamines are critical in oncogenesis and therefore present a therapeutic target for the treatment and prevention of recurrence of NB and other types of cancer. This study will focus on the use of DFMO in high risk neuroblastoma patients that are in remission as a strategy to prevent recurrence.

3.0 Study Objectives

3.1 Primary Objective:

3.1.1 To evaluate the preventative activity of DFMO as a single agent in patients that are in remission based on: Event free survival (EFS)

3.2 Secondary Objectives:

3.2.1 Correlation of urinary polyamine levels with progression of disease in neuroblastoma.

3.2.2 To evaluate the preventative activity of DFMO as a single agent in patients with neuroblastoma who are in remission based on:

- Overall Survival (OS)

3.2.3 To continue to determine the safety and tolerability of DFMO as a single agent and in pediatric and young adult patients with high risk neuroblastoma that is in remission.

3.2.4 To evaluate the pharmacokinetics (PK) of new DFMO formulation in a minimum of 10 patients.

3.2.5 Biological Correlates to minimally include: 1) Urine: polyamine levels and blood inflammatory markers, 2) Blood- microRNA analysis as predictor of DFMO effect; ODC SNP analysis in DNA isolated from nucleated cells; circulating tumor cell analysis, and explorative biomarker analysis 3) Bone Marrow- flow cytometry of minimal residual disease of tumor; explorative biomarker analysis

4.0 Study Design

This study is an open label, single agent, multicenter, study for patients with neuroblastoma that is in remission.

4.1 DFMO Treatment

In this study subjects will receive twenty-seven (27) cycles of oral DFMO at a dose of 500 to 1000 mg/m^2 BID (per dosing chart in section 6.1.2) on each day of a 28 day cycle. DFMO is provided as 250mg tablets.

4.2 Response Evaluations

At the times indicated in Section 7.0 and the Table of Procedures and Assessments, scans will be obtained to evaluate response for subjects enrolled in this study. Response will be assessed to evaluate the potential benefit of DFMO in this patient population.

4.3 Pharmacokinetic Evaluations

Plasma concentrations of DFMO will be determined for a minimum of 10 currently enrolled subjects on their last day of taking the DFMO tablet formulation and on Days 2-5 post start of taking the new formulation at hours 0 (pre dose), 30min, 1 hour, 3 hours, and 6 hours post-dose. Change from old formulation to new formulation may take place on any day of any cycle after subject consents to new formulation.

4.4 Biological Studies

These studies will evaluate the level of polyamines and inflammatory markers in urine. Subjects will have an opportunity to participate in additional correlative biological studies on a voluntary basis to evaluate the level of polyamines and inflammatory markers in blood. These voluntary studies will evaluate microRNA levels in blood and ODC activity in white blood cells. They will also evaluate minimal residual disease in bone marrow. These biologic studies may be able to contribute to our knowledge of molecular determinants of response to therapy and/or the development of biomarkers to help guide future therapy.

5.0 Patient Selection

Eligibility:

5.1 Inclusion Criteria:

1. Age: 0-21 years at the time of diagnosis.
2. Diagnosis: histologic verification at either the time of original diagnosis or a previous relapse of high risk neuroblastoma.
3. Disease Status: Neuroblastoma that is in remission
4. Greater than 30 days from completion of cytotoxic and antibody therapy and less than 120 days from previous therapy
5. A negative serum or urine pregnancy test is required for female subjects of child bearing potential (onset of menses or ≥13 years of age).
6. Both male and female post-pubertal study subjects need to agree to use one of the more effective birth control methods during treatment and for six months after treatment is stopped. These methods include total abstinence (no sex), oral contraceptives ("the pill"), an intrauterine device (IUD), levonorgestrol implants (Norplant), or medroxyprogesterone acetate injections (Depo-provera shots). If one of these cannot be used, contraceptive foam with a condom is recommended.
7. ANC > 500/µl and platelet count >50,000/µl
8. Organ Function Requirements: Subjects must have adequate liver function as defined by:
   a. AST and ALT <10x upper limit of normal
   b. Serum bilirubin must be ≤ 2.0 mg/dl
   c. 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	05
1 to < 2 years	0.6	0.6
2 to < 6 year	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

9. Informed Consent: All subjects and/or legal guardians must sign informed written consent. Assent, when appropriate, will be obtained according to institutional guidelines

5.2 ***Exclusion Criteria:***

1. Lansky score < 60%
2. BSA (m^2) of <0.25
3. Investigational Drugs: Subjects who are currently receiving another investigational drug are excluded from participation.
4. Anti-cancer Agents: Subjects who are currently receiving other anticancer agents are not eligible. Subjects must have fully recovered from the effects of prior chemotherapy (hematological and bone marrow suppression effects).
5. Infection: Subjects who have an uncontrolled infection are not eligible until the infection is judged to be well controlled in the opinion of the investigator.
6. Subjects who, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study, or in whom compliance is likely to be suboptimal, should be excluded.

Additional criteria:

Subjects willing to participate in the correlative biologic studies will sign an additional consent form to provide bone marrow and blood for analysis.

- Stratum 1: Subjects that are in remission at the end of upfront therapy defined as chemotherapy (5-7 cycles), surgery as indicated, consolidation therapy as indicated, radiation therapy as indicated, anti-GD2 antibody therapy with retinoic acid up to 6 cycles.

- Stratum 2: Subjects that are in remission after any previous relapse or refractory therapy

6.0 STUDY DRUG ADMINISTRATION

6.1 DFMO

6.1.1 Description, Formulation, and Storage of DFMO

DFMO (eflornithine hydrochloride is an inhibitor of ornithine decarboxylase (ODC) designated chemically as 2-(difluoromethyl)-DL-ornithine monohydrochloride monohydrate.

The dosage form to be used in this study are provided as a convex tablet containing 250 mg of eflornithine HCl, monohydrate. The tablets are packaged and sealed in opaque white HDPE bottles, and each bottle contains 100 tablets. The DMFO tablets are supplied by KC Pharma.

The tablets are to be stored at room temperature (20-25^{0}C).

6.1.2 DFMO Dosing Table Per Weight Group:

The following table will be used to determine dispensing of DFMO.

DFMO Dose:

BSA (m2)	Tablets to be Dispensed for each dose	Total Tablets Per Day	Actual Mg/m2
>1.5	Four (4) tablets orally twice a day	Eight (8)	625 and down per dose
0.75 to 1.5	Three (3) tablets orally twice a day	Six (6)	500 to 1000 per dose
0.5 to < 0.75	Two (2) tablets orally twice a day	Four (4)	675 to 1000 per dose
0.25 to < 0.5	One (1) tablet orally twice a day	Two (2)	500 to 1000 per dose
<0.25	Not eligible for trial		

6.1.3 Administration of DFMO

Treatment will be administered on an outpatient basis unless hospitalization is required for another reason.

Each entire dose of DFMO tablets should be swallowed.

Subjects will be advised to maintain a low Polyamine diet during the duration of this study. A handout will be provided to subjects with foods they should avoid while on this study.

Subjects will be given a 'patient instruction sheet' for home administration.

Subjects will be given a 'dosing diary' to keep track of doses given at home.

6.1.4 Subjects Unable to Swallow Tablets

For subjects unable to swallow tablets, DFMO tablets may be chewed or crushed in a teaspoon and administered with a small amount of liquid. Note, tablets may not completely dissolve.

Recommended liquids for mixing (to cover taste)- Lemonade, Apple, Cranberry, Grape, or Pineapple

DFMO may NOT be mixed in high polyamine juices such as orange or grapefruit juice.

Crushed DFMO tablets may also be mixed in 1-2 tablespoons of chocolate syrup or apple sauce. The DFMO tablets will not dissolve, and this is acceptable. This technique is simply to mask the flavor.

6.1.5 Missed Doses

If a subject vomits or misses a dose they should skip the missed dose and continue the drug at the next dose. Do not make up missed doses.

6.1.6 DFMO Clinical Pharmacology

DFMO (Eflornithine hydrochloride) is a member of the following drug classes: 1) inhibitor of ornithine decarboxylase (ODC), 2) hirsutism (excess hair growth) retardant, and 3) antiprotozoals. Eflornithine is FDA approved as a cream for treatment of female hirsutism, and in intravenous form for treatment of trypanosomiasis. The oral tablet form is not available outside of the clinical trial setting in the U.S., and the formulation used in this trial is similar to that used in the Phase III colon adenoma clinical trial in combination with sulindac (Meyskens *et al.,* 2008).

Contraindications: Prior hypersensitivity to eflornithine.

Common side effects:

Overall, the most frequently reported AEs in NCI, DCP-sponsored chemoprevention studies were diarrhea (9.0%), headache (7.5%), nausea (6.5%), hearing loss (5.6%), tinnitus (4.3%) and asthenia (4.7%). Other common toxicities (each accounting for 2 to 3% of all AEs reported) were epigastric pain, flatulence, dyspepsia, anemia, dizziness and skin rash. Less common toxicities (each accounting for 1 to 2% of all AEs reported) were: stomatitis, rhinitis, insomnia, infections, vomiting, vasodilation, dry mouth, constipation, dry skin, menstrual disorders, pharyngitis, emotional lability, pruritis, myalgia, and pain (miscellaneous). The most significant adverse effects associated with clinical administration of DFMO in chemoprevention trials are loss of hearing acuity and tinnitus. These effects have generally been found to be reversible when DFMO treatment is stopped. Patients who receive a cumulative oral dose of DFMO below 150 g/m2 experience minimal ototoxicity.

Infrequent side effects: Hearing loss/change by audiometry testing has been reported in 8.4% of patients on high dose eflornithine. Rash and alopecia have been reported in 3% of patients. Anorexia and abdominal pain have been reported in 2% of patients treated with eflornithine.

Rare but serious side effects include dizziness (1%), headaches (2%), and seizures (8%), have been reported in patients on intravenous eflornithine. Myelosuppression (including leukopenia, [37%], anemia [55%], and thrombocytopenia [14%]) has been reported at high intravenous doses, but does not usually occur at the low dose (500 mg) utilized in this study.

Pregnancy and Lactation: Pregnancy category C. It is unknown if eflornithine crosses the placenta. Case reports in humans along with animal studies (mice, rats) indicate potential for fetotoxicity. Experiments in rodents indicate that eflornithine blocks yolk sac formation and trophoblast differentiation, affecting processes such as vasculogenesis and steriodgnesis (Lopez-

Garcia, *et al.*, 2008). The World Health Organization has not determined a breast feeding rating for eflornithine due to insufficient data. The Thompson lactation rating is that infant risk cannot be ruled out. No studies investigating the safety of lactation after eflornithine administration have been conducted, nor are there data to determine drug levels in breast milk after drug administration.

Toxicity for DFMO Potential Risk:

Likely Happens to 10-30 patients out of every 100	**Less Likely** Happens to 3-10 patients out of every 100	**Rare** Happens to fewer than 3 patients out of every 100

• Fewer red and white blood cells
  ○ a low number of red blood cells can make you feel tired and weak and may require transfusion.
  ○ a low number of white blood cells can make it easier to get infections
• Decrease in the number of platelets made in the bone marrow
	• Nausea
	• Hearing Loss
	• Ringing in ears
	• Diarrhea
	• Headache
	• Weakness
		• Loss of appetite
		• Abdominal Pain
		• Flatulence (gas)
		• Dizziness
		•
		• Skin Rash
		• Seizures
		• Sores in the mouth
		• Runny nose
		• Difficulty sleeping
		• Infections
		• Dry mouth
		• Constipation
		• Dry skin
		• Menstrual disorders
		• Sore throat
		• Vomiting
		• Vasodilation (the relaxation of blood vessels possibly causing low blood pressure)
		• Emotional ups and downs
		• Itchiness
		• Body aches
		• Pain

Four particular areas of concern have been identified with regard to the safety of subjects participating in this study and an attempt to address each of them is outlined below: The main considerations are: thrombocytopenia, hearing loss, gastrointestinal and non-G.I. side effects.

a) DFMO Specific Adverse Events

(1) Hearing loss.

Although hearing loss has been a problem at higher doses (see below), clinical changes in hearing have been uncommon (one of 123 subjects in the phase IIb trial) and reversible in the doses proposed for this trial (62). An extended analysis of these observations is in press (65). There was no statistically significant shifts in distortion product otoacoustic emission levels. For

auditory pure tone thresholds, there was a subtle 2-3 dB decrease in hearing sensitivity for the two higher DFMO doses (0.2 and 0.4 gm/M^2/day), but only for the two lowest frequencies at 250 and 500 HZ. However in two phase I trials using lower doses of DFMO, done by other investigators, no audiometric changes were seen after approximately 6 months of DFMO therapy at 0.50Gm/m^2/d (total dose 90Gm) although some changes were seen at higher doses (79). Hearing loss may occur in association with DFMO administration at high doses. In a meta-analysis of previous studies, (64), it was reported that less than 10% of the subjects who received cumulative doses below 150 Gm/m^2 developed a demonstrable hearing deficit, while hearing losses were observed in up to 75% of subjects who received cumulative doses above 250 Gm/m^2. This side effect was thought to be totally reversible upon drug discontinuation. Some study participants taking DFMO at doses similar to those used in this study have experienced mild decreases in hearing soft sounds. These changes have been uncommon and usually subclinical (that is, noticeable on special hearing tests called audiograms, but not in normal conversation or daily activities). Although an affected participant's level of hearing usually returns to its usual state when drug is stopped, in a small percentage of cases (occurring in less than 5% of participants taking the drug) effects have persisted even after drug was stopped. These changes are most likely not reversible in all subjects. However, in a recently completed phase IIb trial of DFMO audiologic changes that were clinically significant were not detected, even in the highest dose group (0.4Gm/m^2/day) (extended analysis, 65). It is probable that at low doses of DFMO ongoing recovery of inner ear polyamines occurs and clinical hearing loss will be rarely, if ever, seen. Since hearing loss is usually totally reversible after drug discontinuation, this approach appears safe and cost effective.

(2) Thrombocytopenia (low platelets)

Thrombocytopenia has been reported predominantly in studies using "therapeutic" doses of DFMO (>3Gm/m^2/day) and primarily in cancer patients who had previously undergone chemotherapy or patients with compromised bone marrow. Other side effects ascribed to DFMO have been rare and, to date, seen only at high doses.

(3) Other

Skin rash, anemia, and neutropenia have also been seen with DFMO administration

(4) GI

Abdominal pain, loss of appetite, diarrhea have been reported.

6.2 Guidelines for Dose Modifications

Toxicities and dose modifications (refer to section 6.3) will be monitored in all cycles. Adjustments to the doses of study drug will be based upon toxicity, graded according to the NCI Common Toxicity Criteria (CTC), Version 4.0, if these were normal at baseline. Events that are not described in the NCI criteria will be assigned grades according to the criteria provided in Section 8.0. Criteria for determining the relatedness of clinical adverse events to treatment (Section 8.0) should be utilized to determine the relationship of adverse events to the treatment.

6.3 Dose Modification

Patients experiencing any toxicity specified below attributable to DFMO or any intolerable toxicity will have their dose of DFMO held until toxicities have reverted to ≤Grade 2 toxicity (with the exceptions below for neutropenia, thrombocytopenia and transaminases). Upon resolution of the toxicity, subjects will receive a dose reduction of DFMO to one step down on the dosing table in section 6.1.2 (one less tablet per dose than their current dose). Subjects that are currently only taking one tablet per dose BID will be dose reduced to one tablet per day (QD). Subjects will be allowed to dose reduce for subsequent toxicities defined here as many times as they can until they reach the one tablet per day (QD) dosing. At that point if they experience another dose reducing toxicity (as defined here) they will be required to go off protocol therapy.

- Grade 4 neutropenia or thrombocytopenia that persists for 7 days or longer after holding study drug. (DFMO should be held as soon as a Grade 4 related neutropenia is discovered. Subject will only dose reduced if toxicity persists at a Grade 4 for longer than 7 days after the hold starts. If subject resolves before 7 days of hold they will resume at previous dose. If subject does not resolve after 14 days of a hold they will be discontinued from study).
- >10 X elevation of transaminases that persists for 7 days or longer after holding study drug. (DFMO should be held as soon as a 10X related elevation is discovered. Subject will only dose reduced if toxicity persists at >10X ULN or higher for longer than 7 days after the hold starts. If subject resolves before 7 days of hold they will resume at previous dose. If subject does not resolve to <10x ULN after 14 days of a hold they will be discontinued from study).
- Any other Grade 3 non-hematologic toxicity, excluding alopecia, nausea, vomiting, and diarrhea that does not adequately respond to treatment.

If there is no resolution of above toxicity by 14 days, DFMO should be discontinued, and subjects should be discontinued from the study.

6.4 Study Drug Accountability

Study drug must only be used for subjects enrolled in the trial. The investigational site staff must maintain a careful inventory of study drug. Drugs will be distributed to investigational site staff and patients using FDA guidelines for distribution of investigational agents. Study drug use will be recorded on a Study Drug Inventory Form. This form will contain the following information:

- Subject number and initials for each subject receiving study drug
- Date and quantity of DFMO received by the site
- Date and quantity of DFMO dispensed
- Date and quantity of DFMO returned

At each monitoring visit, the clinical monitor will reconcile with the actual inventory of study drug at each site.

6.5 Concomitant Medications and Treatments

All intercurrent medical conditions will be treated at the discretion of the Investigator according to acceptable community standards of medical care. All concomitant medications and treatments will be documented on the appropriate case report form.
The following medications are not permitted during the trial:

- Any cytotoxic chemotherapy
- Any other investigational treatment
- Any other systemic anti-neoplastic therapy including, but not limited to, immunotherapy, hormonal therapy, targeted therapies, anti-angiogenic therapies, or monoclonal antibody therapy
- Any radiotherapy, including systemically administered radioisotopes, unless administered with palliative intent

Erythropoietin, blood products, anti-emetics, steroids, and transfusions may be administered at the discretion of the Investigator based on established criteria

7.0 STUDY PROCEDURES AND ASSESSMENTS

7.1 Enrollment of Patients

Prior to consent of the subject, the research coordinator at the lead site will be contacted (via e-mail). This coordinator will then reply with study space availability. If the subject fits all enrollment criteria, the site will again contact the coordinator at the lead site for official enrollment confirmation and unique subject identifier assignment. In addition, a study enrollment form will be faxed to the coordinator at the lead site. This determination will be made based on subject data (subject qualifies for study) and current subjects enrolled in trial (i.e. how many subjects are currently enrolled). A subject may NOT be enrolled on trial until official approval from the lead site is received.

All subjects (or patients' legal representatives) must provide written informed consent before any study specific assessments may be performed.

7.2 Screening

The Investigator is responsible for keeping a record of all subjects screened for entry into the study and subsequently excluded.

The following screening procedures must be performed within 14 days prior to the first dose of study drug (7 days extra may be requested from the study chair in exceptional cases). Studies must be done *after* last previous treatment for malignancy:

1. Signed informed consent form. All subjects (or patients' legal representatives) must provide written informed consent before any study specific assessments may be performed. Signed informed consent form for voluntary participation in correlative biologic analysis will also be obtained.
2. CT or MRI to confirm remission status
3. MIBG scan to confirm remission status. Consider PET scan for non MIBG avid subjects.
4. Audiogram
5. Bone marrow aspirate and biopsy
6. Additional Optional Bone Marrow-for subjects with additional informed consent bone marrow samples for biological correlatives per section 12.0)

The following screening procedures must be performed up to 5 days prior to the first dose of study drug.

1. Complete medical and surgical history, including documentation of the histologic evidence of malignancy and prior treatments for cancer. Include all other pertinent medical conditions and a careful history of all prior medical treatments;
2. Demographics;
3. Physical examination (including height and weight), noting all abnormalities including baseline dermatologic and neurologic exam,
4. BSA calculation (from body weight and height);
5. Vital signs, including temperature, pulse rate, and blood pressure
6. ECOG Performance status/Lansky Play status (Appendix I);
7. CBC with differential;
8. Serum electrolytes, blood urea nitrogen (BUN), creatinine, bilirubin, LDH, ALT, AST and ferritin;
9. C-reactive protein (CRP*)* and Erythrocyte sedimentation rate *(*ESR*)*
10. Urine for Vanillylmandelic Acid (VMA) & Homovanillic Acid (HVA)
11. Urine or serum pregnancy test for female subjects of child bearing potential (onset of menses or ≥13 years of age);
12. Concomitant medications/therapies including documentation of steroid use and dose;
13. Confirmation of inclusion and exclusion requirements
14. Urine for Biological Correlates per section 12.0

Following completion of all required screening procedures and certification of all inclusion and exclusion criteria by the Investigator, the lead site will be contacted (via e-mail or phone call), at

which time the subject will be enrolled in the trial and a unique subject number assigned. The lead site will act as the central coordinating body. The subject may not start on study until the lead site has provided official approval of enrollment.

7.3 Treatment Phase – Cycle 1

The first cycle will be 28 days in duration. The following procedures must be completed:

Cycle 1 Day 1 (may be performed up to 5 days <u>prior</u> to DFMO administration unless otherwise indicated)

1. Medical History and Physical examination (including body weight), including documentation of an update of all previous abnormalities, any new abnormalities.
2. Vital signs, including temperature, pulse rate, blood pressure (sitting) (to be done on Cycle 1 Day 1);
3. Review and recording of concomitant medications; (to be done on Cycle 1 Day 1)
4. Monitoring and documentation of all AEs and review of concurrent illnesses (to be done on Cycle 1 Day 1)
5. Urine for Biological Correlates per section 12.0 (to be done on Cycle 1 Day 1 in addition to screening sample that was sent in section 7.2)
6. Optional: Blood for Biological Correlates per section 12.0 (additional consent required)
7. Optional: Blood for Biological Correlates (Circulating Tumor Cells to Houston, TX) per section 12.0 (additional consent required).
8. Dispense drug dosing diary

Cycle 1 Day 15 (+/- 3 day window)

Subjects will return to the clinic on Cycle 1 Day 15 for evaluations. The following evaluations will be conducted:

1. History and Physical exam
2. Vital signs, including temperature, pulse rate, and blood pressure (sitting)
3. CBC with differential;
4. Serum electrolytes, BUN, creatinine, bilirubin, ALT, AST; and LDH
5. Review and recording of concomitant medications;
6. Monitoring and documentation of all AEs and review of concurrent illnesses
7. Urine for Biological Correlates per section 12.0
8. Optional: Blood for Biological Correlates per section 12.0 (additional consent required)

7.4 Treatment Phase – Cycles 2-12

All Cycles will be 28 days in duration without interruption. The following procedures must be completed:

Cycle 2-12 Day 1

The following procedures may be performed up to +/- 5 days from Day 1:

1. Medical History and Physical examination (including body weight), including documentation of an update of all previous abnormalities, any new abnormalities.
2. Vital signs, including temperature, pulse rate, blood pressure (sitting);
3. Review and recording of concomitant medications;
4. Monitoring and documentation of all AEs and review of concurrent illnesses
5. BSA calculation (from body weight and height);
6. ECOG Performance status/Lansky Play status (Appendix I);
7. CBC with differential;
8. Serum electrolytes, blood urea nitrogen (BUN), creatinine, bilirubin, LDH, ALT, and AST;
9. C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR)
10. Urine for Vanillylmandelic Acid (VMA) & Homovanillic Acid (HVA)
11. Urine for Biological Correlates per section 12.0
12. Optional: Blood for Biological Correlates per section 12.0 (additional consent required)
13. Cycles 3, 6, 9, and 12- Optional: Blood for Biological Correlates (Circulating Tumor Cells to Houston, TX) per section 12.0 (additional consent required).
14. Collection of previous cycle drug dosing diary and dispensing of new drug dosing diary
15. Urine or serum pregnancy test for female subjects of child bearing potential (onset of menses or ≥13 years of age);

7.5 Treatment Phase – Cycles 13-27

All Cycles will be 28 days in duration without interruption. The following procedures must be completed:

Cycle 13-27 Day 1

The following procedures may be performed up to +/- 5 days from Day 1:

1. Medical History and Physical examination (including body weight), including documentation of an update of all previous abnormalities, any new abnormalities.
2. Vital signs, including temperature, pulse rate, blood pressure (sitting);
3. Review and recording of concomitant medications;
4. Monitoring and documentation of all AEs and review of concurrent illnesses
5. BSA calculation (from body weight and height);

6. ECOG Performance status/Lansky Play status (Appendix I);
7. Urine for Vanillylmandelic Acid (VMA) & Homovanillic Acid (HVA)
8. Urine for Biological Correlates per section 12.0
9. Urine or serum pregnancy test for female subjects of child bearing potential (onset of menses or ≥13 years of age);
10. Collection of previous cycle drug dosing diary and dispensing of new drug dosing diary

Additionally at the start of cycles 14*, 17, 21*, 24, and at off therapy (end of cycle 27)

Windows- The following should be done within +/-5 days of the start of specified cycle. For off-therapy/end of cycle 27 they should be done within 30 days (+7) after stopping study drug:

1. CBC with differential;
2. Serum electrolytes, blood urea nitrogen (BUN), creatinine, bilirubin, LDH, ALT, and AST;
3. C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR)
4. Optional: Blood for Biological Correlates per section 12.0 (additional consent required)
5. Optional: Blood for Biological Correlates (Circulating Tumor Cells to Houston, TX) per section 12.0 (additional consent required).

* Start of cycles 14 and 21 bloodwork stated above can be done at the same time as the IV is placed for the scans done at the end of cycles 13 and 20 in order to reduce the amount of venipuncture the subject experiences.

End of Cycles 3, 6, 9, 13, 20 and off therapy (cycle 27) – scans may be done more often per institutional standards.

Windows- Scans should be done within +/-14 days of the start of next cycle. Off therapy/end of cycle 27 scans should be done within 30 days (+7) after stopping study drug.

1. MIBG scan (for MIBG avid subjects only). Consider PET scan for non MIBG avid subjects.
2. CT/MRI (use same radiologic method as baseline)
3. Bone marrow biopsy and aspirate is to be performed if the treating physician has concerns for progression (If bone marrow testing is being performed and the subject has signed informed consent for the use of tissue in the correlative biologic study as a post-treatment sample please send additional samples per section 12.0)

For patients in first remission who require evaluations for previous front line treatments that are different than the timing of the above schedule, contact the study chair for approval to accept the subjects previously scheduled evaluations instead of repeating the above studies.

End of Cycles 6, 12, and 27

Windows- The following should be done within +/-14 days of the start of the next cycle. For off-therapy/end of cycle 27 it should be done within 30 days (+7) after stopping study drug.

1. Audiogram

(Audiogram should also be done at any time point for any suspected hearing loss)

7.6 PK's for Currently Enrolled Subjects switching to new Formulation DFMO (approximately 10 subjects):

1. **Last Day of Old DFMO Formulation:** Blood for Pharmacokinetic sampling will be done on last day of taking old formulation*. Subject should be instructed to hold their morning dose of old DFMO. Pre-dose PK sample will be collected, and then subject will take old DFMO in clinic. In total, Samples will be collected pre-dose, (Time 0), as well as at 30min, 1 hour, 3 hours, and 6 hours post-dose (exact time should be noted on CRF). Plasma will be separated and frozen at –80°C until analysis per section 12.1.

   * Change from old formulation to new formulation may take place on any day of any cycle after subject consents to new formulation and does not need to be on day 1 of a cycle.

2. Start new Formulation of DFMO- Subject should start new formulation at second dose of this day in clinic once all PK's are drawn, this will be Day 1 of new drug formulation.
3. **One day during Days 2-5 of taking on new formulation.** Blood for Pharmacokinetic sampling on one day during days 2-5 of taking the new formulation. Subject should be instructed to hold their morning dose of new DFMO. Pre-dose PK sample will be collected, and then subject will take new DFMO in clinic. In total, Samples will be collected pre-dose, (Time 0), as well as at 30min, 1 hour, 3 hours, and 6 hours post-dose (exact time should be noted on CRF). Plasma will be separated and frozen at –80°C until analysis per section 12.1.

7.7 Other Schedules:

1. Additional imaging or assessments may be done if clinically indicated. Type of imaging, type of assessment and timing should be recorded as well as reason for imaging and/or assessment.
2. Survival will be monitored on an ongoing basis during the study, then every 6 months from the time the subject is off-treatment for a period of 2 years, then yearly for up to five years off therapy.

7.8 Protocol Treatment Completion

Subjects who receive 27 total 28-day treatment cycles will be considered as having completed protocol (total of 756 days).

7.9 Off-Therapy / 30 Day Follow-up Visit

Subjects will return to the clinic 30 (+7) days after the last dose of DFMO, and the following evaluations will be conducted:

1. Medical History and Physical examination (including body weight), including documentation of an update of all previous abnormalities, any new abnormalities, and a detailed neurological exam;
2. ECOG Performance status/Lansky Play status (Appendix I);
3. Vital signs, including temperature, pulse rate, blood pressure (sitting);
4. Review and recording of concomitant medications;
5. Monitoring of AEs and review of concurrent illnesses
6. Collect previous cycles drug dosing diaries (if not collected already)

Any subject with a suspected study drug-related toxicity at the follow-up visit must be followed until all current adverse events have resolved to baseline or ≤ grade 2. This may require additional clinical assessments and laboratory tests. The follow-up results will be recorded on the appropriate page of the CRF, as well as in the subject's source documentation. Subjects that have started a new anti-cancer treatment since going off DFMO will be censored from any further AE collection at the date of starting the new therapy.

7.10 Survival Follow-up

Subjects will be followed for long term survival by contact with parent or treating institution to confirm survival at the following time points (time from last dose of study drug):

- 6 months
- 1 year
- 18 months
- 2 years
- Yearly after (up to 5 years total)

Follow up will continue for five (5) years off-therapy or until subject death or subject is lost to follow up (per definition in section 9.0). The follow-up results including follow-up status, remission status, and date of death will be recorded on the appropriate page of the CRF, as well as in the subject's source documentation.

8.0 Adverse Event Reporting

8.1 Definitions

8.1.1 Adverse Event

An ***adverse event*** is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An untoward medical event which occurs outside the period of follow-up as defined in the protocol will not be considered an adverse event unless related to study drug. Worsening of a medical condition for which the efficacy of the study drug is being evaluated will not be considered an adverse event.

8.1.2 Unexpected Adverse Event

An ***unexpected adverse event*** is one for which the nature or severity of the event is not consistent with the applicable product information, e.g., the investigator's brochure.

8.1.3 Serious Adverse Event

A ***serious adverse event*** is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Other important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

The term "severe" is often used to describe the intensity (severity) of an event; the event itself may be of relatively minor medical significance (such as a severe headache). This is not the same as "serious", which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning.

8.2 Documenting Adverse Events

The Investigator should elicit information regarding the occurrence of adverse events through open-ended questioning of the patient, physical examination and review of laboratory results.

All Grade 2 or higher (per CTCAE version 4.0) adverse events, whether serious or not, will be described in the source documents and the adverse event page of the case report form. All new events (Grade 2 or higher), as well as those that worsen in intensity or frequency relative to baseline, which occur after administration of study drug through the period of protocol-specified follow-up, must be captured.

Information to be reported in the description of each adverse event includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded)
- The date of onset of the event
- The date of resolution of the event and whether the event is serious or not
- Action taken; drug treatment required; non-drug treatment required; hospitalization or prolongation of hospitalization required; diagnostic procedure performed; patient discontinued from the study
- Outcome: complete recovery or return to baseline; unknown/lost to follow-up; adverse event persisting; patient died (notify lead site immediately, and complete the Serious Adverse Event page and the Final Visit section of the case report form)

Adverse events, regardless of suspected cause, will be collected for 30 days following the last dose of DFMO and until all current adverse events have resolved to baseline or ≤ grade 2.

All adverse events will be reviewed by the safety officer.

8.3 Expedited Reporting of Adverse Events

All fatal or life–threatening adverse events must be reported to the lead site immediately by telephone, fax, or e-mail within 24 hours of discovery as well as to the appropriate regulatory authorities (local IRB and FDA if required and KC Pharma as appropriate.). The lead site will then report directly to the safety officer. If full information is not known, additional follow-up by the Investigator will be required.

All other serious adverse events must be reported to the lead site by telephone, fax, or e-mail within 5 days as well as to the appropriate regulatory authorities (local IRB and FDA and KC Pharma as required and appropriate). Sponsor will aid site in determining FDA reporting requirements for any SAE. All medwatches must be submitted by the sponsor holding the IND and should not be submitted locally by the participating site to the FDA.

The Investigator must report all serious adverse events reported to regulatory authorities in an expedited manner to the local IRB or IEC. All serious adverse events must be followed until resolution or stabilization.

8.4 Grading and Relatedness of Adverse Events

8.4.1 Grading of Severity of an Adverse Event

Each adverse event (Grade 2 or higher) will be graded for severity per the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE V 4.0), and these criteria must be used in grading the severity of adverse events. The criteria can be found at: http://ctep.cancer.gov/reporting/ctc.html.

Grading of Severity of an Adverse Event Not Listed in Published Criteria:
For those adverse events which are not listed as part of the NCI CTCAE V 4.0, the same grading system should be used, where:

- **Mild** corresponds to an event not resulting in disability or incapacity and which resolves without intervention
- **Moderate** corresponds to an event not resulting in disability or incapacity but which requires intervention
- **Severe** corresponds to an event resulting in temporary disability or incapacity and which requires intervention
- **Life-threatening** corresponds to an event in which the patient was at risk of death at the time of the event
- **Fatal** corresponds to an event that results in the death of the patient

8.5 Relatedness to Study Drug

The Investigator must attempt to determine if an adverse event is in some way related to the use of the study drug and define an attribution category. This relationship should be described as follows:

RELATIONSHIP	ATTRIBUTION	DESCRIPTION
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly **NOT** related* to the intervention. The event is clearly due to causes distinct from the use of the study drug, such as a documented pre-existing condition, the effect of a concomitant medication, or a new condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unrelated to the use of the study drug.
	Unlikely	The AE *is **doubtfully related*** to the intervention. Adverse event does not have temporal relationship to intervention, could readily have been produced by the subject's clinical state, could have been due to environmental or other interventions, does not follow known pattern of response to intervention, does not reappear or worsen with reintroduction of intervention.
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention. The event follows a reasonable temporal sequence from administration of the study drug and the event follows a known response pattern to the study drug BUT the event could have been produced by an intercurrent medical condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug OR the event could be the effect of a concomitant medication.
	Probable	The AE ***is likely related*** to the intervention. The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug AND the event cannot have been reasonably explained by an intercurrent medical condition OR the event cannot be the effect of a concomitant medication.
	Definite	The AE ***is clearly related*** to the intervention. The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug and based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug. The adverse event improves upon discontinuation of the study drug and reappears upon repeat exposure.

9.0 Patient Withdrawal and Trial Discontinuation

9.1 Criteria for Subject Off-Therapy

Subjects may be withdrawn from the study treatment for the following reasons:

- Subject completes all protocol defined therapy- 27 cycles
- Relapsed neoplastic disease
- Subject or guardian withdraws consent to continue study drug
- Subject develops an intercurrent illness that precludes further participation, or requires a prohibited concomitant treatment
- The Investigator withdraws the subject in the subject's best interests
- Subject is lost to follow-up (defined as the inability to contact the subject on 3 separate occasions over a period of 2 weeks)
- Administrative reasons (e.g., the subject is transferred to hospice care)
- An adverse event, which in the opinion of the Investigator, precludes further trial participation or fulfills the protocol requirements for withdrawal (e.g., the development of dose limiting toxicity despite a reduction in protocol therapy for a previous episode of dose limiting toxicity)
- Death

9.2 Criteria for Subject Off-Study

Subjects may be withdrawn from the study completely which includes survival follow-up for the following reasons:

- Subject or guardian withdraws consent to continue in the trial
- Subject is lost to follow-up (defined as the inability to contact the subject on 3 separate occasions over a period of 2 weeks)
- Subject completes all protocol defined therapy including all follow-up time points.
- Death

9.3 Trial Discontinuation

The lead site may discontinue the trial as a whole or at an individual investigational site at any time. Reasons for early trial discontinuation may include, but are not limited to, unacceptable toxicity of study drug, a request to discontinue the trial from a regulatory authority, protocol violations at an investigational site, violations of good clinical practice at an investigational site, or poor enrollment. The lead site will promptly inform all Investigators in the event of premature study discontinuation and provide all Investigators with instructions regarding the disposition of subjects still on study. Should the study be terminated prematurely, all unused study drug, case report forms and any other study material will be returned to the lead site.

10.0 DATA ANALYSIS

10.1 Data Quality Assurance

Electronic case report forms (CRF) will be checked for correctness against source document data by the monitor. If any entries into the CRF are incorrect, incomplete or illegible, the monitor will ask the Investigator or the study site staff to make appropriate corrections.

10.2 Statistical Analysis

This is an open label, single agent, multicenter, study for patients with neuroblastoma that are in remission.

The following data sets will be used in this study:

- All enrolled and eligible subjects (ITT) population: All eligible subjects who have a signed informed consent form.
- All treated and eligible subjects (Safety evaluable) population: All subjects who received at least one dose of study drug
- All eligible subjects treated to first evaluable time point with evaluation completed (generally 3 cycles) (as Treatment Efficacy) population, unless subjects have reached the study endpoint of progression of disease at an earlier time point.

Efficacy analyses will be performed on the Treatment evaluable population. Safety analysis will be performed on the Safety and Efficacy evaluable population.

10.3 All baseline patient characteristics will be summarized in a tabular format. Safety data will be described for all subjects receiving at least one dose of DFMO. Safety data will include values for hematology, serum chemistry, vital signs, and adverse events. The proportion of subjects experiencing adverse events, serious adverse events, dose limiting toxicities and treatment delays will be summarized. Enrollment to study will not pause at interim analysis time points.

Sample Size and Analysis:

Stratum 1:

A 70% 2-year EFS rate was selected as the Phase I baseline rate. The baseline 70% EFS rate at 2-years was based upon published data by Yu et al. (82). It is hypothesized that DFMO will increase the 2-year EFS rate to 80% which represents an approximately 10% increase in the duration of the EFS rate at 2-years. Assuming a directional 5% one population binomial test, a sample size of n = 127 patients will be required to achieve an 80% power to detect this 2-year difference in EFS (70% vs 80%).

The comparison of the biomarker prevalence rates assumes that the Stratum 1 cohort will experience a relapse rate of 30% and thus a 70% non-relapse rate at up to two years of follow-up. It is also assumed that the corresponding prevalence of an elevated biomarker will be 60% for the relapse group and 30% in the non-relapse group, respectively. This hypothesized prevalence difference will be detectable with 85% power with an overall sample size of N=127 patients (38 relapse and 89 non-relapse) using a Fisher's Exact Test and a non-directional Type I Error level of 5%.

The sequential biomarker data over the course of treatment for each patient will be classified as elevated or not based upon the following *a priori* study criteria and blinded to patient progression status. Elevation of the biomarker is defined as diacetylspermine levels > 500nmol/gm creatinine on 2 consecutive urine levels. The biomarker prevalence rates for the patients who progress (estimated to be n= 38) and those who do not progress (estimated n=89) over the two years of the trial treatment will be compared using a Fisher's exact test of the equality of the two EFS prevalence rates implemented with a 5% two-tailed Type I error level. A 95% exact confidence interval for the absolute difference in prevalence rates along with a 95% exact confidence interval for the odds ratio for the resulting prevalence rates will be obtained to complement the formal hypothesis testing. Since OS will also be a secondary outcome for comparing the utility of the biomarker prevalence, time-to-event analyses will also be implemented using the Kaplan-Meier approach initially using the *a priori* biomarker definition. These initial time-to-event analyses will then be supplemented with a Cox proportional hazard modeling effort using the quantitative biomarker assessment to allow for an exploratory analysis of other cut-point definitions that can be used to contrast with that of the original *a priori* definition. Potential confounding clinical measures that are identified for the recurrent and non-recurrent patients will be incorporated in a limited fashion using Cox proportional hazard models that also include the biomarker effect. This exploration of confounders will need to be limited due to the anticipated small sample sizes. Data from this stratum will guide the statistical design for a Phase II study in this patient population.

Stratum 2:

Based upon tabled median EFS times all patients presented by Santana et al. (Cancer, June 15, 2008) for first and second recurrence times, a median first recurrence time of 8.7 months is equivalent to a 14.8% EFS at two years, and the median time to a subsequent second recurrence of 3.8 months is equivalent to an EFS rate of 1.3% at two years assuming an exponential time-to-event model. Assuming that two thirds of the relapse patient population will have had only a single relapse and that the other third of the patients will have had two or more relapses, then a weighted average of these two EFS rates (14.8% and 1.3%) equals 10.3%. A 10% historical EFS rate for two years was assumed for simplicity. Examination of an increase in EFS will require a sample size of n = 33 subjects in Stratum 2 to test whether treatment with DFMO can prolong the overall estimated EFS at two years to 30% from the 10% two years estimate for the above historical data in this patient population. The sample size is based upon a one sample binomial test of proportions with a power 80% and a 5% two -tailed Type I error level.

An observation of 7 or more patients with at least a two year EFS rate out of the n = 33 patients will result in the rejection of the null hypothesis of a 10% EFS in favor of the alternative of a 30% two year EFS rate. An exact binomial 95% confidence interval for the two year estimated EFS will complement the prior formal two-stage hypothesis test. Since the actual EFS time-to-event data is derived from the times to recurrence, a Kaplan-Meier analysis of the time-to-event data analysis will be conducted, and the median time to recurrence and a 95% confidence for this value will be obtained to supplement this EFS point estimate. Since this group of Stratum 2 patients will consist of patients with prior single and multiple recurrence histories, EFS and time to event data will be examined by prior recurrence histories. In particular, the impact of prior recurrence history on the time to recurrence will be explored using Kaplan-Meier analysis and Cox proportional hazard models. The response to DFMO therapy and subsequent recurrences will also be explored in combination with prior recurrence histories using Cox models.

10.4 Event Free Survival

The event free survival (EFS) is defined as the period from the first day of administration of study drug until the first occurrence of relapse, progressive disease, secondary cancer, or death or, if none of these events occurred, until the last contact with the subject.

Progression is defined as the appearance of any new lesions >10mm on CT/MRI, any new lesions on MIBG or new disease present in the bone marrow.

10.5 Overall Survival

Overall Survival (OS) will be defined as the first day of administration of study drug until death or will be censored at the last contact with the subject if death did not occur during the study. Overall survival will be monitored on an ongoing basis during the study, then every 6 months from the time the subject is off-study treatment up to a period of 2 years, then yearly for up to five years total.

11.0 ADMINISTRATIVE PROCEDURES

11.1 Patient Informed Consent

No study related procedures will be performed until a patient or a patient's legal representative has given written informed consent. The lead site will provide to the site Investigators a sample informed consent document that conforms to all the requirements for informed consent according to ICH GCP and US FDA guidelines (21 CFR 50). However, it is up to each site Investigator to provide a final informed consent that may include additional elements required by the Investigator's institution or local regulatory authorities. The IRB/EC for each investigational site must approve the consent form document prior to study activation; changes to the consent form during the course of the study may also require IRB/EC approval. The informed consent document must clearly describe the potential risks and benefits of the trial, and each prospective participant must be given adequate time to discuss the trial with the Investigator or site staff and to decide whether or not to participate. Each patient who agrees to participate in the trial and

who signs the informed consent will be given a copy of the signed dated and witnessed document. The original copy of the signed, dated, and witnessed informed consent document will be retained by the Investigator in the study files.

The Investigator must also obtain authorization from the patient to use and/or disclose protected health information in compliance with the Health Insurance Portability and Accountability Act (HIPAA). Written HIPAA authorization may be obtained as part of the informed consent process.

11.2 Ethical Conduct of the Study and IRB/IEC Approval

The study will be conducted according to the principles of the 2004 version of the Declaration of Helsinki, the International Conference on Harmonization Guidance on Good Clinical Practice and the requirements of all local regulatory authorities regarding the conduct of clinical trials and the protection of human subjects.

The Investigator will submit the protocol, the Investigator's Brochure, the informed consent and any other material used to inform patients about the trial to the local IRB/IEC for approval prior to enrolling any patient into the trial. The IRB/IEC should be duly constituted according to applicable regulatory requirements. Approval must be in the form of a letter signed by the Chairperson of the IRB/IEC or the Chairperson's designee, must be on IRB/IEC stationary and must include the protocol by name and/or designated number. If an Investigator is a member of the IRB/IEC, the approval letter must stipulate that the Investigator did not participate in the final vote, although the Investigator may participate in the discussion of the trial. The Investigator will also inform the IRB/IEC of any serious adverse events that are reported to regulatory authorities and will provide to the IRB/IEC a final summary of the results of the trial at the conclusion of the trial.

Any amendments to the protocol will be done at the lead site, and will be submitted to the IRB/IEC for review and written approval before implementation. These approved changes will then be forwarded to sites for review by their local IRB/IEC. Written approval from sites will be forwarded to the lead site.

11.3 Data Safety Monitoring Board (DSMB)

An independent Data Safety and Monitoring Board (DSMB) will oversee the conduct of the study. The members of this Board will receive database summaries, including adverse event reports, and will convene either in person or via teleconference every 6 months. The Board will be responsible for decisions regarding possible termination and/or early reporting of the study.

11.4 Monitoring and Auditing

A clinical monitor will make regularly scheduled trips to the investigational site to review the progress of the trial as defined in the Monitoring Plan. The actual frequency of monitoring trips will depend on the enrollment rate and performance at each site. At each visit, the monitor will review various aspects of the trial including, but not limited to, screening and enrollment logs; compliance with the protocol and with the principles of Good Clinical Practice; completion of

case report forms; source data verification; study drug accountability and storage; facilities and staff data quality; regulatory documentation; and study integrity. In addition the site may be audited by KC Pharma government inspectors who must be allowed access to CRFs, source documents and other study files. The site must promptly notify the study chair of any inspections scheduled by regulatory authorities, and also forward copies of the inspection reports to the study chair. The study chair will promptly forward this information to KC Pharma.

During scheduled monitoring visits, the Investigator and the investigational site staff must be available to meet with the study monitor in order to discuss the progress of the trial, make necessary corrections to case report form entries, respond to data clarification requests and respond to any other trial-related inquiries of the monitor.

11.5 Pre-Study Documentation

Prior to initiating the trial, the Investigators at each site will provide to the Lead site the following documents:

- A signed FDA Form 1572
- A current curriculum vitae for the Principal Investigator and each sub-investigator listed on the FDA Form 1572
- A copy of the Investigator's medical license from the state in which the study is being conducted
- A letter from the IRB or EC stipulating approval of the protocol, the informed consent document and any other material provided to potential trial participants with information about the trial (e.g., advertisements)
- A copy of the IRB- or EC-approved informed consent document
- Current IRB membership list for IRB's without a multiple project assurance number or an IRB organization number under the Federal Wide Assurance program (www.ohrp.osophs.dhhs.gov).
- A signed Investigator Protocol Agreement
- A completed financial disclosure form for each person listed in box 6 of the FDA form 1572.
- Current laboratory certification for the reference laboratory
- A list of current laboratory normal values for the reference laboratory

11.6 Confidentiality

It is the responsibility of the investigator to insure that the confidentiality of all subjects participating in the trial and all of their medical information is maintained. Case report forms and other documents submitted must never contain the name of a trial participant. Each subject in the trial will be identified by a unique identifier that will be used on all CRF's and any other material submitted to the lead site. All case report forms and any identifying information must be kept in a secure location with access limited to the study staff directly participating in the trial.

The results of the study may be presented in reports, published in scientific journals or presented at medical meetings; however, subject names will never be used in any reports about the study.

11.7 Source Documents

The Investigator will maintain source records separate from the case report forms in the form of clinical charts, medical records, original laboratory, radiology and pathology reports, pharmacy records, etc. The Investigator will document in the clinic chart or medical record the name and number of the trial and the date on which the subject signed informed consent prior to the subject's participation in the trial. Source documents must completely reflect the nature and extent of the patient's medical care, and must be available for source document verification against entries in the case report forms when the monitor visits the investigational site. All information obtained from source documents will be kept in strict confidentiality.

11.8 Record Retention

The Investigator will retain the records of the study for 15 years, or for 2 years following the date that a marketing application for the study drug is approved, or if no marketing application is filed, or if such an application is not approved, for 2 years after the IND has been closed. The lead site will notify Investigators when retention of study records is no longer required. All study records must be maintained in a safe and secure location that allows for timely retrieval, if needed.

Study records that must be retained include copies of case report forms, signed informed consents, correspondence with the IRB or IEC, study drug dispensing and inventory records, source documents, clinic charts, medical records, laboratory results, radiographic reports) and screening/enrollment logs.

Should the Investigator relocate or retire, or should there be any changes in the archival arrangements for the study records, the lead site must be notified. The responsibility for maintaining the study records may be transferred to another suitable individual, but the lead site must be notified of the identity of the individual assuming responsibility for maintaining the study records and the location of their storage.

12.0 Biological Evaluation

A major challenge in the development of cancer therapeutics is an absence of understanding the relationship of the disease to response to therapy, and the ability to predict which subjects are most likely to respond to any particular agent. Emerging technologies including ability to establish primary tumor cells in culture, evaluate pharmacogenomics and explore biomarkers may provide a way to explore relationships between clinical benefit and treatment.

Urine Sample Analysis –

Decarboxylated S-adenosylmethionine (dcSAM) Performed by Dr. Bachmann (section 12.1.1):
Adenine and its derivatives are known to react with 2-chloroacetaldehyde to form highly fluorescent tricyclic derivatives. This reaction gives a sensitive and specific method for measuring dc-SAM in urine and plasma samples. The reaction mixture will be incubated at 40°C overnight. An aliquot of this mixture will be injected onto an Altex Ultrosphere column for chromatographic separation. Detection will be accomplished using a Perkin-Elmer LS-4 spectrofluorometer, as described by others.[77]

Polyamines Performed by Dr. Bachmann (section 12.1.1)
High performance liquid chromatography (HPLC) and other methods will be used, as per previous studies ([4, 77]), to detect putrescine, spermidine, spermine, monoacetylspermidine and monoacetylspermine and diacetylspermine. Samples will be adjusted to 0.2N perchloric acid and analyzed directly. Acid hydrolysis methods will be employed to remove acetyl groups, and thus measure diacetylated amines. The detection level will be 1-10 pmol. Sources of error associated with these measures in colonic tissue have been previously reported ([78]). Urinary creatinine levels will also be determined, using a commercial kit (Oxford Biochemical Research), to normalize urinary polyamines. In the method, picric acid reacts with creatinine and other urinary

components to produce an orange color, which can be quantified spectrophotometrically at 490 nm at alkaline pH. The creatinine reaction degrades rapidly when acidified. The difference in optical density is a direct measure of the creatinine concentration.

Blood and Bone Marrow Samples Analysis

ODC SNP analysis –Performed by Pediatric Oncology Translational Research Laboratory (Section 12.1.2)
The ODC G316A single nucleotide polymorphism (SNP) was associated with polyamine contents in prostate and colorectal mucosal biopsies. The lowest levels of polyamines are found in colorectal mucosal tissues from individuals homozygous for the A allele, with highest levels observed among carriers of the GG-genotype. There is no relationship between ODC G316A allele genotype and colorectal content of histamine, an amine not dependent on ODC for its synthesis. We wish to determine the effects of DFMO treatment on polyamine levels in patients with each ODC genotype. Results from our recent clinical trial suggest that ototoxicity associated with DFMO therapy is restricted to a small fraction of people with the ODC 316AA genotype. These clinical trial results are corroborated by clinical translational studies that are based on molecular epidemiology investigations and have been replicated by three independent groups in humans showing that a polymorphism affecting the expression of ODC, the DFMO target protein, is highly associated with metachronous colon adenomas and sporadic breast cancer. In addition, two independent groups have reported that this same polymorphism is associated with prostate cancer progression and colon cancer survival. In order to develop algorithms predicting who will benefit, and who will have side effects of DFMO treatment, we will determine the ODC G263T and G316A types of all study participants, by analyzing DNA obtained from nucleated blood cells using established methods. We will also assess levels of micro RNAs in serum (Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A. Serum microRNAs are promising novel biomarkers PLoS One. 2008 Sep 5;3(9):e3148) as predictive markers of DFMO effect. We have evidence in both human cancer cells and apparently normal rectal tissue from humans that DFMO modulates cell and tissue contents of specific micro RNAs.

Biomarker Analysis –Performed by Pediatric Oncology Translational Research Laboratory (Section 12.1.2)
Subject biomarkers will be evaluated in blood plasma using antibody array analysis in the laboratory of Dr. Sholler. Antibody arrays will be generated to target biomarker candidates derived from gene expression, proteomic, and glycomic studies of tumor tissue (obtained from related biological studies). We will use these arrays to quantify the various protein levels in order to identify proteins or panels of proteins that differentiate patients with poor prognosis from patients with good prognosis. Specific carbohydrate levels also will be characterized on each protein to determine the associations of those measurements with prognosis.

Circulating Tumor Cell Analysis –Performed by Dr. Li (Section 12.1.2)
Circulating tumor cells (CTC) are emerging as novel tools in the detection and prognosis of several types of metastatic cancers. At present, CTC markers are limited to epithelial cancers and there are no specific markers available to detect mesenchymal and epithelial-mesenchymal

transformed (EMT) CTCs. We have discovered a cell surface specific marker for detection of mesenchymal CTC using an antibody 84-1. Utilizing our cell surface marker, we can enumerate and isolate CTCs that aid in the early detection of tumors, metastasis, and relapse and will contribute to the development of specific targeted therapies, an ultimate goal of personalized medicine. The samples thus collected will be utilized to monitor the therapeutic outcome in patients.

Bone Marrow –Performed by Dr. Kidd (Sections 12.1.3)
Immunophenotyping of bone marrow samples using six-color analysis of the CD81(PEdye), NCAM antigen CD56(APC dye), CD9(perCP-Cy5.5 dye) and possible stem cell antigen CD34 (PE-CY7 dye) and the absence of leukocytic antigen CD45 (APC-Hy dye) for evaluation of minimal residual disease present in bone marrow.

12.1 Pharmacokinetic Sample Instructions

Blood samples for the pharmacokinetic assay of DFMO and metabolites will be collected from approximately 10 currently enrolled subjects on their last day of taking the old DFMO formulation and on one day during Days 2-5 of taking the new formulation. Samples will be collected prior to administration of DFMO (Time 0) and at the following times: 30min, 1 hour, 3 hours, and 6 hours post-dose (exact time should be noted on CRF).

The blood samples will be processed to plasma, aliquoted and stored frozen at -80ºC until assayed.

PK parameters (C_{max}, T_{max}, AUC_{0-24}, $AUC_{0-\infty}$, $t_{1/2}$, Cl, and Vd) will be determined as appropriate using standard noncompartmental procedures (WINNONLIN software). Individual subject measurements, as well as summary statistics (e.g., mean, standard deviation, range, %CV) will be reported.

Procedures for obtaining, storing and shipping of the pharmacokinetic samples are as follows:

1. One 2cc whole blood specimen will be collected in a purple top tube (EDTA) for each time point.
2. Place samples immediately in ice water bath until ready to process (no longer than 24 hours).
3. Specimen will be spun in a centrifuge at 2,000g for 15 minutes
4. Following centrifugation; using a clean pipette, the plasma from EDTA tube will be aliquoted into a plastic screw capped cryovial.
5. Specimens must be labeled with the participants unique study identifier, PK specific time point (include cycle, day, and time point) and date and time of specimen collection
6. Specimens will then be stored in a -80 freezer until ready to be shipped.

PK Specimen shipping:

- All specimens will be shipped on dry ice following the federal IATA hazardous Specimen Shipping Guidelines
- Specimens should be batch shipped (after each subject completes all PK time points) on a Monday-Thursday.
- PK Specimens will be shipped to the following address:

Ping Zhao
Pediatric Oncology Translational Research Laboratory
Coopers Landing
1345 Monroe Ave, Suite 121
Grand Rapids, MI 49505
Ph: 616-486-8645
Ping.zhao@helendevoschildrens.org

12.2 Biological Correlative Studies Sample Instructions

Subjects will have the following required urine samples sent.

12.2.1 Required Urine Polyamines Collection:

At screening, on Days 1 and 15 of Cycle 1, and on Day 1 of cycles 2-27, urine polyamines will be collected.

A. First morning void urines will be collected using a clean catch method in containers and placed at 4^{o}C until transported to the laboratory. Collection of 15ml is ideal, but lab can accept amounts down to a minimum of 1 (one) ml.

B. Specimens will be transferred to 15ml polypropylene conical tubes (use multiple 15ml tubes as needed) and stored at -80^{o}C until analysis for the dc-SAM and polyamines. Samples should be kept at 4^{o}C (either in refrigerator or on ice pack during shipping) until frozen and must be frozen within 48 hours from collection time. If unable to freeze within 48 hours please note this on the container along with time before frozen.

C. Samples will be batch shipped on a Monday (send after approximately 20 samples are collected) and sent on dry ice to:

Ping Zhao
Pediatric Oncology Translational Research Laboratory
Coopers Landing
1345 Monroe Ave, Suite 121
Grand Rapids, MI 49505
Ph: 616-486-8645
Ping.zhao@helendevoschildrens.org

Subjects will have the following optional (recommended) blood and bone marrow samples sent.

12.2.2 Optional Blood Collection

1. **Sample One, Two, and Three**-

   A. Sample 1- On Day 1: 2 mls of blood will be collected in an EDTA tube (s). At the time of collection, the tube should be gently inverted 6-8 times.

   B. Sample 2- On Days 1 and 15 of Cycle 1, Day 1 of cycles 2 through 12, and 14, 17, 21, 24 and off -therapy- 5 mls of blood will be collected in an EDTA tube(s). At the time of collection, the tube should be gently inverted 6-8 times.

   C. Sample 3- On Days 1 and 15 of Cycle 1, Day 1 of cycles 2 through 12, and 14, 17, 21, 24 and off-therapy- 5 mls of blood will be collected in an EDTA tube(s). At the time of collection, the tube should be gently inverted 6-8 times.

   D. Shipment/Storage will be as follows:
   Specimens A-C above should be placed on ice immediately upon draw and sent on an ice pack immediately via FED-EX overnight. (If Friday collection site may store in refrigerator and ship on Monday). Ship to:

   Ping Zhao
   Pediatric Oncology Translational Research Laboratory
   Coopers Landing
   1345 Monroe Ave, Suite 121
   Grand Rapids, MI 49505
   Ph: 616-486-8645
   Ping.zhao@helendevoschildrens.org

2. A 7.5cc whole blood specimen will be collected in a BD CPT Vacutainer tube(s) containing the anti-coagulant Sodium Heparin (BD Cat no. REF362753, 8mL draw capacity).

A. At screening and then start of cycles 3, 6, 12, 14, 17, 21, 24 and off-therapy.

B. Specimen Handling:

1. If BD CPT Vacutainer tube is not available at the site, collect blood in a Green top heparin tube (do not use glass tubes). Do not follow steps 2-5- Ship without processing in the green top tube. Skip to C below for shipping instructions.
2. After collection, store tube upright at room temperature until centrifugation. Blood samples should be centrifuged within two hours of blood collection.
3. Centrifuge tube/blood sample at room temperature (18-25° C) in a horizontal rotor for a minimum of 15 minutes
4. at 1500 to 1800 RCF (Relative Centrifugal Force).
   NOTE: Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times. Also, check to see that the tube is in the proper centrifuge carrier/adapter.
5. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer. These tubes are now ready for transportation. If not transporting immediately, they can be stored at room temperature until transportation. Samples should be shipped at room temperature.

C. Ship samples the same day as collection. Samples will be sent **ambient temperature** to:

Xueqing Xia
Dept. Pediatrics—Research, MOD2.016
UT MD Anderson Cancer Center
7777 Knight Rd.
Houston, TX 77054
713-563-9617

12.2.3 Optional Bone Marrow Collection

If subject agrees to optional biology portion of study, additional bone marrow samples should be sent at the following time points:
(Please note that if subject has also signed to protocol NMTRC 00B, these samples should be sent under that study instead):

All Subjects- At Enrollment, Any time a bone marrow is done during protocol therapy, Early Withdrawal (if indicated), and per institutional standards after that.

Sample Collection-

Send green top (sodium heparin) tube(s) with a minimum of 2cc and preferably 5cc of bone marrow aspirate in them. The bone marrow samples should be shipped room temperature- but needs to be sent out same day priority overnight (must get there within 24 hours of the draw). Shipments are only accepted Monday through Friday, so Bone Marrow draw needs to be done and shipped out on Monday through Thursday only please.
Please contact the lab at the number below to let them know of pending arrival.

Bone marrow 2-5cc in green top (sodium heparin) tube(s) at room temperature and will be shipped FED-EX overnight to:

Flow Cytometry Lab of Spectrum Health
Attn: Pamela Kidd, MD
Lemmen-Holton Cancer Pavilion
145 Michigan Street NE Suite 6201
Grand Rapids, MI 49503
phone: 616-486- 6270

12.3 Sample Storage and Destruction

Samples collected for any studies performed in this protocol may be stored indefinitely to research scientific questions related to cancer and/or study drugs. The subject retains the right to have the sample material destroyed at any time by contacting the principal investigator.

Appendix I: Performance Status/Scores

Performance Status Criteria Karnofsky and Lansky performance scores are intended to be multiples of 10					
ECOG (Zubrod)		**Karnofsky**		**Lansky***	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

*The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.

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