Protocol DFMO Phase I/II

NMTRC 010B

A Phase I/II Trial of DFMO in Combination with Bortezomib in Patients with Relapsed or Refractory Neuroblastoma

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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 review 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

PROTOCOL SYNOPSIS

PROTOCOL TITLE	A Phase I/II Trial of DFMO in Combination with Bortezomib in Patients with Relapsed or Refractory Neuroblastoma
PROTOCOL NUMBER	NMTRC010B
PHASE OF DEVELOPMENT	Phase I/II
OBJECTIVES	**Primary- Phase I** • To determine the safety and tolerability of DFMO in combination with bortezomib at 3 dose levels of DFMO: 1500mg/m2 twice daily, 2000mg/m2 twice daily, and 2500mg/m2 twice daily in subjects with relapsed or refractory neuroblastoma who receive one full cycle of this dose. **Primary- Phase I and II** To evaluate the activity of DFMO in combination with bortezomib in relapsed or refractory neuroblastoma based on: ⬧ Progression free survival (PFS) ⬧ Overall response rate (ORR) **Secondary- Phase I and II** • To determine the safety and tolerability of DFMO in combination with bortezomib in pediatric subjects with refractory or recurrent neuroblastoma. • For subjects followed with PET scan: comparison of changes in PET activity and correlation with PFS • Correlation of urinary polyamine levels with response and progression of disease in neuroblastoma. • Biological Correlates to evaluate or explore minimally include: 1) Urine: polyamine levels 2) Blood: Inflammatory markers (ESR, CRP, IL-6), Biomarkers for Let 7 and SNPS, and explorative biomarker analysis, 3) Bone Marrow/Tumor biopsies (if biopsied per standard of care): research studies including Immunohistochemistry/Western Blot/qRT-PCR of bone marrow tumor cells or tumor to study Let-7, ODC, Lin28, MYCN, OKT-4, BMI1, HMGA2, GLUT-4, p-AKT, NF-kB, SOCS3 will be performed in an explorative biomarker analysis. Studies will measure thymidine and polyamine levels. 4) In vitro research studies of tumor cells collected above will include: Genomic analysis of cells pre- and post- treatment (when applicable), cell viability assays to determine subject IC50 and correlation of *in vitro* response to *in vivo* response. • To determine the dose effect of DFMO on biological correlates

STUDY DESIGN	NMTRC010B is an open label, multicenter, study to evaluate the efficacy of DFMO in combination with Bortezomib in patients with relapsed or refractory neuroblastoma. **Phase 1** This portion of the study uses a standard 3+3 design in which cohorts of 3 patients will receive oral DFMO at a starting dose of 1500 mg/m^2 BID of a 21-day cycle. Subjects that complete one cycle will be considered as having completed Phase I. The dose escalation scheme for subsequent cohorts and modifications for dose limiting toxicities (DLT) are detailed in the protocol. The MTD of single agent therapy will be defined as the dose level below which DLTs are seen in ≥ two of six subjects dosed. **Cycle 1 through 6: DFMO + Bortezomib** Phase I: Subjects will receive oral DFMO twice daily at their assigned dose level on each day of this 21-day cycle. **DFMO Dose Level** / **Dose** 1 / 1500 mg/m^2 BID 2 / 2000 mg/m^2 BID 3 / 2500 mg/m^2 BID Phase II: The highest tolerated DFMO dose from the Phase I dose escalation will be used for the Phase II portion of the study. Subjects will receive oral DFMO twice daily on each day of this 21-day cycle. All Phases: Bortezomib will be given at 1.3mg/m2/dose IV Push on Days 1, 4, and 8 of each 21-day cycle **Phase I Subjects:** The Phase I subjects on this study will be the first pediatric subjects to receive DFMO in combination with Bortezomib. If a Dose Limiting Toxicity (DLT) is seen in 2/6 subjects, this portion of the study will allow for a -1 dose level reduction (1000mg/m2/dose). If the DLT is seen in 2/6 subjects at the -1 dose level, a further reduction to dose level -2 (500mg/m2/dose) will be allowed. Enrollment will be held at the end of this phase of the study until all subjects within the cohort have completed one cycle and a safety analysis has been completed by the study committee and Data Safety and Monitoring Board (DSMB). The study committee will then decide whether the study can continue to the next cohort and subsequently to the Phase II portion of the trial.

	Phase I: At least three and up to six subjects per cohort, for an estimated three cohorts (based on safety) may be enrolled for a projected total of 12 to 24 subjects. Phase II: A total of 32-38 relapsed/refractory neuroblastoma subjects will be enrolled to ensure that there will be 32 subjects evaluable for efficacy. Phase 1 subjects assigned to the final dosing level that complete at least 2 cycles and re-evaluation will be eligible to continue onto the Phase II portion of the study and will be included in the total enrollment numbers. **Response** Response will be evaluated at the end of cycles 2, 4, and 6 with: • CT and/or MRI- same as baseline • MIBG or PET scan- same as baseline • Bone Marrow- if positive at study entry **Maintenance Cycles** Subjects that complete 6 cycles will be considered as having completed the protocol defined therapy, however they may continue on cycles of DFMO (at their current dose level) and bortezomib for as long as they are tolerating it well and not having progression. Subjects in maintenance cycles will have response evaluations done as per standard of care.
ELIGIBILITY	**Inclusion Criteria** 1. Age: ≤ 21 years at the time of diagnosis. 2. Diagnosis: Histologic verification at either the time of original diagnosis or relapse of neuroblastoma. 3. Disease Status: For the purposes of this study, aggressive multidrug chemotherapy is defined as chemotherapy including 2 or more agents that must include an alkylating agent and a platinum-containing compound. Patients must have ONE of the following: 1) Any episode of recurrent disease following completion of aggressive multi-drug frontline therapy. 2) Any episode of progressive disease during aggressive multi-drug frontline therapy. 3) Primary resistant/refractory disease detected at the conclusion of at least 4 cycles of aggressive multidrug induction chemotherapy on or according to a high-risk neuroblastoma protocol (examples include Children's Oncology Group trials: A3973, ANBL0532, ANBL09P1, etc.).

	4. Measurable or evaluable disease, including at least one of the following: Measureable tumor by CT or MRI; or A positive MIBG or PET; or Positive bone marrow biopsy/aspirate. 5. Current disease state must be one for which there is currently no known curative therapy or no additional therapies proven to prolong survival with an acceptable quality of life. 6. A negative serum or urine pregnancy test is required for female subjects of child bearing potential (onset of menses or ≥13 years of age). 7. Organ Function Requirements a. Subjects must have adequate liver function as defined by: • AST and ALT <5x upper limit of normal • Serum bilirubin must be ≤ 2.0 mg/dl b. Subjects must have adequate Bone Marrow function defined as: For patients <u>without</u> bone marrow involvement: • Peripheral absolute neutrophil count (ANC) ≥ 750/μL • Platelet count ≥ 50,000/μL (transfusion independent, defined as not receiving platelet transfusions within a 7 day period prior to enrollment. Exception: Patients that are platelet dependent due to previous extensive treatment- e.g. - MIBG therapy). • Hemoglobin ≥ 8.0 g/dL (may receive RBC transfusions) Patients known to have bone marrow involvement with neuroblastoma are eligible provided that minimum ANC and platelet count criteria are met but are not evaluable for hematological toxicity. Patients that do not have bone marrow involvement but that are transfusion dependent at study entry are eligible provided that minimum ANC and platelet count criteria are met but are not evaluable for platelet toxicity.

c. Subjects must have adequate renal function defined as:

- 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

8. 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

9. Lansky score <50%
10. BSA (m2) of <0.25
11. Prior Therapy- Patients must have fully recovered from the acute toxic effects of all prior anti- cancer chemotherapy and be within the following timelines:

    a. Myelosuppressive chemotherapy: Must not have received within 2 weeks of enrollment onto this study (6 weeks if prior nitrosourea).

    b. Hematopoietic growth factors: At least 5 days since the completion of therapy with a growth factor.

    c. Biologic (anti-neoplastic agent): At least 7 days since the completion of therapy with a biologic agent. For agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time during which adverse events are known to occur. The duration of this interval must be discussed with the Study Chair.

    d. Immunotherapy: At least 6 weeks since the completion of any type of immunotherapy, e.g. tumor vaccines.

    e. Monoclonal antibodies: At least 7 days or 3 half-lives, whichever is longer, must have elapsed since prior treatment with a monoclonal antibody.

    f. XRT: At least 14 days since the last treatment except for radiation delivered with palliative intent to a non-target site.

    g. Stem Cell Transplant or Rescue: No evidence of active graft vs. host disease and ≥ 2 months must have elapsed since transplant.

12. Investigational Drugs: Subjects who have received another investigational drug within the last 14 days are excluded from participation.
13. 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. 14. 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, blood, urine, and tumor (if available) for analysis.
ESTIMATED NUMBER OF SUBJECTS/GEOGRAPHIC REGIONS	A projected total of 12 to 24 subjects for the Phase I and 32- 38 subjects for the Phase II will be enrolled. A total of 38-62 subjects will be enrolled to complete the dose escalation and achieve 32 evaluable subjects for the Phase II. Up to 16 sites in the United States will participate.
LENGTH OF STUDY	Accrual to this study is estimated to be 2 years, with follow up for five years after last subject completion.
INVESTIGATIONAL PRODUCT DOSE/ROUTE/REGIMEN	DFMO is an oral agent that will be administered twice daily on every day of each 21-day cycle in combination with bortezomib given IV on Days 1, 4, and 8 of each 21-day cycle.
STUDY ASSESSMENTS	Refer to Table of Assessments for timing of study procedures.
CRITERIA FOR EVALUATION	Safety Measures: • Safety analysis will be conducted on all subjects who have received at least one dose of study drug, and will include the frequency and grade of all adverse events. Response Measures: • **Overall Response Rate (ORR)** ♦ To determine the overall response rate (ORR) by the presence of radiologically assessable disease by cross-sectional imaging and in MIBG or PET scans. Response will be defined by criteria in section 9.0; or disappearance of abnormal MIBG or PET foci. ♦ Duration of response, defined as the period of time from when measurement criteria are met for complete response (CR) or partial response (PR), whichever is first recorded, until the first date that recurrent or progressive disease (PD) is objectively documented (taking as reference for PD the smallest measurements recorded since the treatment started) ♦ The assessment of response will include the initial measurable targets and will be performed after the second cycle, then after every other cycle. Serial results of bone marrow aspirates, and

	urinary catecholamines will be followed to confirm clinical response in subjects with stable disease. • A subject will be defined as a responder if CR/PR is observed at any time during the treatment. • Time to progression, defined as the period from the start of the treatment until the criteria for progression are met. • Survival will be recorded at all times during the study, and follow-up will be every three months for one year and then annually for five years.
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 all grade 2 or greater adverse events and reporting of DLT's and MTD. The proportion of subjects experiencing adverse events, serious adverse events, and treatment delays will be summarized for each dosing cohort. Response data will be summarized as the proportion of subjects experiencing progressive disease, stable disease, partial responses or complete responses summarized in tabular format and supplemented with exact confidence intervals. Progression free survival and duration of any responses will also be summarized based on the Kaplan-Meier approach. **Sample Size:** **Phase I: A** 3+3 design with 3 dose levels will be used. At least three and up to six subjects per cohort, for an estimated three cohorts (based on safety) may be enrolled for a projected total of 12 to 24 subjects. **Phase II:** Primary endpoint of ORR, a sample size of n=32 (6 from Phase I MTD) will be required to test whether treatment with DFMO can result in an ORR of 10% (Reference Value set at ORR <= 1%) The sample size to detect this difference uses a two-stage test with 5% Type I error level and 80% power. Examination of the secondary endpoint of PFS will require a sample size of n = 23 subjects to test whether treatment with DFMO can prolong PFS by 48 days (from 42 days based on historical data in this subject population to 90 days). The sample size to detect this difference uses a two-stage test with 5% Type I error level and 80% power.

Procedures and Assessments

	Screen	Cycle 1					Cycles 2 through 6					Subsequent Cycles	Follow Up
		Day 1	Day 4	Day 8	Day 15	Day 16-21	Day 1	Day 4	Day 8	Day 15	Day 16-21		
Informed consent	X												
Medical & surgical history, demographics histologic evidence of malignancy	X	X											
Prior therapy for malignancy		X											
Tumor Tissue (Pharmacogenomics)[a]	X												
Interval Medical History & Physical Exam		X	X	X	X		X		X	X		X	X
Vital signs including O_2 saturation	X	X	X	X	X		X	X	X	X		X	X
ECOG or Lansky play status	X						X					X	X
CBC and differential	X			X	X		X		X	X		X	X
Serum chemistries	X			X	X		X			X		X	X
CRP, ESR, IL-6 (IL-6 is recommended)	X						X						
Pregnancy test (urine)	X						X					X	
Urinary VMA/HVA	X						X					X	X
BSA calculation	X						X					X	
Administration of Bortezomib		X	X	X			X	X	X			X	
Administration of DFMO		X	→	→	→	→	→	→	→	→	→	→	
Dispense (and collect) drug dosing diary		X					X						
AE monitoring		X	X	X	X		X	X	X	X		X	X
Concomitant medications	X	X	X	X	X		X	X	X	X		X	X
CT or MRI	X										X[b]	X[b]	X[b]
MIBG or PET scan	X										X[b]	X[b]	X[b]
Bone Marrow aspirate and biopsy ^ *(if + at enrollment)*	X										X[b]	X[b]	X[b]
Audiogram	X										X[c]	X[c]	X[c]
Urine for biological correlates[a]		X			X		X						
Blood for biological correlates[a]		X			X		X						
Survival													X

a *Optional additional informed consent required*

b At the end of cycle 2, 4, and 6, then every other cycle

c At the end of cycle 2 and 6, then yearly

^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.

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. We have shown ODC inhibition reverses the LIN28/Let7 pathway, an important pathway in cell differentiation and regulation of glycolytic metabolism. In studying this pathway, it was found to be regulated as well by the NFkB pathway. We have therefore studied the combination of DFMO with Bortezomib and showing synergy of these medications in neuroblastoma in vitro and in vivo. This study will address this concept in children with relapsed or refractory neuroblastoma.

Background and Preliminary Data

1.1 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).

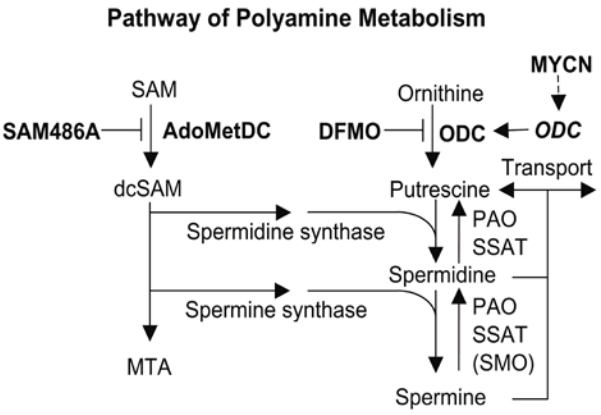


Figure 1. Pathway of polyamine metabolism showing two target enzymes of the polyamine inhibitors DFMO and SAM486A. By inhibiting ODC, DFMO depletes putrescine (Put) and spermidine (Spd) pools, whereas it only modestly affects spermine (Spm) pools. By inhibiting AdoMetDC, SAM486A depletes Spd and Spm, 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).

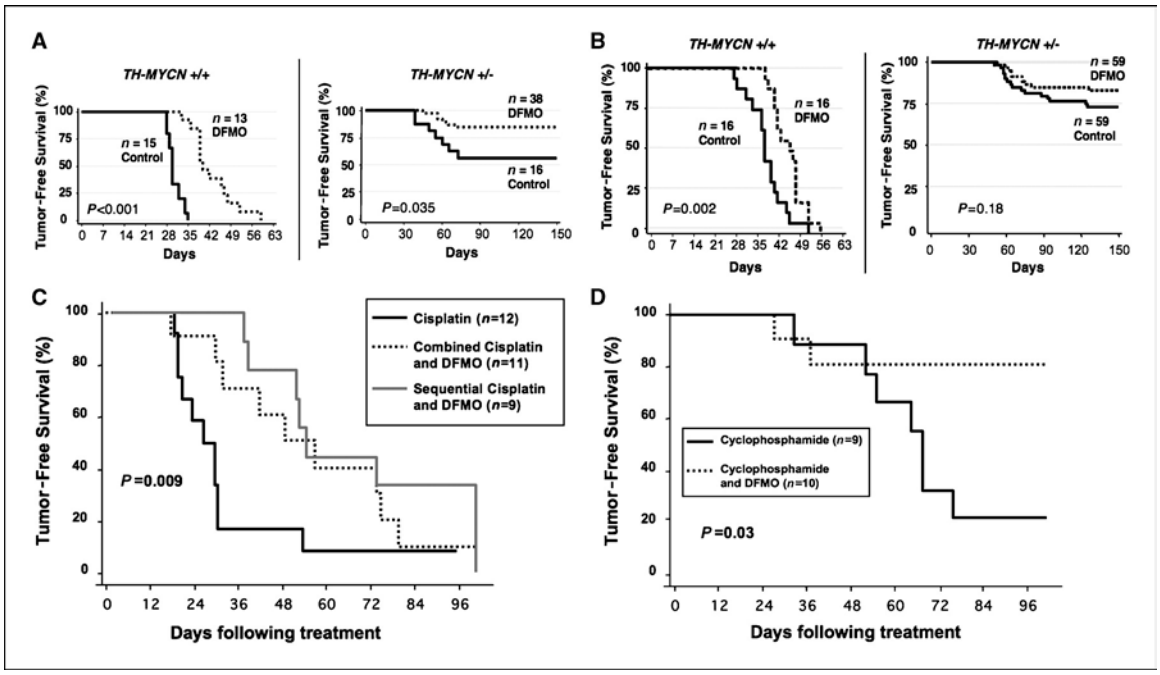


Figure 2. Extended tumor-free survival in neuroblastoma-prone mice treated with DFMO. **A,** tumor-free survival curves for homozygous (*TH-MYCN* +/+) or hemizygous (*TH-MYCN* +/-) mice stratified by DFMO therapy. DFMO-treated mice (dashed lines) received DFMO from birth onward (preemptive treatment 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).

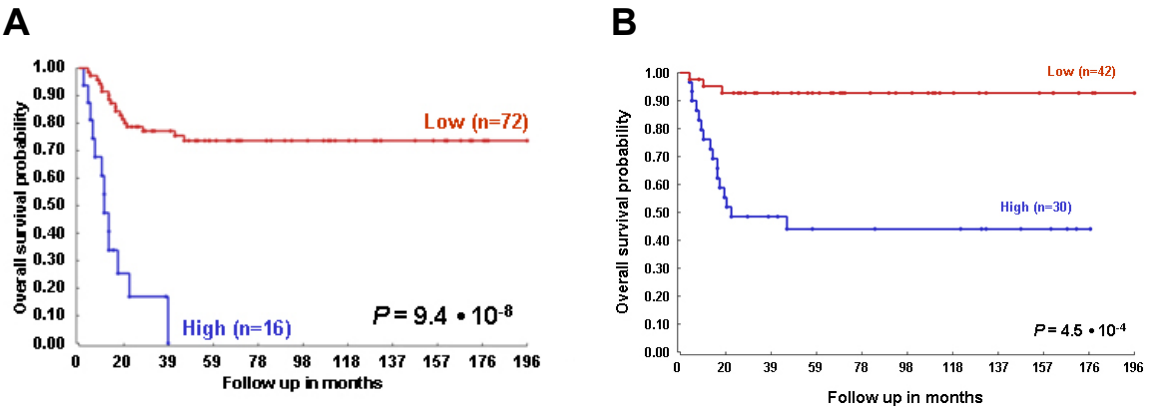


Figure 3. Correlation of ODC gene expression with NB patient survival prognosis. **A,** Kaplan-Meier graphs representing the survival prognosis of 88 NB patients based on high or low expression levels of ODC. Survival probability of NB patients (follow-up over 196 months) with low ODC expression is significantly 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).

Combination of DFMO and Bortezomib targeting LIN28/Let7 axis and glycolytic metabolism.

Overexpression of LIN28 correlates with poor outcome in neuroblastoma (NB) [1]. The LIN28/Let-7 axis affects many cellular processes including cell differentiation and glycolytic metabolism [2-4]. The LIN28/Let-7 axis also affects MYCN, which is often overexpressed in high risk NB [5, 6]. Studies in colon cancer have shown that ornithine decarboxylase (ODC) inhibition decreases LIN28 levels, thereby reversing the LIN28/Let-7 axis [7].

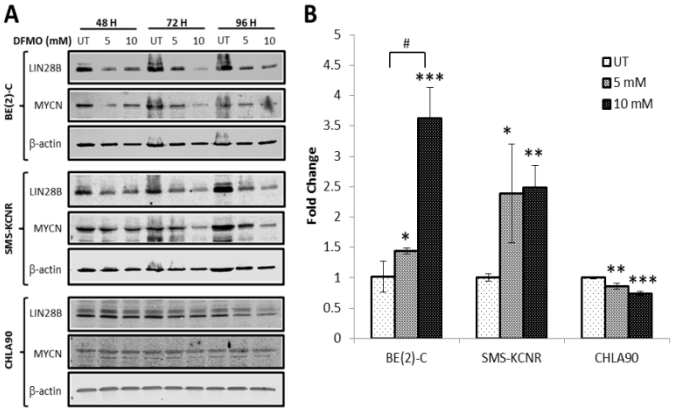


Figure 4: DFMO treatment reverses the LIN28/Let-7 axis in NB. A) Western blot analysis showed that DFMO treatment reduces LIN28B and MYCN protein levels in NB cells lines. B) qRT-PCR analysis showed increased Let-7 miRNA levels in BE(2)-C and SMS-KCNR cell lines with DFMO treatment.

Our preliminary studies demonstrate that therapy targeting ODC reverses the LIN28B/Let-7 axis in NB and decreases MYCN protein expression (Figure 4). Our studies show that cells overexpressing LIN28B also have greater sensitivity to difluoromethylornithine (DFMO) treatment, which inhibits ODC and decreases cellular polyamines [8].

Many cancer cells are reprogramed to produce ATP primarily via glycolytic metabolism as opposed to oxidative phosphorylation, a phenomenon termed the "Warburg effect" [9-13]. This phenomenon is well documented in NB [14]. Our studies showed a decrease in ATP/cell with DFMO treatment in NB, indicating a decrease in glycolytic metabolic activity (data not shown). Patient observations in our Phase I study with DFMO and *in vivo* xenograft studies using PET scans further validated the findings that DFMO treatment reduces glycolytic metabolism in NB (Figure 2).

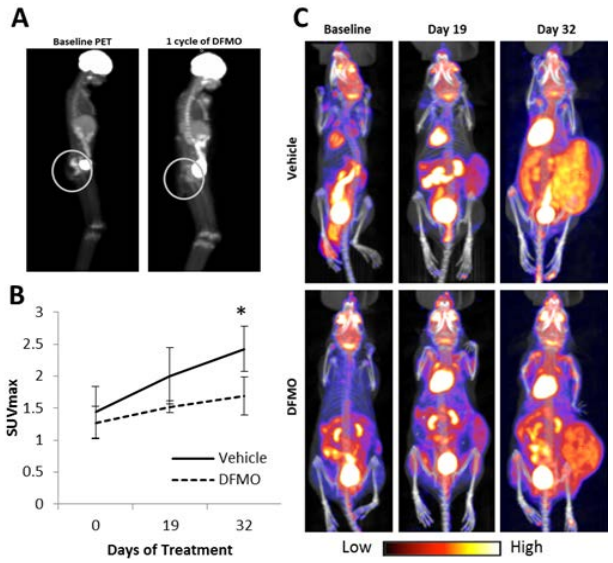


Figure 5: DFMO treatment reduces glycolytic metabolism in NB. A) Patient PET scans before and after one cycle of DFMO treatment. B) Average SUVmax (measure of metabolism) was lower in mice treated with DFMO compared to untreated. C) PET scans of one untreated and one DFMO-treated mouse at baseline, day 19, and day 32 of treatment.

Recent studies have shown that the LIN28/Let-7 and metabolism pathways are important in cancer stem cell (CSC) regulation. Our collaborator, Dr. Max Wicha, has demonstrated the importance of these pathways in breast CSCs. His studies show that elevated glycolytic metabolism is a characteristic of CSCs [15] and suggests that the LIN28/Let-7 axis is of critical importance in CSCs [16]. Additionally, his research has demonstrated the importance of SOCS3 and the NF-kB/IL-6 inflammatory loop in CSCs [17]. The NF-kB/IL-6 inflammatory loop is linked to the LIN28/Let-7 axis [3]; therefore, to further inhibit this pathway, we studied bortezomib (Velcade). Bortezomib inactivates NF-kB through proteasome inhibition. NF-kB binds and enhances expression at the LIN28 promoter [3], therefore inhibition by bortezomib reduces LIN28. Downstream effects of this include inhibition of the insulin receptor and GLUT-4 and decrease in glycolytic metabolism [2]. Proteosome inhibition also results in an increase in SOCS3 which in turn inhibits the JAK/STAT pathway and decreases IL-6 [17] (Figure 3). Our preliminary studies show synergy between DFMO and bortezomib in NB.

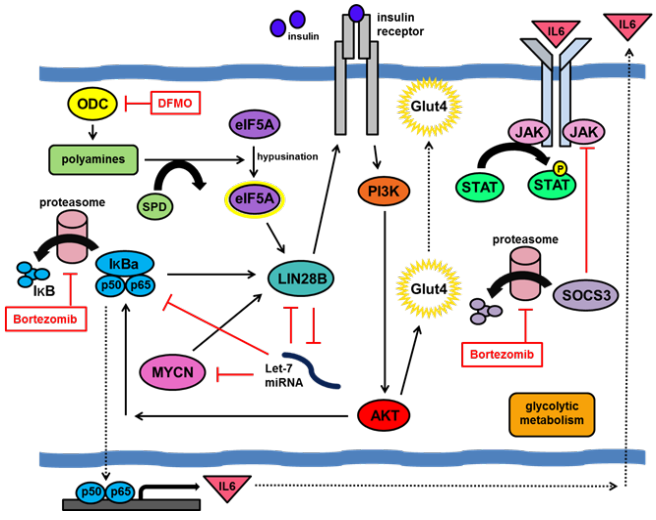


Figure 3: Pathways of importance in CSCs. Combined treatment with DFMO and Bortezomib targets multiple points of the ODC-LIN28 pathways and IL-6 inflammatory loop.

This combination was tested in *in vitro* and *in vivo* studies and shown to be well tolerated and effective together. A mice xenograft model treated with the combination with DFMO and bortezomib resulted in a decrease in tumor growth (Figure 5).

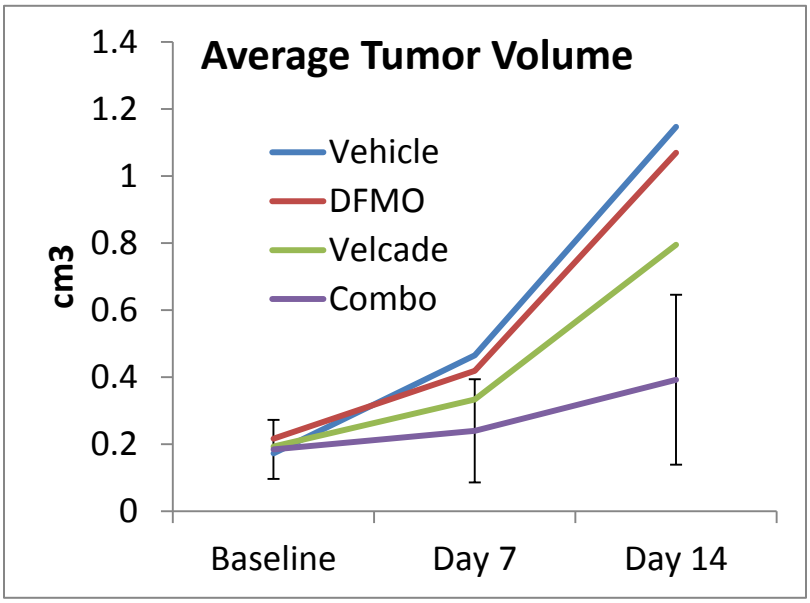


Figure 5. SMS-KCNR Xenograft treated with DFMO and Botrezomib

1.2 Clinical Work

Summary of Phase I Neuroblastoma Trial (NTC01059071)

NTC01059071 was a multi-center, investigator initiated dose escalation study that was completed in 2013. The following dose levels of DFMO (CPP-1X) were investigated: 500 mg/m^2, 750 mg/m^2, 1000 mg/m^2 and 1500 mg/m^2. Cycles were 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. Subjects 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. Subjects who did not complete 2 cycles were replaced in the cohort. Subjects 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.

At the close of the study 21 subjects had received at least one dose of DFMO, with 3 evaluable subjects at 500 mg/m^2, 3 evaluable subjects at 750 mg/m^2, 3 evaluable subjects at 1000 mg/m^2, and 6 evaluable subjects at 1500 mg/m^2. All subjects were multiple relapsed/refractory, with 14 males and 7 females, mean age 9, range (1-17), and 60% White/Caucasian.

There were no DLT's reported during this study and no serious adverse events. The most prevalent adverse events were low hemoglobin, neutropenia and thrombocytopenia. The majority of the events were considered unrelated to treatment.

Nine subjects completed at least 5 cycles of therapy therefore completing study defined protocol therapy. Time on study ranged from 1 to 43 cycles, with 4 subjects on therapy 1 year or more.

Summary of Phase II Neuroblastoma Prevention Trial

NCT01586260 is a multi-center, open label, single agent study that was opened in 2012 for children with neuroblastoma that is in remission. The dose level of DFMO being investigated is: 1000 mg/m^2 twice daily. Cycles are 28 days, with DFMO given days 1-28 for all cycles. Subjects are considered to have completed the study if they have received 27 cycles of treatment (2 years) without a remission.

To date 51 subjects had received at least one dose of DFMO, with 44 subjects remaining on study at this time. All subjects had neuroblastoma that is in remission, with 33 males and 18 females, mean age 9, range (1-17), and 69% White/Caucasian. There have been no related SAE's reported during this study to date.

Summary of Dosing and Schedule Development for Bortezomib

Bortezomib (Velcade®, IND #58443) is an FDA approved proteasomal Inhibitor for the treatment of multiple myeloma and mantle cell lymphoma. Pre-clinical studies have demonstrated anti-tumor activity of bortezomib in pediatric cancers, including leukemia, non-Hodgkin lymphoma, neuroblastoma, rhabdomyosarcoma, and Ewing's sarcoma. Bortezomib has been studied in one phase 1 and three phase 2 Children's Oncology Group (COG) studies and is currently under investigation in a phase 3 study. The Phase 1 study was combined with Vorinostat and the MTD for bortezomib was 1.3mg/m2 IV on D1, 4, 8 and 11 of a 21 day cycle. On this trial, one child developed grade 4 neuropathy late in treatment. Due to this, the phase 2 trials have used bortezomib at either 1.2 mg/m2 or 1.3mg/m2 on D1, 4 and 8 of a 21 day cycle. The one trial using the 1.2mg/m2 combined bortezomib with more myelosuppressive therapy (Ifosfamide/Vinorelbine). The remaining COG trails, including the current phase 3 trial, have use the dose of Bortezomib at 1.3mg/m2 IV on D1, 4 and 8 of a 21 day cycle. This dose and schedule of Bortezomib is chosen for this study as the standard of care dose in pediatrics.

Summary

In summary, our studies show that DFMO reverses the LIN28/Let-7 pathway, reverses MYCN overexpression and decreases cellular ATP and PET activity in NB cells. These pathways, in addition to the proteasome pathway, have been shown to be important in CSCs but have yet to be linked to CSCs in NB. Given the high rate of relapse in NB, targeting of the CSC is important to prevent relapse. We believe that our preliminary study lays the framework for an investigation of the effects of ODC-Lin 28/Let-7 inhibition in NB using DFMO and bortezomib in children with relapsed/refractory neuroblastoma.

2.0 Study Objectives

2.1 Primary Objective- Phase I

2.1.1 To determine the safety and tolerability of DFMO in combination with bortezomib at 3 dose levels of DFMO: 1500mg/m2 twice daily, 2000mg/m2 twice daily, and 2500mg/m2 twice daily in subjects with relapsed or refractory neuroblastoma who receive one full cycle of this dose.

2.2 Primary- Phase I and II

2.2.1 To evaluate the activity of DFMO as a single agent and in combination with bortezomib in relapsed or refractory neuroblastoma based on:

- Progression free survival (PFS)
- Overall response rate (ORR)

2.3 Secondary Objectives:

2.3.1 To determine the safety and tolerability of DFMO in combination with bortezomib in pediatric subjects with refractory or recurrent neuroblastoma.

2.3.2 For subjects followed with PET scan: comparison of changes in PET activity and correlation with PFS

2.3.3 Correlation of urinary polyamine levels with response and progression of disease in neuroblastoma.

2.3.4 Biological Correlates to evaluate or explore minimally include: 1) Urine: polyamine levels 2) Blood: Inflammatory markers, Biomarkers for Let 7 and SNPS, and explorative biomarker analysis, 3) Bone Marrow/Tumor biopsies (if biopsied per standard of care): research studies including Immunohistochemistry/Western Blot/qRT-PCR of bone marrow tumor cells or tumor to study Let-7, ODC, Lin28, MYCN, OKT-4, BMI1, HMGA2, GLUT-4, p-AKT, NF-kB, SOCS3 will be performed in an explorative biomarker analysis. Studies will measure thymidine and polyamine levels. 4) In vitro research studies of tumor cells collected above will include: Genomic analysis of cells pre- and post- treatment (when applicable), cell viability assays to determine subject IC50 and correlation of in vitro response to in vivo response.

2.3.5 To determine the dose effect of DFMO on biological correlates

3.0 Study Design

NMTRC010B is an open label, multicenter, study to evaluate the efficacy of DFMO in combination with bortezomib in subjects with relapsed or refractory neuroblastoma.

3.1 Cycles 1-6

3.1.1 DFMO- Phase I

Subjects will receive six (6) cycles of oral DFMO twice daily at their assigned dose level on each day of this 21-day cycle.

DFMO Dose Level	Dose
1	1500 mg/m^2 BID
2	2000 mg/m^2 BID
3	2500 mg/m^2 BID

DFMO is provided as 250mg tablets.

3.1.2 DFMO- Phase II

The highest tolerated DFMO dose from the Phase I dose escalation will be used for the Phase II portion of the study. Subjects will receive oral DFMO twice daily on each day of this 21day cycle.

DFMO is provided as 250mg tablets.

3.1.3 Bortezomib

Bortezomib will be given at 1.3mg/m2/dose IV Push on Days 1, 4, and 8 of each 21-day cycle

The Phase I subjects on this study will be the first pediatric subjects to receive DFMO in combination with Bortezomib. If a Dose Limiting Toxicity (DLT) is seen in 2/6 subjects, this portion of the study will allow for a -1 dose level reduction (1000mg/m2/dose). If the DLT is seen in 2/6 subjects at the -1 dose level, a further reduction to dose level -2 (500mg/m2/dose) will be allowed. Enrollment will be held at the end of this phase of the study until all subjects within the cohort have completed one cycle and a safety analysis has been

completed by the study committee and Data Safety and Monitoring Board (DSMB). The study committee will then decide whether the study can continue to the next cohort and subsequently to the Phase II portion of the trial.

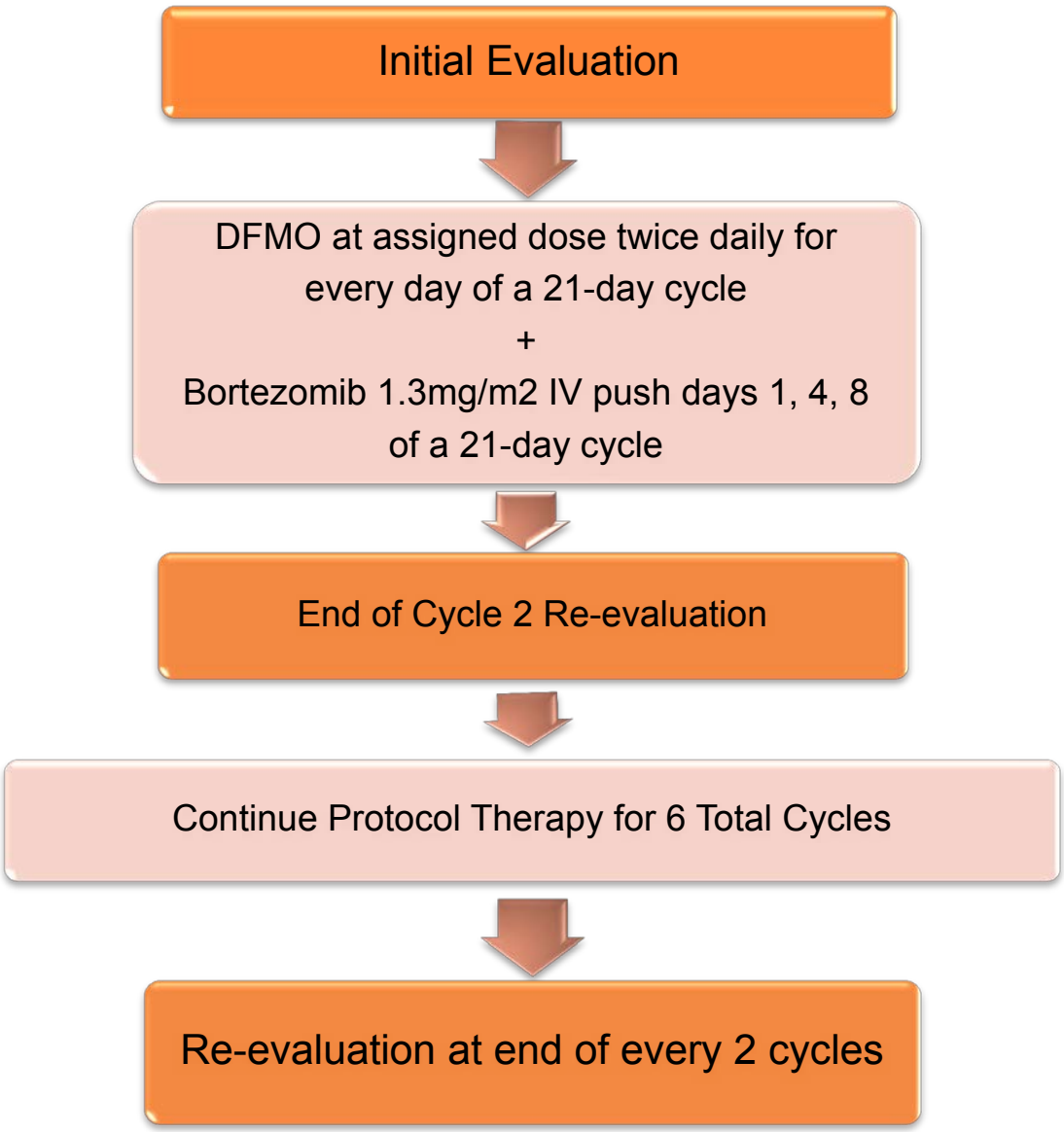
3.2 Response Evaluations

At the times indicated in Section 6.0 and the Table of Procedures and Assessments, scans will be obtained to evaluate response for subjects enrolled in this study. Response will be assessed according to criteria outlined in Section 7.0 to evaluate the potential benefit of DFMO in this subject population.

3.3 Biological Studies

Subjects will have an opportunity to participate in additional correlative biological studies on a voluntary basis collecting urine, blood, bone marrow and tumor (if a standard of care sample is being collected). These samples will be sent to the POTRL at Helen DeVos Children's Hospital and University of Arizona for studies described in Section 12. The studies will evaluate the effect of DFMO and bortezomib on neuroblastoma. These studies will include evaluation of the ODC-Lin28/Let-7 pathway, glycolytic metabolism and inflammation. These biologic studies may be able to contribute to our knowledge of molecular profiles or biomarkers of response to therapy to help guide future treatment studies.

Protocol Design Schema



4.0 Subject Selection

4.1 *Inclusion Criteria:*

1. Age: ≤ 21 years at the time of diagnosis.
2. Diagnosis: Histologic verification at either the time of original diagnosis or relapse of neuroblastoma.
3. Disease Status: For the purposes of this study, aggressive multidrug chemotherapy is defined as chemotherapy including 2 or more agents that must include an alkylating agent and a platinum-containing compound. Patients must have ONE of the following:

   1) Any episode of recurrent disease following completion of aggressive multi-drug frontline therapy.

   2) Any episode of progressive disease during aggressive multi-drug frontline therapy.

   3) Primary resistant/refractory disease detected at the conclusion of at least 4 cycles of aggressive multidrug induction chemotherapy on or according to a high-risk neuroblastoma protocol (examples include Children's Oncology Group trials: A3973, ANBL0532, ANBL09P1, etc.).
4. Measurable or evaluable disease, including at least one of the following: Measureable tumor by CT or MRI; or A positive MIBG or PET scan; or Positive bone marrow biopsy/aspirate.
5. Current disease state must be one for which there is currently no known curative therapy or no additional therapies proven to prolong survival with an acceptable quality of life.
6. A negative serum or urine pregnancy test is required for female subjects of child bearing potential (onset of menses or ≥13 years of age).
7. Organ Function Requirements:
   a. Subjects must have adequate liver function as defined by:
      - AST and ALT <5x upper limit of normal
      - Serum bilirubin must be ≤ 2.0 mg/dl

   b. Subjects must have adequate Bone Marrow function defined as:

      For patients without bone marrow involvement:
      - Peripheral absolute neutrophil count (ANC) ≥ 750/μL
      - Platelet count ≥ 50,000/μL (transfusion independent, defined as not receiving platelet transfusions within a 7 day period prior to enrollment. Exception: Patients that are platelet dependent due to previous extensive treatment- e.g. - MIBG therapy).
      - Hemoglobin ≥ 8.0 g/dL (may receive RBC transfusions)

      Patients known to have bone marrow involvement with neuroblastoma are eligible provided that minimum ANC and platelet count criteria are met but are not evaluable for hematological toxicity.

      Patients that do not have bone marrow involvement but that are transfusion dependent at study entry are eligible provided that minimum ANC and platelet count criteria are met but are not evaluable for platelet toxicity.

c. Subjects must have adequate renal function defined as:
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

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

4.2 ***Exclusion Criteria:***

1. Lansky score <50%
2. BSA (m2) of <0.25
3. Prior Therapy- Patients must have fully recovered from the acute toxic effects of all prior anti- cancer chemotherapy and be within the following timelines:

   a. Myelosuppressive chemotherapy: Must not have received within 2 weeks of enrollment onto this study (6 weeks if prior nitrosourea).

   b. Hematopoietic growth factors: At least 5 days since the completion of therapy with a growth factor.

   c. Biologic (anti-neoplastic agent): At least 7 days since the completion of therapy with a biologic agent. For agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time during which adverse events are known to occur. The duration of this interval must be discussed with the Study Chair.

   d. Immunotherapy: At least 6 weeks since the completion of any type of immunotherapy, e.g. tumor vaccines.

   e. Monoclonal antibodies: At least 7 days or 3 half-lives, whichever is longer, must have elapsed since prior treatment with a monoclonal antibody.

   f. XRT: At least 14 days since the last treatment except for radiation delivered with palliative intent to a non-target site.

   g. Stem Cell Transplant or Rescue: No evidence of active graft vs. host disease and ≥ 2 months must have elapsed since transplant.
4. Investigational Drugs: Subjects who have received another investigational drug within the last 14 days are excluded from participation.

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, blood, urine and tumor (if available) for analysis.

5.0 STUDY DRUG ADMINISTRATION

5.1 DFMO

5.1.1 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).

5.1.2 DFMO Dosing: Phase I

Subjects will be given oral DFMO twice daily at their assigned dose level on each day of this 21-day cycle. Upon enrollment subjects will be assigned a DFMO dose level by NMTRC staff. Dosing will be per Dose level charts below. Doses are rounded to the nearest whole tablet.

The dose of DFMO will be recalculated at the beginning of each cycle based on BSA using a weight and height obtained up to 5 days prior to the cycle start date.

DFMO Dose Level	Dose
1	1500 mg/m^2 BID
2	2000 mg/m^2 BID
3	2500 mg/m^2 BID

DFMO Dose Table for Dose Level 1 - 1500 mg/m^2 BID:

BSA (m2)	Tablets to be Dispensed for each dose	Total Tablets Per Day	Actual Mg/m2
>1.5	Nine (9) tablets orally twice a day	Eighteen (18)	1500 and down per dose
1.3 to 1.5	Eight (8) tablets orally twice a day	Sixteen (16)	1539 to 1333 per dose
1.1 to 1.2	Seven (7) tablets orally twice a day	Fourteen (14)	1458 to 1591 per dose
1.0	Six (6) tablets orally twice a day	Twelve (12)	1500 per dose
0.8 to 0.9	Five (5) tablets orally twice a day	Ten (5)	1389 to 1563 per dose
0.6 to 0.7	Four (4) tablets orally twice a day	Eight (8)	1429 to 1667 per dose
0.5	Three (3) tablets orally twice a day	Six (6)	1500 per dose
0.3 to 0.4	Two (2) tablets orally twice a day	Four (4)	1250 to 1667 per dose

DFMO Dose Table for Dose Level 2 - 2000 mg/m^2 BID:

BSA (m2)	Tablets to be Dispensed for each dose	Total Tablets Per Day	Actual Mg/m2
1.5 to >1.5	Twelve (12) tablets orally twice a day	Twenty-four (24)	2000 and down per dose
1.4	Eleven (11) tablets orally twice a day	Twenty-two (22)	1964 per dose
1.2 to 1.3	Ten (10) tablets orally twice a day	Twenty (20)	1923 to 2083 per dose
1.1	Nine (9) tablets orally twice a day	Eighteen (18)	2046 per dose
1.0	Eight (8) tablets orally twice a day	Sixteen (16)	2000 per dose
0.9	Seven (7) tablets orally twice a day	Fourteen (14)	1944 per dose
0.7 to 0.8	Six (6) tablets orally twice a day	Twelve (12)	1875 to 2143 per dose
0.6	Five (5) tablets orally twice a day	Ten (10)	2083 per dose
0.5	Four (4) tablets orally twice a day	Eight (8)	2000 per dose
0.4	Three (3) tablets orally twice a day	Six (6)	1875 per dose
0.3	Two (2) tablets orally twice a day	Four (4)	1667 per dose

DFMO Dose Table for Dose Level 3 - 2500 mg/m^2 BID:

BSA (m2)	Tablets to be Dispensed for each dose	Total Tablets Per Day	Actual Mg/m2
1.5 to >1.5	Fifteen (15) tablets orally twice a day	Thirty (30)	2500 and down per dose
1.4	Fourteen (14) tablets orally twice a day	Twenty-eight (28)	2500 per dose
1.3	Thirteen (13) tablets orally twice a day	Twenty-six (26)	2500 per dose
1.2	Twelve (12) tablets orally twice a day	Twenty-four (24)	2500 per dose
1.1	Eleven (11) tablets orally twice a day	Twenty-two (22)	2500 per dose
1.0	Ten (10) tablets orally twice a day	Twenty (20)	2500 per dose
0.9	Nine (9) tablets orally twice a day	Eighteen (18)	2500 per dose
0.8	Eight (8) tablets orally twice a day	Sixteen (16)	2500 per dose
0.7	Seven (7) tablets orally twice a day	Fourteen (14)	2500 per dose
0.6	Six (6) tablets orally twice a day	Twelve (12)	2500 per dose
0.5	Five (5) tablets orally twice a day	Ten (10)	2500 per dose
0.4	Four (4) tablets orally twice a day	Eight (8)	2500 per dose
0.3	Three (3) tablets orally twice a day	Six (6)	2500 per dose

5.1.3 DFMO Dosing: Phase II

The highest tolerated DFMO dose from the Phase I dose escalation will be used for the Phase II portion of the study. Subjects will receive oral DFMO twice daily on each day of this 21-day cycle per dosing chart above for appropriate dose level. Doses are rounded to the nearest whole tablet.

5.1.4 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 (but not required) 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.

5.1.5 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, applesauce, or other food. The DFMO tablets will not dissolve, and this is acceptable. This technique is simply to mask the flavor.

5.1.6 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.

5.1.7 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.

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 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.

5.2 Bortezomib

5.2.1 Dose Calculation and Administration of Bortezomib- All Phases

Bortezomib will be given at 1.3mg/m2/dose IV Push on Days 1, 4, and 8 of each 21-day cycle.

Doses should be rounded per institutional standard.

The dose of bortezomib will be recalculated at the beginning of each cycle based on BSA using a weight and height obtained up to 5 days prior to cycle start date.

5.2.2 Bortezomib (Velcade)

Formulation and Stability: Bortezomib is a commercially available product and will not be supplied for this study. Bortezomib should be stored and dispensed per institutional standards and product insert.

Source and Pharmacology: Bortezomib (PS - 341) is a reversible inhibitor of the chymotrypsin -like activity of the 26S proteasome (a multicatalytic protease present in all eukaryotic cells). The 26S proteasome is a large protein complex that degrades proteins that have been conjugated to ubiquitin. The ubiquitin-proteasome pathway plays an essential role in regulating the intracellular concentration of specific proteins, and constitutes the major mechanism for intracellular protein degradation (80%). Those intracellular proteins which maintain homeostasis within cells include numerous regulatory proteins involved in cellular integrity, such as cell-cycle control, cellular apoptosis, transcription factor activation, and tumor growth via ATP-dependent processes. Inhibition of the 26S proteasome prevents this targeted proteolysis, which can affect multiple signaling cascades within the cell. This disruption of normal homeostatic mechanisms can lead to cell death. The binding of bortezomib to human plasma proteins averages 83% over a concentration range of 100 to 1000 ng/mL. The mean elimination half-life of bortezomib after multiple dosing ranged from 40 to 193 hours after the 1 mg/m 2 dose and 76 to 108 hours after the 1.3mg/m2 dose. In vitro studies with human liver microsomes and human cDNA-expressed cytochrome P450 isozymes indicate that bortezomib is primarily oxidatively metabolized via cytochrome P450 enzymes 3A4, 2C19, and 1A2. Bortezomib metabolism by CYP 2D6 and 2C9 enzymes is minor. The major metabolic pathway is deboronation to form 2 deboronated metabolites that subsequently undergo hydroxylation to several metabolites. Deboronated bortezomib metabolites are inactive as 26S proteasome inhibitors.

Bortezomib is metabolized by multiple cytochrome P450 (CYP) enzymes, with CYP3A4 being the primary contributor, and with only minor contributions of CYPs 2C19, 1A2, 2C9 and 2D6. It is a weak inhibitor of CYP2C19 and does not inhibit CYP1A2, 2C9, 2D6, and 3A4 at clinically relevant concentrations (IC50 > 30 μM). Co-administration of strong CYP3A4 inducers should be discussed with pharmacy prior to use throughout therapy with bortezomib (refer to Appendix II for a list of strong inducers). Co-administration of strong and clinically relevant moderate CYP3A4 inhibitors should be avoided until 72 hours beyond administration of Day 8 bortezomib for each course (exception: administration of prophylactic fluconazole may begin ≥ 24 hours after the Day 8 bortezomib dose). Similarly, patients receiving strong and clinically relevant moderate inhibitors should discontinue use of these agents 72 hours prior to start of Day 1 bortezomib in all courses (refer to Appendix II for a list of strong and clinically relevant moderate inhibitors). Additional inducers or inhibitors of CYP450 enzymes can be found at http://medicine.iupui.edu/clinpharm/ddis.

In vitro and in vivo studies showed that green tea compounds, ascorbic acid (vitamin C) and other antioxidants, have the potential to significantly inhibit the activity of bortezomib. To avoid the risk of any possible interaction it is recommended that green tea containing products and any supplemental products containing vitamin C, flavonoids or other antioxidants (eg, vitamins, herbal supplements) be discontinued from at least 24 hours prior to the initiation of bortezomib through 72 hours after the last bortezomib dose. Injectable multivitamins used as a component of parenteral nutrition should also be avoided during this time period to minimize the risk of direct

vitamin C inactivation of bortezomib. In addition, it is recommended that the total dietary intake of vitamin C not exceed the RDA for age. Normally balanced diets are acceptable; supplementation with high doses of vitamin C or injectable vitamin C should be avoided.

Bortezomib Risks:

Risks and side effects related to Bortezomib include those which are:

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

• **A feeling of weakness and/or tiredness**
• **Nausea and/or vomiting**
• **Constipation or diarrhea**
• **Loss of appetite**
• **Fever**
• **Fewer red blood cells and platelets in the blood**
  ○ **a low number of red blood cells can make you feel tired and weak**
  ○ **a low number of platelets causes you to bruise and bleed more easily**
• **Fluid retention and build-up in the arms and legs leading to swelling and an increase in weight**
• **Infection**
• **Nerve damage that may cause pain, burning, numbness, and tingling in the hands and feet and may affect the ability to perform tasks that require fine movements**
• **Nerve damage that may cause muscle weakness or paralysis in the hands and feet and may affect the ability to perform tasks that require fine movements**
	• **Low blood pressure**
	• **Dizziness**
	• **Fainting**
	• **Difficulty sleeping or falling asleep**
	• **Chills including shaking chills**
	• **Skin rash with the presence of macules (flat discolored area) and papules (raised bump)**
	• **Excessive loss of water from the body**
	• **Acid or upset stomach (heartburn)**
	• **Pain which may be in the abdomen (belly), back, bone, head, joints, arms and legs, muscles, and nerves**
	• **Headache or head pain**
	• **Cough**
	• **Shortness of breath**
	• **Fewer white blood cells in the blood**
	  ○ **a low number of white blood cells can make it easier to get infections**
	• **Low numbers of white blood cells called lymphocytes that may last a long time and make it easier to get infections which may be life threatening**
	• **A stoppage (or blockage) of the intestine which may require treatment**
	• **Inflammation and/or sores in the mouth and/or throat that may make swallowing difficult and are painful (painful mouth sores)**
	• **Bleeding in the gut that may show in the stools**
	• **Nose bleed**
	• **Fever with a low white blood cell count which could indicate infection and may require hospitalization and treatment with antibiotics**
	• **Infection caused by hard to treat bugs including bacteria, virus, and fungus**
	• **Muscle weakness of the whole body**
	• **Anxiety**
	• **Blurred vision**
	• **Fluid build-up in the lungs that can make you feel short of breath**
		• **A hole in the intestines which would cause leakage into the abdomen (belly) with pain and infection**
		• **Damage to the brain associated with high blood pressure which may lead to difficulty thinking, carrying out normal tasks, headache, seizures (convulsions), difficulty seeing, blindness, or other visual changes, which if caught early can be reversed**
		• **Severe damage to the brain tissue which could lead to difficulty carrying out normal daily tasks or could lead to coma**
		• **Severe kidney damage (which may be permanent)**

5.3 Guidelines for Dose Modifications

Toxicities and dose modifications 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 8.5) should be utilized to determine the relationship of adverse events to the treatment.

5.4 Dose Delay

Subjects without bone marrow metastases must have an ANC > 500/µl before starting Day 1 of each cycle. Subjects experiencing any related toxicity specified in 5.5 or any intolerable toxicity on a scheduled treatment day will have their dose of study drug held per Sections 5.6 and 5.7. If toxicity is encountered on Day 1 of any cycle, the day of resolution and administration of the dose will be considered Day 1 of that cycle. Treatment may be delayed no more than 14 days to allow recovery from related toxicity.

5.5 Definition of Dose-Limiting Toxicity (DLT)

DLT will be defined as any of the following events that are possibly, probably or definitely attributable to DFMO or bortezomib.

Dose limiting hematologic and non-hematologic toxicities are defined differently.

5.5.1 Non-Hematologic Dose-Limiting Toxicity

5.5.1.1 Any Grade 3 or Grade 4 non-hematological toxicity attributable to the investigational drug with the specific exclusion of:

- Grade 3 nausea, vomiting, diarrhea unless > 3 days despite optimal treatment with antiemetics
- ALT/AST elevation > 10x ULN that returns to Grade ≤ 2 or baseline prior to the time for the next treatment cycle. Note: For the purposes of this study the ULN for ALT/AST is defined as 50 U/L.
- Direct Bilirubin level > 1.5x – 3x upper limit of normal
- Grade 3 fever
- Grade 3 infection
- Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia responsive to oral supplementation.
- Grade 2 allergic reactions that results in discontinuation of study drug

5.5.1.2 A peripheral neuropathy that results in a discontinuation of the bortezomib dose (See Section 5.7).

5.5.1.3 Non-hematological toxicity that causes a delay of >14 days between treatment cycles.

5.5.2 Hematological dose limiting toxicity (for bone marrow negative patients only).

Hematological dose limiting toxicity is defined as:

- Grade 4 neutropenia for > 7 days
- Grade 4 thrombocytopenia for > 14 days (excluding special populations defined in 8.4.2)
- Myelosuppression that causes a delay of >14 days between treatment cycles.

****Any suspected or confirmed dose-limiting toxicity should be reported immediately (within 24 hours of discovery of the event) to the NMTRC and Study Chair.****

5.6 DFMO Dose Modification

Subjects experiencing any dose limiting toxicity (DLT) specified above (Section 5.5) attributable to DFMO or any other intolerable toxicity will have their dose of DFMO held until toxicities have reverted to ≤ Grade 2 toxicity. Upon resolution of the toxicity, subjects will receive a -1 dose reduction of DFMO to one step down on the dosing table in section 5.1.2 Example- If the subject is currently taking 3 tabs twice a day they will step down to the weight range/dose directly below the one they are at and take 2 tabs twice a day. 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 one more time to a -2 dose level. At that point if they experience another dose reducing toxicity (as defined here) they will be required to go off protocol therapy.

If there is no resolution of above toxicity attributable to DFMO to ≤Grade 2 by 14 days, DFMO should be discontinued, and subjects should be discontinued from the study.

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For subjects entering this trial with bone marrow disease and for subjects entering this trial with platelet transfusion dependency, modifications will not be made based on platelet count.

5.7 Bortezomib Dose Modification

Subjects experiencing any dose limiting toxicity (DLT) specified above (Section 5.5) attributable to bortezomib or any other intolerable toxicity will have their dose of bortezomib held until toxicities have reverted to ≤ Grade 2 toxicity. Upon resolution of the toxicity, subjects will have bortezomib restarted per recommendations below.

Bortezomib-Related Hepatotoxicity

Subjects with mild hepatic impairment do not require dose adjustment of bortezomib. Subjects with severe hepatic impairment should receive bortezomib at modified doses as outlined below:

	Direct Bilirubin Level	Bortezomib Dose Modification
Moderate*	> 1.5x – 3x upper limit of normal * Will not be considered a DLT	Reduce bortezomib to 0.7 mg/m^2 for all doses in the cycle in which hepatotoxicity is present. Consider dose escalation to 1 mg/m^2 if transaminitis resolves and is not possibly, probably or definitely related to bortezomib.
Severe	> 3x upper limit of normal	

-No adjustment necessary for elevated SGOT (ALT).

Bortezomib-Related Peripheral Neuropathy

Subjects with bortezomib-related neurotoxicity will remain on protocol therapy but have bortezomib dose de-escalated or held as specified below. Peripheral neuropathy will be closely monitored during each course of treatment and toxicities graded using CTCAE v 4.0.

Peripheral sensory neuropathy grading should be based on the maximum toxicity occurring during the previous course. All dose modifications should be based on the worst preceding toxicity. Bortezomib dose will be decreased for sensory peripheral neuropathy as follows:

Severity of peripheral sensory neuropathy	**Bortezomib modification**
Grade 1 without pain	None
Grade 1 with pain or Grade 2 without pain*	Decrease bortezomib to the next lower dose level. For patients receiving 1.0 mg/m2/day bortezomib decrease to 0.7 mg/m2/day. * Will not be considered a DLT
Grade 2 with pain or Grade 3*	Hold bortezomib treatment until symptoms have resolved to < Grade 1. When toxicity resolves, reinitiate bortezomib at the next lower dose level. For patients receiving 1.0 mg/m2/day bortezomib, decrease to 0.7 mg/m2/day. *Will not be considered a DLT
Grade 4*	Discontinue bortezomib *DLT

If Grade 3 peripheral sensory neuropathy persists for > 2 weeks, bortezomib should be discontinued. Subjects who discontinue bortezomib will be off protocol therapy.

If Grade 3 peripheral sensory neuropathy recurs despite bortezomib dose reduction to 1 mg/m^2, the subject should discontinue bortezomib. Subjects who discontinue bortezomib will be off protocol therapy.

Pulmonary Toxicity

There have been rare reports of acute diffuse infiltrative pulmonary disease of unknown etiology such as pneumonitis, interstitial pneumonia, lung infiltration and acute respiratory distress syndrome (ARDS) in subjects receiving bortezomib. For this reason, pulmonary toxicity will be monitored carefully during the study. **Bortezomib should be held in subjects with ARDS that may be bortezomib related.**

Monitoring

Pulmonary function will be monitored by pulse oximetry. Measurement of pulse oximetry should be documented within 12 hours prior to each dose of bortezomib. A chest radiograph should be performed with the development of respiratory symptoms. In the absence of identifiable causes of respiratory stress, all subjects should demonstrate oxygen saturation on room air of ≥ 94% at sea level (> 90% at high altitude) prior to each bortezomib dose. Pulmonary toxicity due to bortezomib is frequently delayed and can occur up to 3 weeks after the final dose of bortezomib is administered.

Pulmonary symptoms such as increased respiratory rate may indicate toxicity related to bortezomib and pulse oximetry should be checked for persistent, unexplained hyperpnea. Respiratory symptoms such as cough, hypoxia and dyspnea should be aggressively evaluated.

Dose Reductions and Discontinuation of Bortezomib for Pulmonary Toxicity

Bortezomib will be dose reduced to 1 mg/m2 for any resolving Grade 3 pulmonary toxicity (excluding hiccups or voice changes/laryngitis), including hypoxia that is possibly, probably or definitely related to bortezomib. Subjects that experience a Grade 4 pulmonary toxicity possibly, probably or definitely related to bortezomib (excluding hiccups and voice changes/laryngitis) will discontinue bortezomib. Subjects who discontinue bortezomib will be removed from protocol therapy.

Subjects re-experiencing the same bortezomib-related qualifying Grade 3 pulmonary toxicity following a single bortezomib dose reduction will be removed from protocol therapy.

Dose Reduction for Other Bortezomib-Associated Toxicities

Bortezomib will be dose reduced to 1 mg/m2 for non-hematological severe toxicities (other than those described above) that are possibly, probably or definitely related to bortezomib. A severe toxicity is defined as any Grade 3 or 4 toxicity as described in Section 5.5.

For subjects entering this trial with stable platelets between 50,000-100,000/ µl (those with poor bone marrow recovery after previous treatment) study drug should be held if platelets are Grade 3 as defined in Section 8.4.2. For subjects entering this trial with platelet transfusion dependent, modifications will not be made based on platelet count.

Bortezomib doses may not be re-escalated in subjects with dose limiting toxicity.

If a specific bortezomib-related toxicity recurs despite one dose reduction, subject will discontinue study.

5.8 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 subjects 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.

5.9 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; another site of disease must be available for response evaluation.

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

6.0 STUDY PROCEDURES AND ASSESSMENTS

6.1 Enrollment of Subjects

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 and dose level 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 subjects' legal representatives) must provide written informed consent before any study specific assessments may be performed.

6.2 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 21 days prior to the first dose of study drug. Studies must be done *after* last previous treatment for malignancy:

1. Signed informed consent form. All subjects (or subjects' 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 of measurable disease sites;
3. MIBG or PET scan;
4. Audiogram;
5. Bilateral 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. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation;
4. BSA calculation (from body weight and height);
5. ECOG Performance status/Lansky Play status (Appendix I);
6. CBC with differential;
7. Serum electrolytes, blood urea nitrogen (BUN), creatinine, bilirubin, LDH, ALT, AST and ferritin;
8. C-reactive protein (CRP*)* and Erythrocyte sedimentation rate *(*ESR*)* and IL-6 level (IL-6 recommended).
9. Urine for Vanillylmandelic Acid (VMA) & Homovanillic Acid (HVA);
10. Serum or Urine pregnancy test for female subjects of child bearing potential (onset of menses or ≥13 years of age);
11. Concomitant medications/therapies;
12. Confirmation of inclusion and exclusion requirements;

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 and drug dose 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.

6.3 Treatment Phase – Cycle 1

The first cycle will be 21 days in duration. The following procedures must be completed:

Cycle 1 Day 1 (procedures listed here must be done on Day 1):

1. Physical examination (including body weight), including documentation of an update of all previous abnormalities, any new abnormalities, and a detailed neurological exam
2. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation;
3. Review and recording of concomitant medications;
4. Monitoring and documentation of all AEs and review of concurrent illnesses
5. Optional: Urine for Biological Correlates per section 12.0 (additional consent required);
6. Optional: Blood for Biological Correlates per section 12.0 (additional consent required);
7. Dispense drug dosing diary;
8. DFMO dosing begins;
9. Bortezomib administration.

Cycle 1 Day 4 (+/- 1 day window)

Subjects will return to the clinic on Cycle 1 Day 4 for Bortezomib and evaluations. The following evaluations will be conducted:

1. History and Physical exam;
2. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation;
3. Monitoring and documentation of all AEs and review of concurrent illnesses;
4. Review and recording of concomitant medications;
5. DFMO dosing continues daily;
6. Bortezomib administration.

Cycle 1 Day 8 (+/- 1 day window)

Subjects will return to the clinic on Cycle 1 Day 8 for evaluations. The following evaluations will be conducted:

1. History and Physical exam;
2. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation;
3. CBC with differential;
4. Serum electrolytes, BUN, creatinine, bilirubin, ALT, AST; and LDH;
5. Monitoring and documentation of all AEs and review of concurrent illnesses;
6. Review and recording of concomitant medications;
7. DFMO dosing continues daily;
8. Bortezomib administration.

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, blood pressure, and O2 saturation;
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. DFMO dosing continues daily;
8. Optional: Urine for Biological Correlates per section 12.0 (additional consent required);
9. Optional: Blood for Biological Correlates per section 12.0 (additional consent required).

6.4 Treatment Phase – Cycles 2-6

All Cycles will be 21 days in duration. The following procedures must be completed:

Cycle 2-6 Day 1 (+/- 3 day window to start Day 1 <u>treatment</u>)

The following <u>procedures</u> may be performed up to 5 days prior to starting treatment/Day 1 unless otherwise indicated:

1. History and Physical examination (including body weight), including documentation of an update of all previous abnormalities, any new abnormalities, and a detailed neurological exam (same day);
2. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation (same day);
3. Review and recording of concomitant medications;
4. Monitoring and documentation of all AEs and review of concurrent illnesses (same day);
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*),* IL-6 level (IL-6 recommended);
10. Urine for Vanillylmandelic Acid (VMA) & Homovanillic Acid (HVA);
11. Optional: Urine for Biological Correlates per section 12.0 (additional consent required);
12. Optional: Blood for Biological Correlates per section 12.0 (additional consent required);
13. Collection of previous cycle drug dosing diary and dispensing of new drug dosing diary;
14. Serum or Urine pregnancy test for female subjects of child bearing potential (onset of menses or ≥13 years of age);
15. DFMO dosing continues daily;
16. Bortezomib administration.

Cycle 2-6 Day 4 (+/- 1 day window)

Subjects will return to the clinic on Cycle 1 Day 4 for Bortezomib and evaluations. The following evaluations will be conducted:

1. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation;
2. Monitoring and documentation of all AEs and review of concurrent illnesses;
3. Review and recording of concomitant medications;
4. DFMO dosing continues daily;
5. Bortezomib administration.

Cycle 2-6 Day 8 (+/- 1 day window)

Subjects will return to the clinic on Cycle 1 Day 8 for evaluations. The following evaluations will be conducted:

1. History and Physical exam;
2. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation;
3. CBC with differential;
4. Monitoring and documentation of all AEs and review of concurrent illnesses;
5. Review and recording of concomitant medications;
6. DFMO dosing continues daily;
7. Bortezomib administration.

Cycle 2-6 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, blood pressure, and O2 saturation;
3. CBC with differential;
4. Serum electrolytes, BUN, creatinine, bilirubin, ALT, AST;
5. Review and recording of concomitant medications;
6. Monitoring and documentation of all AEs and review of concurrent illnesses.
7. DFMO dosing continues daily.

End of Cycles 2, 4, and 6, then every 2 cycles thereafter while on study drug

1. MIBG or PET scan (use same as baseline)
2. CT/MRI (use same radiologic method as baseline);
3. Bone marrow biopsy and aspirate if positive at study entry (If the subject has signed informed consent for the use of tissue in the correlative biologic study please send additional samples per section 12.0);

End of Cycles 2, 6, then yearly thereafter

1. Audiogram

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

6.5 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 3 months from the time the subject is off-treatment for a period of 1 year, then yearly for up to five years.

6.6 Protocol Treatment Completion

Subjects who receive 6 total 21-day treatment cycles will be considered as having completed the protocol. Additional treatment cycles may be delivered in a maintenance setting if there are no safety concerns and there is no disease progression. Maintenance monitoring will be conducted as described for Subsequent Cycles (Section 6.7).

6.7 Subsequent (Maintenance) Cycles (for subjects that have completed the 6 protocol treatment cycles)

Drug administration will be according to guidelines with premedication as specified. The following evaluations will be performed on the days of each cycle as indicated. Evaluations will be performed prior to dosing unless otherwise indicated.

All Cycles will be 21 days in duration. The following procedures must be completed:

Day 1 (+/- 3 day window to start Day 1 treatment)

The following procedures may be performed up to 5 days prior to starting treatment/Day 1 unless otherwise indicated:

1. History and Physical examination (including body weight), including documentation of an update of all previous abnormalities, any new abnormalities, and a detailed neurological exam (same day);
2. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation (same day);
3. Review and recording of concomitant medications;
4. Monitoring and documentation of all AEs and review of concurrent illnesses (same day);
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. Urine for Vanillylmandelic Acid (VMA) & Homovanillic Acid (HVA);
10. Serum or Urine pregnancy test for female subjects of child bearing potential (onset of menses or ≥13 years of age).
11. DFMO dosing continues daily;
12. Bortezomib administration.

Day 4 (+/- 1 day window)

Subjects will return to the clinic on Cycle 1 Day 4 for Bortezomib and evaluations. The following evaluations will be conducted:

1. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation;
2. Monitoring and documentation of all AEs and review of concurrent illnesses;
3. Review and recording of concomitant medications;
4. DFMO dosing continues daily;
5. Bortezomib administration.

Cycle Day 8 (+/- 1 day window)

Subjects will return to the clinic on Cycle 1 Day 8 for evaluations. The following evaluations will be conducted:

1. History and physical exam;
2. Vital signs, including temperature, pulse rate, blood pressure, and O2 saturation;
3. CBC with differential;
4. Monitoring and documentation of all AEs and review of concurrent illnesses;
5. Review and recording of concomitant medications;
6. DFMO dosing continues daily;
7. Bortezomib administration.

6.8 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. 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, and O2 saturation;
4. CBC with differential;
5. Serum electrolytes, BUN, creatinine, bilirubin, LDH, ALT, AST;
6. Urine for Vanillylmandelic Acid (VMA) & Homovanillic Acid (HVA);
7. Review and recording of concomitant medications;
8. Monitoring of AEs and review of concurrent illnesses;
9. Collect previous cycles drug dosing diaries (if early withdrawal);
10. MRI/CT; MIBG or PET scan; **if not already obtained within the previous two cycles.**
11. Bone marrow biopsy and aspirate (if positive at study entry); **if not already obtained within the previous two cycles;**
12. Audiogram (if clinically indicated).

Any subject with a suspected study drug-related toxicity at the follow-up visit must be followed until the related event(s) has 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 new AE collection at the date of starting the new therapy but will continue survival follow up.

6.9 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):

- 3 months
- 6 months
- 9 months
- 1 year
- Then Yearly after (up to 5 years total)

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

7.0 Efficacy Assessments

7.1 Tumor Assessments/Scans

Tumor assessments/imaging studies must be obtained at baseline and at the end of the second and fourth cycles, and again after every other cycle. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

All radiological images must be available for source verification. Images may be submitted for extramural review for final assessment of antitumor activity.

Subjects who come off study for any reason should have a final end of study disease-specific assessment done.

7.2 Scan Submission:

All required study scans (CT's, MRI's, and MIBG's) will be reviewed by central radiology. All study required scans should be sent on disc. Scans will be sent as a batch at the end of each subject's therapy or sooner if any concern for progression to:

Alyssa VanderWerff
NMTRC
Clinical Program Coordinator
100 Michigan Avenue NE MC 272
Grand Rapids, MI 49503
Tel: (616) 267-0327
E-Mail: Alyssa.VanderWerff@helendevoschildrens.org

7.3 Response Criteria

Overall response rate (ORR) in subjects with radiologically assessable disease will be determined by CT or MRI by cross-sectional imaging, by MIBG or PET scans, and/or bone marrow assessment.

Response Assessment: Each subject will be classified according to their "best response" for the purposes of analysis of treatment effect. Best response is determined from the sequence of the objective status described below.

Response Criteria for Subjects with Solid Tumors: This study will use the (RECIST) Response Evaluation Criteria in Solid Tumor (version 1.1) from the NCI. Key points are that a maximum of 5 target lesions are identified and that changes in the ***largest*** diameter (uni-dimensional measurement) of the tumor lesions are used in the RECIST v1.1 criteria.

- Measurable disease: The presence of at least one lesion that can be accurately measured in at least one dimension with the longest diameter at least 10 mm (CT scan slice thickness no greater than 5 mm). The investigator will identify up to 5 measurable lesions to be followed for response. Previously irradiated lesions must demonstrate clear evidence of progression to be considered measurable.
- Measurable Disease Response will be assessed at end of Cycle 2 and then at least every other cycle
- Serial measurements of lesions are to be done with CT or MRI, using the same method of assessment is to be used to characterize each identified and reported lesion at baseline and during follow-up.
- Quantification of Disease Burden: The sum of the longest diameter (LD) for all target lesions will be calculated and reported as the disease measurement.
- **Complete Response (CR):** Disappearance of all target and non-target lesions.
- **Very Good Partial Response (VGPR):** Greater than 90% decrease of the disease measurement for CT/MRI lesions, taking as reference the disease measurement done to confirm measurable disease at study entry. Non-target CT/MRI lesions stable to smaller in size.
- **Partial Response (PR):** At least a 30% decrease in the disease measurement, taking as reference the disease measurement done to confirm measurable disease at study enrollment. No new lesions or progression of any non-target measurable lesion.
- **Stable Disease (SD):** Neither sufficient decrease to qualify for PR or sufficient increase to qualify for PD taking as reference the smallest disease measurement since the treatment started.
- **Progressive Disease (PD):** At least a 20% increase in the sum of the disease measurements for measurable lesions, taking as reference the smallest disease measurement recorded since the start of treatment (nadir), or the appearance of one or more new lesions.

Response Criteria for Subjects with Bone Marrow Disease:

- Those subjects with morphologic evidence of neuroblastoma by routine H and E staining (NSE staining only is not evaluable) will be evaluable to assess bone marrow response.
- **Complete response:** No tumor cells detectable by routine morphology on two consecutive bilateral bone marrow aspirates and biopsies done at least three weeks apart after study entry.
- **Progressive disease**: Tumor seen on morphology on two consecutive bilateral bone marrow aspirates and biopsies done at least three weeks apart in subjects who had NO tumor in bone marrow at study entry. (Note: Subject may be declared as progressive disease in bone marrow after only one diagnostic bone marrow at the discretion of the treating physician after discussion with the study chair.)
- **Stable disease**: Persistence of any amount of tumor in the bone marrow by morphology that does not meet criteria for either complete response or progressive disease.

Response Criteria for Subjects with MIBG/PET Positive Lesions

- Subjects who have a positive MIBG or PET scan at the start of therapy will be evaluable for MIBG/PET response. The use of I123 for MIBG imaging is recommended for all scans. If this radioisotope is unavailable at the treating institution, the use of the same radioisotope for all MIBG scans for an individual subject is strongly encouraged.
- **Complete response** = complete resolution of all MIBG/PET positive lesions
- **Partial response** = resolution of at least one MIBG/PET positive lesion, with persistence of other MIBG/PET positive lesions.
- **Stable disease** = no change in MIBG/PET scan in number of positive lesions (includes subjects who have same number of positive lesions but decreased intensity)
- **Progressive disease** = Development of new MIBG/PET positive lesions

The intensity of MIBG/PET uptake is not to be considered in the above institutional evaluation.

Duration of response:

Duration of response is defined as the period of time from when measurement criteria are met for complete response (CR) or partial response (PR), whichever is first recorded, until the first date that recurrent or progressive disease (PD) is objectively documented (taking as reference for PD the smallest measurements recorded since the treatment started)

The assessment of response will include the initial measurable targets and will be performed after the first and second cycle, then after every other cycle. Serial results of bone marrow aspirates, biopsies and urinary catecholamines will be reviewed for responding subjects to confirm response or lack of progression.

8.0 Adverse Event Reporting

Common Terminology Criteria for Adverse Events (CTCAE)
This study will utilize the version 4.0 of the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the CTCAE version 4.0 can be downloaded from the NCI website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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 subject 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 subject 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 subject/event outcome or action criteria usually associated with events that pose a threat to a subject'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 subject, 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; subject discontinued from the study
- Outcome: complete recovery or return to baseline; unknown/lost to follow-up; adverse event persisting; subject 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 treatment or until a new treatment is started. Suspected study drug-related toxicity at the 30 day follow-up visit must continue to be followed until resolution to baseline or ≤ Grade 2 or stabilization of the event.

8.3 Expedited Reporting of Adverse Events

All fatal, life–threatening, or dose limiting 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). The lead site, NMTRC, will then report directly to the safety officer, FDA if required, and KC Pharma as appropriate. 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 of discovery as well as to the appropriate regulatory authorities (local IRB). The lead site will then report directly to the safety officer, FDA if required, and KC Pharma as appropriate.

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.

NMTRC Reporting Contact

Please report all expedited reports to:

Alyssa VanderWerff and Genevieve Bergendahl, RN
NMTRC
Tel: (616) 267-0327
E-Mail: Alyssa.VanderWerff@helendevoschildrens.org
E-Mail: Genevieve.Bergendahl@helendevoschildrens.org

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 subject was at risk of death at the time of the event
- **Fatal** corresponds to an event that results in the death of the subject

8.5 Grading of Hematologic Adverse Events in special populations

Subjects who are platelet transfusion dependent at study entry will not have grading of platelet levels.

Subjects who have bone marrow involvement at study entry will not have grading of any hematologic levels.

8.6 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 Subject 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- 6 cycles
- Progressive 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 withdrawal from 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 subjects with relapsed or refractory neuroblastoma.

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 2 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.

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:

The primary outcome measure is the Overall Response Rate (ORR). A two-stage minimax test for a single binomial population using a directional 5% Type I error level of the null hypothesis that the Overall Response Rate is <=1% versus the alternative hypothesis of the Overall Response Rate being 10% will require 32 evaluable subjects to achieve an 80% power for each of the subject stratum.. The two-stage decision rule for each strata is as follows:

Stage 1- When 20 evaluable subjects have been entered into the study and observed for Overall Response, the study will terminate and accept the null hypothesis of a <=1% Overall Response Rate if none (0) of these 20 subjects show a Complete Response or Partial Response (i.e. stop due to futility). If 1 or more of the 20 evaluable subjects show an Overall Response, then the study will continue to Stage 2.

Stage 2- When the full 32 evaluable subjects have been entered into the study and observed for an Overall Response, the study would terminate and accept the null hypothesis of an Overall Response Rate <=1% if 1 or fewer of the 32 subjects show a Complete Response or Partial Response. If 2 or more of the 32 evaluable subjects show an Overall Response, the study will terminate and will result in the rejection of the null hypothesis and conclude that the 10% Overall Response Rate is more likely.

Total subjects: Assuming that approximately 10% of subjects will be non-evaluable, the actual sample size needed will be n =35 per stratum.

The secondary outcome measure is Progression Free Survival (PFS) at 100 days. The sample size discussion that follows mimics the two-stage design approach used for the primary outcome measure, ORR. However, since this sample size and decision rule description relate to PFS at 100 days, this section is illustrative only and will not be used to impact on study accrual.

Assuming an exponential model with the null hypothesis median Progression Free Survival of 42 days and an alternative median Progression Free Survival of 82 days, then one would expect the proportion of subjects who are progression free at 100 days of follow-up to be19.2% under the null value of 42 days and 42.9% under the alternative value of 82 days. Shorter follow-up time frames would increase the proportion of progression free subjects while a longer follow-up would decrease these proportions.

Rationale for the null hypothesis effect size is derived from recent Children's Oncology Group reports in multiply relapsed NB patients. Forty-two days was selected as the standard for comparison because it is the median time to progression for a control population of NB patients recently treated on other Phase I and II trials (CCG-8607, ANBL00321, NANT-0103, CCG-0926, and CCG-0961).

Progression Free Survival (PFS) is defined as the period from the first day of administration of study drug (DFMO) until the criteria for progression are met taking as reference the screening measurements. The definition of "progression" as applied to this control population will include deaths from any cause. From the control patients in the COG study (n=136), the 42 day PFS rate was 50% ± 4%. Day 42 corresponds to the time at which most patients will have completed 2 courses of study therapy (assuming no delays, 21 days per course for 2 courses is 42 days). An assessment of disease will be required after the completion of 2 courses. Therefore, the standard for comparison, which DFMO/Velcade is anticipated to exceed, has been set at a median time to progression of 42 days.

Using a one sample exact binomial test of the 19.2% versus the 42.9% progression free survival values at 100 days, the overall sample size would be n = 23 using a two-stage minimax test with directional 5% Type I error level and 80% power
The two-stage decision rule is as follows:

Stage 1- When 18 evaluable subjects have been entered into the study and observed for at least 100 days, the study will terminate and accept the null hypothesis of 19.2% progression free

survival if 5 or fewer of the 18 subjects are progression free at 100 days of follow-up (i.e. stop due to futility). If 6 or more of the 18 evaluable subjects are progression free, then the study will continue to Stage 2.

Stage 2- When 23 evaluable subjects have been entered into the study and observed for at least 100 days, the study would terminate and accept the null hypothesis of 19.2% progression free survival if 7 or fewer of the 23 subjects are progression free at 100 days of follow-up. If 8 or more of the 23 evaluable subjects are progression free, the study will terminate and will result in the rejection of the null hypothesis and conclude that the 42.9% progression free survival at 100 days is more likely.

It is thus clear that the analysis of PFS will be able to progress without difficulty if the full sample size of 32 evaluable subjects per stratum is achieved for the ORR primary outcome evaluation. It is also clear that even with an early termination for futility for the ORR outcome at n = 20 subjects at Stage I in either of the subject strata, that the PFS secondary outcome should be testable although with slightly lower power.

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 or for any study drug <u>related</u> Grade 5 toxicity occurring on study, whichever occurs first. The Board will be responsible for recommendations regarding possible termination and/or early reporting of the study.

Study will be on hold for safety monitoring by DSMB review when:

1. Any deaths deemed related to the study drug by the treating PI while on study or occurring less than 30 days after medications ended or 2 serious adverse events possibly related to protocol within 60 days.
2. Any Life threatening hemorrhage requiring hospitalization
3. Any intracerebral hemorrhage
4. Any other reason that the NMTRC feels it is in the best safety interest of the subjects.

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 representatives of KC Pharma and/or 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 as required.

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.

Neuroblastoma Tumor Cell Analysis –Performed by Pediatric Oncology Translational Research Laboratory (Section 12.1.2) Studies will be performed to determine the dose effect of DFMO on biological correlates. Biological Correlates will include an analysis of neuroblastoma cells isolated from bone marrow and from tumor biopsies. Cells will be grown in culture as well as in mice xenograft models. Research studies will include immunohistochemistry, western blot, qRT-PCR of bone marrow tumor cells or tumor to study Let-7, ODC, Lin28, MYCN, OKT-4, BMI1, HMGA2, GLUT-4, p-AKT, NF-kB, SOCS3. Studies will measure thymidine and polyamine levels. In vitro research studies of tumor cells collected above will include: Genomic analysis of cells pre- and post- treatment, cell viability assays to determine subject IC50 and correlation of *in vitro* response to *in vivo* response.

12.1 Biological Correlative Studies Sample Instructions

12.1.1 Optional Urine Polyamines Collection:

On Days 1 and 15 of Cycle 1 and on Day 1 of cycles 2-6 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 4oC (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.

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

12.1.2 Optional Blood Collection

1. **Sample One**- 5ml of blood in purple-top (EDTA) tube(s) will be collected

   A. On Days 1 and 15 of Cycle 1 and on Day 1 of cycles 2-6

   B. The specimen should be placed on ice immediately upon draw and sent on an ice pack immediately via FED-EX overnight along with sample 2 below. (If Friday collection site may store in refrigerator and ship on Monday)-

   Tube(s) will go 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. **Sample Two**- 5ml of blood in purple-top (EDTA) tube(s) will be collected

   A. On Days 1 and 15 of Cycle 1 and on Day 1 of cycles 2-6

   B. The specimen 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)-

   Tube(s) will go 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

12.1.3 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 may be sent under that study instead):

All Subjects- At Enrollment, End of cycle 2, 4, and 6, and Early Withdrawal (if indicated).

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:

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

12.1.4 Optional Tumor Sample Collection

If subject agrees to optional biology portion of study, and undergoes a standard of care tumor biopsy, tumor samples should be sent as follows:

Sample Collection-

Contact Ping Zhao as below for collection materials (kit). Collection instructions can be found in REDCap. To be shipped out on Monday through Thursday only.

A. Viable, fresh tumor > 0.2 grams should be placed in tissue culture media using sterile technique for cell line generation- ambient.
B. Remaining Tumor collected in RNA later- ambient.
C. Remaining Tumor flash frozen samples in cryovials on dry ice.

Record sample information on sample collection form found in REDCap and ship form along with samples (2 packages- one ambient, one on dry ice) via FED-EX overnight 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

12.2 Sample Storage and Destruction

Samples collected for any studies performed in this protocol may be stored in a safe and confidential laboratory area indefinitely to research future scientific questions related to cancer and/or study drugs. Samples will be frozen and stored in a carefully controlled deep freezer. The samples that are stored for future research may only be used by Dr. Sholler and the other investigators who are participating in this study or other NMTRC studies. There is no way to predict exactly what research tests will be performed with the stored samples at this time. Because these tests are for research only, results will not be shared with the subject or their physician. In the unlikely event that the research testing finds important information about the subject's current or future health, the researchers may contact the primary treating oncologist about what the research test results might mean. Only the primary treating oncologist will be notified and the information will not become part of the subject's medical record. The primary treating oncologist may discuss this unexpected finding with the subject, and may

recommend that they see a genetic counselor and/or repeat testing in a clinical (not research) laboratory if needed.

Samples will be stored with a unique identifier and date of collection only; samples will not contain names or any other identifying information. No information that identifies the subject will be used for any of the future research tests and studies. Samples will not be traceable from the lab to the subject. Only the enrolling site will hold the key to the sample identification between the unique code and the subject. 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.

APPENDIX II: LIST OF STRONG AND CLINICALLY RELEVENT MODERATE CYP3A4 INDUCERS AND INHIBITORS

Strong CYP3A4 Inducers:	
Generic Name	*Common Trade Name*
Carbamazepine	Tegretol
Phenobarbital	Luminal
Phenytoin	Dilantin
Rifampin	Rifadin
St. John's wart	N/A
Systemic dexamethasone	Decadron
Strong* and clinically relevant moderate CYP3A4 Inhibitors:	
Generic Name	*Common Trade Name*
Aprepitant	Emend
Clarithromycin*	Biaxin
Erythromycin	Eryc, EryPed
Fluconazole	Diflucan
Grapefruit and its juice	N/A
Itraconazole*	Sporanox
Ketoconazole*	Nizoral
Voriconazole	VFend
Posaconazole*	Noxafil

A list of additional, less potent inducers or inhibitors of CYP450 isoenzymes can be found at http://medicine.iupui.edu/clinpharm/ddis/

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