Protocol Nifurtimox Phase II

V0706

A Phase II Trial of Nifurtimox for Refractory or Relapsed Neuroblastoma or Medulloblastoma.

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INVESTIGATOR SIGNATURE SHEET

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

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

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

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

_____ _____

Investigator's Signature Date

_____

Investigator's Printed Name

_____

Investigational Site Name

A Phase II Trial of Nifurtimox for Refractory or Relapsed Neuroblastoma or Medulloblastoma.

1.0 PROTOCOL CONCEPT

There is currently no curative treatment for children with relapsed/refractory neuroblastoma, and for these children the 5-year survival rate is <10%. Although there have been reports that the survival rate of children with chemosensitive relapsed medulloblastoma can approach 40% following intensive chemotherapy combined with autologous stem cell support, more recent data looking at survival of all patients relapsing after modern combination chemotherapy and radiation is also on the order of 10-15%. As such, new therapeutic approaches are needed to treat these children.

This phase II clinical trial is specifically designed to assess the antineoplastic effect of the antiparasitic drug nifurtimox when given in combination with cyclophosphamide and topotecan to children with relapsed or refractory neuroblastoma or medulloblastoma.

A recently completed phase I trial of nifurtimox in subjects with relapsed/refractory neuroblastoma established a maximum tolerated dose of 30 mg/kg/day. Preclinical work has shown that single-agent nifurtimox has both *in vitro* and *in vivo* cytotoxicity against both neuroblastoma and medulloblastoma cell lines and xenograft models. In the phase I study, nifurtimox was initially given alone, and then combined with topotecan and cyclophosphamide; responses were seen in both phases of therapy.

The present study is a phase II trial of nifurtimox with the addition of standard doses of cyclophosphamide, and topotecan, in subjects with either neuroblastoma or medulloblastoma that is in first or subsequent relapse, or refractory to standard initial therapy. Our primary objective is to evaluate the efficacy of nifurtimox in combination with these chemotherapy agents. Our secondary aims will be to:

- evaluate the peak serum levels of nifurtimox in these subjects and correlate peak level with response;
- correlate tumor genomic analysis with response;
- evaluate quality of life of subjects receiving this drug combination;

2.0 OBJECTIVES

2.1. Primary Objective:

2.1.1. Test the efficacy (best response) and safety of nifurtimox in children with relapsed or refractory neuroblastoma or medulloblastoma, in combination with cyclophosphamide/topotecan

2.1.2. Evaluate Mean Time to Progression

2.2. Secondary Objectives:

2.2.1. Evaluate the correlation between the pharmacologic serum levels of nifurtimox with tumor response.

2.2.2. Quality of life and neurocognitive evaluation/questionnaire.

2.2.3. Biology studies to include: genomic analysis of cells pre- and post-treatment, correlation of in vitro response to in vivo response, flow cytometry of tumor burden in bone marrow and biomarker development.

3.0 BACKGROUND AND PRELIMINARY DATA

3.1. Clinical background

This Phase II trial is designed to evaluate the anti-tumor efficacy of nifurtimox in children with neuroblastoma and medulloblastoma. Nifurtimox is a synthetic nitrofuran compound that has been used safely since the 1970's for the treatment of Chagas' Disease in both adults and children. The efficacy and toxicity of nifurtimox in a cohort of 62 children with Chagas' Disease has recently been reviewed[2]. In 2002, a patient with progressive refractory neuroblastoma who was being treated with cyclophosphamide and topotecan acquired Chagas' Disease from a blood transfusion, and was started on the recommended dose of nifurtimox (15-20 mg/kg/day); her tumor subsequently regressed[1]. Subsequently 6 multiply relapsed neuroblastoma subjects were treated with nifurtimox (20 mg/kg/day) combined with cyclophosphamide and topotecan through compassionate release. Five of the six subjects showed a response to treatment and two of these subjects attained a complete remission on this therapy and remain without evidence of disease for more than one year after beginning nifurtimox.

A Phase I trial of nifurtimox in children with multiply relapsed/refractory neuroblastoma has been completed. The primary objective was to determine the maximum tolerated dose of this medication, which was found to be 30 mg/kg/day divided in 3 doses. The non-dose-limiting toxicities were mainly nausea and neuropathy. The dose limiting toxicities of two patients at 40 mg/kg/day were a grade 3 pulmonary hemorrhage and a grade 3 neuropathy (reversible). The Phase I trial also evaluated tumor response.

Fourteen patients were evaluable for efficacy following nifurtimox treatment alone. According to RECIST criteria, 6 patients displayed stable disease, and 8 had progressive disease. In addition to RECIST criteria, patients were also evaluated for a clinical response as defined by radiological stable disease with either a 50% decrease in urinary catecholamines, clearance of bone marrow disease, or resolution of MIBG activity. A clinical response was seen in 2 (33%) of the 6 patients with stable disease. Of these patients, one had resolution of bone marrow disease, which was confirmed on subsequent bone marrow biopsies, and one had greater than 50% decrease in urinary catecholamines.
Of the ten patients that were evaluable for efficacy after 3 cycles with nifurtimox, cyclophosphamide and topotecan, 3 showed a partial response, 5 had stable disease, and 2 had progressive disease according to RECIST

criteria. Of the 5 patients with stable disease, a clinical response was seen in 3 (60%) of them. One patient continued to have clearance of bone marrow disease and a greater than 50% decrease in urinary catecholamines. The other two patients had a decrease in urinary catecholamines by over 50%. Two patients were non-evaluable for their response to combination therapy. One patient died from progressive disease prior to receiving combination therapy, and one patient refused further treatment. The mean progression-free survival for all 14 patients who began treatment was 9.1 months (median 4.5 months). The mean progression-free survival varied depending upon the number of previous chemotherapy regimens. In patients that had received < 3 previous chemotherapy regimens, the mean progression-free survival was 12.5 months (median 14.0 months), while in patients that had undergone > 3 prior chemotherapy regimens, the mean progression-free survival was 7.6 months (median 7.6 months). The Mantel p value for this analysis was 0.39. Given the extensive prior treatment these subjects had received, these results are encouraging and form the basis of the proposed study utilizing a nifurtimox dose of 30 mg/kg/day.

The combination of cyclophosphamide and topotecan was chosen for combination therapy because it has shown efficacy in the treatment of relapsed neuroblastoma and medulloblastoma; in an earlier phase II study evaluating this combination conducted by the Pediatric Oncology Group, there was a 34% response rate among patients with relapsed neuroblastoma. We hypothesize that there will be greater response in combination with nifurtimox.

Multiple palliative radiation therapy regimens have been utilized in children with painful bony metastases from neuroblastoma. No systematic review of these schedules has been done, but it has been our observation that short courses of radiation (one to three days) may work as well as more prolonged schedules (up to three weeks) and will be standardized as supportive care for bone pain.

3.2. Preclinical background

Nifurtimox is a nitroheterocyclic compound containing a nitro group that is essential for its efficacy. Nifurtimox has been shown to exert its cytotoxic effect specifically by generating free radicals. The nitro group can be reduced to the nitro anion radical in cell-free systems by interacting with cytochrome P-450 reductase, xanthine oxidase, ascorbate, and catecholamines. Nitro anions can then reduce oxygen to the superoxide anion radical and hydrogen peroxide. In Chagas' disease, the nitro anion free radicals and oxyradicals have been shown to be cytotoxic for the parasite *T. cruzi*. The reduction of the nitro group not only generates anion radicals, but interaction with catecholamines[3] appears also to generate semiquinone free radicals that exacerbate damage to functionally important biomolecules, leading to apoptosis of neuroblastoma cell lines. Neuroblastoma cells are known to contain high levels of catecholamines, thereby potentially leading to relatively specific targeting of these cells.

The importance of the interaction between nifurtimox and catecholamines in neuroblastoma cell lines was confirmed by the reduction of cytotoxicity by pretreatment with AMPT, a tyrosine hydroxylase inhibitor that reduces the total amount of catecholamine stored in cells, also reduces nifurtimox-induced cytotoxicity. In addition, the enhanced sensitivity of sympathetic neurons—but not parasympathetic neurons or non-neuronal cells—to nifurtimox supports this conclusion (Sholler, in submission).

3.3. Nifurtimox Induces Apoptosis of Neuroblastoma Cells in vitro and in vivo.

We tested the effect of nifurtimox on growth of CHLA-90, LA1-55n, LA-N2, SMSKCNR, and SY5Y neuroblastoma cells in culture. Nifurtimox inhibited the growth of all five cell lines in a concentration-dependent manner. After 2 days of exposure to 20 mcg/ml of nifurtimox, the number of cells is decreased to 5.9% of vehicle-treated controls in CHLA-90 ($p<0.001$), 31.3% in LA1-55n ($p<0.001$), 32.9% in LA-N2 ($p<0.001$), 3.6% in SMS-KCNR ($p<0.001$), and 1.8% in SY5Y ($p<0.001$). The concentration required to reach 50% inhibition of growth (IC_{50}) for these cell lines was estimated to be 12.18 mcg/ml for CHLA-90, 13.62 mcg/ml for LA1-55n, 17.17 mcg/ml for LA-N2, 10.98 mcg/ml for SMS-KCNR and 9.15 mcg/ml for SY5Y. In contrast, cytotoxic effects were not observed on normal epithelial cells in culture exposed to nifurtimox.

Concurrent with the decrease in cell viability, nifurtimox treatment produces morphological changes consistent with apoptosis and these morphologic changes occur in a dose-dependent manner. Exposure to 1 µg/ml of nifurtimox caused a decrease in axon length compared to control untreated cells, and at 10 µg/ml nifurtimox this effect was increased significantly with rounding of cells. At 20 µg/ml nifurtimox, neuroblastoma cells were rounded up and floating with only a few cells remaining adherent to the plate.

In addition to its effects *in vitro*, nifurtimox was also able to reduce the growth of neuroblastoma tumors in a xenograft model. Nude mice (*nu/nu*) were injected in the left flank with SMSKCNR cells and allowed to form palpable tumors for 10-14 days. The mice were then fed 5g food pellets daily with or without 150 mg/kg nifurtimox. There were no signs of drug-related toxicity at this dose schedule, which results in serum levels approximately equivalent to a dose of 12 mg/kg in humans. After 28 days of treatment (when control mice reached maximum tumor size of $3.0cm^3$) the mice were sacrificed and their tumors were excised and weighed. Treatment with nifurtimox resulted in inhibition of tumor growth: the average tumor weight in control mice was 1.15 grams compared to an average weight of 0.3 grams in mice receiving nifurtimox ($p=0.03$). Immunohistochemical staining showed that tumors treated with nifurtimox had decreased cell proliferation as evidenced by decrease in Ki67 positive staining (75.3% versus 93.2%, $p<0.001$) and decrease in mitotic index (33.5 versus 50.2, $p=0.012$) as well as an increase in apoptosis as evidenced by an increase in the pyknotic index (63.7 versus 39.5, $p=0.022$).

3.4. Nifurtimox is Cytotoxic to Medulloblastoma Cells in vitro and in vivo

We tested the effect of nifurtimox on growth of D283 and DAOY medulloblastoma cells in culture. Nifurtimox inhibited the growth of these lines in a concentration-dependent manner. After 2 days of exposure to 20 mcg/ml of nifurtimox, the number of cells is decreased to 7.0% of vehicle-treated controls in D283 ($p<0.001$) and 40% in DAOY ($p<0.001$).

A mouse xenograft model was used to more fully evaluate the effect of nifurtimox. Nude mice were injected stereotaxically in the cerebellum with 10^5 D283 cells and fed food with or without nifurtimox (150mg/kg/day) for 7 weeks post-surgery. The animals were then sacrificed, and the tumors removed and evaluated by immunohistochemistry. *In vivo* tumor growth was inhibited by nifurtimox, and histologic evaluation of the tumors showed that nifurtimox treatment resulted in an increase in the apoptotic rate.

3.5. Pharmacokinetic Studies of Nifurtimox

Despite metabolism studies in rats and dogs dating to 1972, there appears to be little published data regarding pharmacokinetics of nifurtimox in humans. The product brochure refers to limited PK studies in healthy adult volunteers and patients with renal failure who received single doses in the range of 10-15 mg/kg. After an oral dose the time to peak plasma concentration was about 2.5 hours, the C_{max} was 0.751 µg/ml (751 ng/ml) and the $T_{1/2}$ was approximately 3.5 hours. There was large inter-patient variability with peak concentrations ranging between 356-1093 ng/ml.

An HPLC assay for serum has been published (Paulos et al, Journal of Chromatography 433:359-362, 1988) and the specifics are outlined below. The sensitivity using detection at 400 nm and separation on a C_{18} column was reported to be 0.077 µg/ml (77 ng/ml) serum, which is approximately 20% of the lowest peak level reported in the studies noted above. Furthermore, with a $T_{1/2}$ of ~ 3.5 hours and TID dosing, the assay appears sensitive enough to do at least limited PK studies on children as small as 10 kg: 1 to 2 ml blood samples obtained at 2 time points (baseline and 180 minutes post-administration) would require a total volume of no more than 4 ml.

3.6. Assay Procedure (adapted from Paulos et al):

A 50 µl aliquot of nitrofurazone solution (30 µg/ml) is added to 1.0 serum sample as an internal standard. Serum proteins are precipitated with 0.6 ml 1 M perchloric acid, and the precipitate removed by centrifugation for 10 minutes at 1000 x g. The liquid phase is aspirated and the acid neutralized with 200 µl of 1 M sodium hydroxide. The nifurtimox (together with the nitrofurazone standard) is extracted into an organic phase of 3.5 ml dichloromethane for 1 hour. A 3 ml aliquot of the organic phase is evaporated to dryness at 40° C, the residue dissolved in 200 µl HPLC mobile phase buffer, of which 20 µl aliquots are used for each HPLC separation.

3.7. HPLC separation:

Chromatography is done on a 15 cm, 3.9 mm ID uBondapak C_{18} column. The mobile phase is methanol-Sorensen phosphate buffer, pH 7.0 (50:50, v/v). The chromatography was done at a rate of 1 ml/min. The standard elutes at approximately 2 minutes and the drug at 3 minutes.

This assay was reported to be linear over the range of 0.077-2.3 μg/ml (77-2300 ng/ml) and the reproducibility based upon a pooled serum sample assayed repetitively over a 2-week period showed an inter-day coefficient of variation of only 4%.

4.0 SUBJECT ELIGIBILITY AND STUDY DESIGN

4.1. Eligibility:

Inclusion Criteria:

4.1.1. Age: 0-21 years at the time of diagnosis.

4.1.2. Diagnosis: Histologic verification at either the time of original diagnosis or relapse of neuroblastoma or medulloblastoma.

4.1.3. Disease Status: Refractory or first or multiple relapsed neuroblastoma, or medulloblastoma that has relapsed after, or is refractory to, a chemotherapy-containing treatment regimen.

4.1.4. Measurable disease, including at least one of the following:

- Measurable tumor >10mm by CT or MRI
- Positive bone marrow biopsy/aspirate.
- For neuroblastoma subjects only, a positive MIBG
- For medulloblastoma subjects only, positive CSF cytology

4.1.5. Current disease state must be one for which there is currently no known curative therapy.

4.1.6. A negative urine pregnancy test is required for female subjects of child bearing potential (onset of menses or ≥13 years of age).

4.1.7. Both male and female study subjects need to agree to use one of the more effective birth control methods during treatment and for six months after treatment is stopped. These methods include total abstinence (no sex), oral contraceptives ("the pill"), an intrauterine device (IUD), levonorgestrol implants (Norplant), or medroxyprogesterone acetate injections (Depo-provera shots). If one of these cannot be used, contraceptive foam with a condom is recommended.

4.1.8. Organ Function Requirements

- Subjects must have adequate liver function as defined by AST or ALT <10x upper limit of normal
- Subjects without bone marrow metastases must have adequate bone marrow function, defined as ANC >500/µl

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

Exclusion Criteria:

4.1.10. Life expectancy <2 months or Lansky score <50%

4.1.11. Subjects that weigh Less than 3.5 kg

4.1.12. Investigational Drugs: Subjects who are currently receiving another investigational drug are excluded from participation.

4.1.13. Anti-cancer Agents: Subjects who are currently receiving other anticancer agents are not eligible. Subjects must have fully recovered from the effects of prior chemotherapy (hematological and bone marrow suppression effects), generally at least 3 weeks from the most recent administration (6 weeks for nitrosoureas).

4.1.14. 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.

4.1.15. 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.

4.2. Stratification

Stratum I –First relapsed and refractory neuroblastoma
Stratum II- Multiply relapsed neuroblastoma
Stratum III- Relapsed/refractory medulloblastoma

4.3. Subject Registration

4.3.1. All sites will complete a "Subject Registration Form." This form will then be scanned or faxed to the Research Coordinator at the Neuroblastoma and Medulloblastoma Translational Research Consortium (NMTRC).

4.3.2. Each subject will be given a unique identifier number. This unique identifier will be kept on a "Subject Identification Log" at each site.

5.0 TREATMENT PLAN

5.1. Nifurtimox Dosing- Cycles 1-6

5.1.1. Neuroblastoma (Stratum 1 and II subjects)- Nifurtimox will be administered at 30 mg/kg/day in three divided doses for every day of the study.

5.1.2. Medulloblastoma (Stratum III subjects)- Nifurtimox will be administered at 20 mg/kg/day in three divided doses for every day of the study.

5.1.3.

5.1.4. Nifurtimox doses will be rounded to the nearest whole tablet (each tablet is provided as 120mg/tablet). There will be no dose interruptions unless toxicity supervenes (see Section 7.0).

5.1.4.1. For subjects ≤ 10 kg or < 1 year of age Nifurtimox contact the NMTRC for dosing.

5.1.4.2. Nifurtimox may be given by G tube or NG tube.

5.1.4.3. Nifurtimox should be given with food

5.1.4.4. Nifurtimox may be crushed or cut and given in food if subject is unable to swallow

5.1.4.5. If subjects vomits after giving Nifurtimox dose:

- If less than or equal to 30 minutes since dose- repeat dose
- If over 30 minutes since dose- do NOT repeat dose

5.1.5. Nifurtimox Dosing Calculation Example (neuroblastoma dose used as example):

Patient weight (kg) x 30 = _____ ÷ 120 = _____ # of pills per day. Round number of pills per day to nearest whole tablet then divide into 3 even (when possible) or uneven doses.
Example: Subject weighs 27kg. 27kg x 30mg/kg/day = 810mg/day. Divide 810mg/day by 120mg/tablet = 6.75 tablets per day. Round up to 7 tablets per day, then divide into 3 doses. Subject should get 3 tabs in am, 2 tabs in afternoon, 2 tabs at night to equal 7 tabs per day.

5.1.6. Nifurtimox Dosing Modification for Subject Weight Changes:
Nifurtimox dose should be adjusted at each refill for weight changes, or sooner at the beginning of each cycle if a noticeable change in weight occurs.

5.2. Combination Therapy:

5.2.1. The combination of cyclophosphamide, and topotecan, along with the nifurtimox, will be used in each cycle.

5.2.2. Nifurtimox will be given orally three times per day per section 5.1.

5.2.3. Each cycle of combination chemotherapy will be given over 5 consecutive days as follows:

5.2.3.1. Prehydration with 500 ml/m^2 D5W ½ NS IV over 30-60 minutes, along with antiemetic therapy as per treating physician preference;

5.2.3.2. Cyclophosphamide, 250 mg/m^2/dose prepared per institutional standards in normal saline, IV, infused over 30 minutes;

5.2.3.3. Topotecan, 0.75mg/m^2/dose, prepared per institutional standards in normal saline, IV, infused over 30 minutes;

5.2.4. This 21 day cycle (+/- 3 days) of nifurtimox, cyclophosphamide, and topotecan will be repeated for a total of 6 times.

5.2.5. Subjects without bone marrow metastases must have adequate bone marrow function, defined as ANC >500/µl before starting any chemotherapy. Subjects with documented bone marrow involvement who have otherwise recovered from prior cytotoxic therapy are eligible to continue therapy. If subjects have been negative for bone marrow metastases, but do not have adequate bone marrow function (as described above), then the bone marrow may be rechecked. If there is no bone marrow involvement then wait to see if ANC recovers; filgrastim or pegfilgrastim can be used to stimulate marrow. If they have not recovered within 2 weeks subject will be taken off protocol therapy, but may remain on nifurtimox under this IND and be monitored per section 9.8

5.3. Subjects with a prior history of seizures will be recommended to start on an anti-seizure medication prior to starting on Nifurtimox.

5.4. At completion of cycle 6 (last cycle), subjects that are benefiting from the study drug may continue to receive nifurtimox under this IND, as single agent or in combination therapy at treating physician's discretion. If subjects experience progression or intolerance at any point thereafter they will be considered resistant to nifurtimox and go off study. Treating physicians will continue to report toxicities as described below and will report response to treatment as subjects are evaluated.

5.5. If subjects are tolerating nifurtimox, but are unable to continue on study for reasons other than progression or dose limiting toxicities (e.g. platelet or ANC counts not recovered within 2 weeks of next cycle start), subjects may remain on nifurtimox under the current IND and continue to be monitored for drug safety and maximal response to nifurtimox.

6.0 STATISTICAL CONSIDERATIONS:

6.1. Sample Size Discussion

The sample size discussion focuses on the primary efficacy outcome measured by the proportion of subjects who, during the 6 cycles of treatment, achieve a best tumor response of either complete response (CR) or partial response (PR) as defined below. An absolute difference in response rates compared to chemotherapy alone of ~15% to 20% would be considered clinically significant. Each stratum of this trial will compare a null hypothesis proportion with an alternative hypothesis proportion and will use a directional 5% Type I error level. Each strata will use an 80% power level. Stratum I consists of neuroblastoma subjects in first relapse, and the null response rate (CR+PR) is taken to be 30% while the alternative response rate (CR+PR) is taken to be 50% as defined above. Stratum II consists of multiply relapsed or refractory neuroblastoma subjects and the null response rate is taken to be 20% while the alternative response rate is taken to be 40%. Stratum III consists of multiply relapsed or refractory medulloblastoma subjects, for whom the null hypothesis response rates are also 5% and so the alternative response rate is 20%. Previous sample size justifications focused upon a single sample size determination for all three strata. Stratum I and II will be modified to implement a two-stage minimax design to accommodate an interim look and stopping for futility due to the larger sample size need while Stratum III will retain the original single stage design given that the current sample size accrual is already at 20 subjects per stratum.

Stratum I

A sample size of n = 39 subjects will be required to test the 30% vs. 50% response rate hypotheses for the Stratum I subject group using a two-stage minimax design with an interim look when the first 19 evaluable subjects are accrued and the final look when 39 evaluable subjects are accrued. The

two-stage decision rule for this design becomes:

> Stage 1: Accept the null hypothesis if the response rate is less than or equal to 6/19 for the 1st 19 subjects. Otherwise continue to stage 2.
> The probability of stopping for futility at Stage 1 is 0.67 when the null hypothesis is true and 0.105 when the alternative is true.
>
> Stage 2: Accept the null hypothesis if the response rate is less than or equal to 16/39. Otherwise reject the null hypothesis.
> The size of the Type I error rate is 4.5% while the actual power is 80.4%.

Stratum II

A sample size of n = 33 subjects will be required to test the 20% vs. 40% response rate hypotheses for the Stratum II subject group using a two-stage minimax design with an interim look when the first 18 evaluable subjects are accrued and the final look when 33 evaluable subjects are accrued. The two-stage decision rule for this design becomes:

> Stage 1: Accept the null hypothesis if the response rate is less than or equal to 4/18 for the 1st 18 subjects. Otherwise continue to stage 2.
> The probability of stopping for futility at Stage 1 is 0.72 when the null hypothesis is true
>
> Stage 2: Accept the null hypothesis if the response rate is less than or equal to 10/33. Otherwise reject the null hypothesis.
> The size of the Type I error rate is 4.6% while the actual power is 80.1%.

Stratum III

A sample size of n = 21 will be required to test the 5% vs. 20% response rate hypotheses for the Stratum III subject groups using a single stage design.

Total patients

Assuming that approximately 5% of Stratum I subjects will be non-evaluable, the actual sample size needed will be n = 41. Assuming that approximately 10% of Stratum II subjects will be non-evaluable, then the actual sample size needed will be n=36. Assuming that approximately 10% of Stratum III subjects will be non-evaluable, then a sample size of n = 23 each will be required for Strata III.

6.2. Statistical Analysis Approaches

Statistical analysis of the primary outcome will be conducted for each of the three subject strata separately Stratum I and II data will utilize a two-stage minimax analysis as outlined in the sample size justification. Stratum III data will be analyzed only when the full sample size for that stratum has been achieved. The number of subjects with complete (CR) and partial (PR) responses, stable disease (SD), and progressive disease (PD), will be summarized relative to the total number of evaluable subjects. An exact binomial model will be used to test the null and alternative hypotheses as specified in the sample size discussion above using the stated directional 5% significance level. In addition, exact 95% confidence intervals for the primary efficacy outcome using the binomial model will be obtained to supplement the p-value calculations and to assist in the interpretation of the observed results at the completion of the full 6 cycles of treatment. Further examination of baseline and treatment-related measures associated with the primary efficacy outcome(CR+PR) rate will utilize a logistic regression model where the outcome of interest will be the group of subjects achieving a CR or PR vs. other subjects.

Statistical analysis of the secondary outcome measures, such as quality of life data, will utilize a within-subjects approach given the repeated measures structure of the data as collected over the multiple observational time points. Time to event analysis using Kaplan-Meier plots and Cox regression models will be used to examine the longer term occurrence of clinical outcomes (such as survival) and the influence of potential covariates. The potential for missing data in any longitudinal study is recognized. Thus, a sensitivity analysis of the impact of missing data will be conducted using a multiple imputation approach if data can be assumed to be missing at random or completely at random. These results will be compared to modeling results based upon linear mixed model results without use of imputations.

7.0 DOSE ADJUSTMENTS

7.1. Nifurtimox Dose Modifications

7.1.1. Any Grade 5 toxicity attributable to Nifurtimox that occurs within 30 days of taking study drug, will require a DSMB meeting to be held within 7 days of reporting the toxicity. Until the time that the DSMB meets, no new subjects may be enrolled on study. The committee will evaluate the relationship of the nifurtimox to the toxicity.

7.1.2. Any subjects who demonstrate Grade 3 or 4 CNS/PNS toxicity attributable to Nifurtimox will have nifurtimox held. If symptoms resolve within 14 days, subjects may resume nifurtimox at a reduced dose of 20 mg/kg/day (15mg/kg/day for medulloblastoma). For subjects that restart at the reduced dose: if they demonstrate a second Grade 3 or 4 CNS/PNS toxicity attributable to Nifurtimox at this point they will have nifurtimox held again. Again, if symptoms resolve within 14 days, subjects may resume nifurtimox at a reduced dose of 15mg/kg/day (10mg/kg/day for

medulloblastoma). If the symptoms do not improve to a Grade 2 or less within 14 days they will be removed from study. For subjects that restart at the 15mg/kg/day (10mg/kg/day for medulloblastoma) reduced dose: if they demonstrate a third Grade 3 or 4 CNS/PNS toxicity attributable to Nifurtimox again at this point they will be removed from study.

7.1.3. Any subject who demonstrates Grade 2 CNS/PNS toxicity attributable to Nifurtimox may have nifurtimox held, at the discretion of their treating physician or the PI, during an evaluation of the symptoms for a period up to 7 days maximum.

7.1.4. Subjects who develop any other Grade 4 toxicity attributable to nifurtimox (other than hematological toxicity or febrile neutropenia) will have the drug held and will be followed until resolution of toxicity to ≤ Grade 2. If symptoms resolve to ≤ Grade 2 within 7 days, subjects may resume nifurtimox at a reduced dose of 20 mg/kg/day (15mg/kg/day for medulloblastoma). If symptoms do not resolve to ≤ Grade 2 within 7 days, subjects will be removed from study and monitored until toxicity is ≤ Grade 2. If subject experiences a second Grade 4 toxicity attributable to nifurtimox they will have the drug held and will be followed until resolution of toxicity to ≤ Grade 2. If symptoms resolve to ≤ Grade 2 within 7 days, subjects may resume nifurtimox at a reduced dose of 15mg/kg/day (10mg/kg/day for medulloblastoma). If symptoms do not resolve to ≤ Grade 2 within 7 days, subjects will be removed from study and monitored until toxicity is ≤ Grade 2. If subject experiences a third Grade 4 toxicity attributable to nifurtimox (other than hematological toxicity or febrile neutropenia) at the reduced dose level of 15mg/kg/day (10mg/kg/day for medulloblastoma), they will be off study. There will be no dose re-escalations allowed.

7.2. <u>Topotecan/Cyclophosphamide Dose Modifications:</u>

7.2.1. Subjects who develop any Grade 4 toxicity that is attributable to cyclophosphamide or topotecan other than the commonly anticipated hematologic or infectious toxicities will be removed from study but may continue on nifurtimox under the current IND as long as no tumor progression is documented. These subjects will continue to be evaluable for toxicity.

7.2.2. Subjects experiencing prolonged (greater than 7 days) Grade 4 hematologic toxicities attributable to cyclophosphamide or topotecan may have their cyclophosphamide and topotecan dose reduce from receiving drug on Days 1 through 5 of each cycle to only receiving drug on days 1 through 3 of each cycle.

7.2.3. Prior to starting each cycle, the ANC should be >500/µl (except in subjects with documented bone marrow involvement); see Sec. 5.2.5, 8.3, 8.4 and 8.5 for guidelines if counts are inadequate.

7.2.4. Subjects experiencing hematuria in excess of sporadic asymptomatic microscopic hematuria may have MESNA added to their chemotherapy/hydration fluids (suggested total MESNA dose is 60% of cyclophosphamide dose). Subjects with gross hematuria, clots, or dysuria despite hydration/MESNA will have topotecan/cyclophosphamide discontinued.

8.0 SUPPORTIVE CARE AND CONCOMITANT THERAPY

8.1. Concurrent anticancer therapy other than cyclophosphamide or topotecan may not be administered to subjects on study EXCEPT for radiation to relieve metastatic bone pain as long as there are other measurable areas of disease to follow for secondary endpoint.

8.1.1. Radiation therapy may be given to sites of painful bone metastases. Prior total body irradiation to doses ≤20 Gy will be permissible, however any prior focal radiation therapy to the symptomatic site will exclude the subject from this standardized radiation therapy plan. Subjects will be treated with three fractions of 3 Gy each. The radiation dose may be prescribed to a point or volume at the discretion of the treating radiation oncologist. Multiple sites may be included and treated per the trial.

8.1.2. Re-treatment radiation therapy techniques and dose schedules will be done at the discretion of the treating radiation oncologist.

8.2. Propofol: In a past study with nifurtimox, a possible interaction between propofol and nifurtimox was observed in two subjects. There is very limited information but a temporal relationship was noted with timing of medications and adverse event occurrence (neurotoxicity), the information is not conclusive at this time. Due to this possible interaction, the following guidelines for propofol use while taking nifurtimox should be followed:

Hold nifurtimox for 24 hours prior to prolonged anesthesia with propofol of >1 hour. If Nifurtimox cannot be held prior to a procedure (for example emergency situations), and alternative agent should be considered. Short duration propofol use (<1 hour) has not elicited any untoward responses in past study subjects, and therefore does not require any change in nifurtimox use prior to procedure.

8.3. Metronidazole: During the course of this study; a possible interaction between metronidazole and nifurtimox has been observed in two subjects. There is very limited information but a temporal relationship was noted with timing of medications and adverse event occurrence (severe neurotoxicity). Due to this possible interaction, the following guidelines for metronidazole use while taking nifurtimox should be followed:

Metronidazole should not be used while taking Nifurtimox. If another alternative agent cannot be used then hold nifurtimox during metronidazole use. Nifurtimox may be restarted 48 hours after completion of last dose of metronidazole.

8.4. **Supportive care:** Appropriate antibiotics, blood products, antiemetics, intravenous fluids, and general supportive care are to be used as deemed clinically necessary by treating physician.
8.5. **For Medulloblastoma Subjects-** If, at start of chemotherapy, platelet count is ≤ 50,000/µl, platelet transfusion recommended to reduce the risk of CNS bleeding.
8.6. If the start of the next cycle of chemotherapy is delayed by >1 week after the anticipated start date, then filgrastim or pegfilgrastim should begin 24-48 hours after the completion of day 5 chemotherapy and continue until neutrophil recovery (administered per treating institution's guidelines).
8.7. Subjects who have a seizure that is likely to be secondary to nifurtimox toxicity will be started on the anti-seizure medication oxcarbazepine. If this medication is not tolerated another anti-seizure medication may be prescribed in consultation with neurology.
8.8. Subjects who develop a Grade 2 or higher nausea or an aversion to swallowing the nifurtimox tablets may split the tablets in 2 and place them in a Size 0 Gelcap, in order to make them easier to tolerate.
8.9. For pain secondary to tumor swelling, dexamethasone at a dose of 5 mg/m^2 BID may be added to regimen.

9.0 REQUIRED OBSERVATIONS

9.1. *Prior* to study entry (within 7 days of start of therapy):
- Physical exam including neurological exam and Clinical Total Neuropathy Scale (TNSc)
- Urine catecholamines (for neuroblastoma only)
- Urine pregnancy test for female subjects of child bearing potential (onset of menses or ≥13 years of age)
- CBC with differential
- Electrolytes, BUN, Creatinine,
- LDH, AST, ALT, Bilirubin, Ferritin
- Neurologic structured interview (see appendix I) and baseline measures on the BRIEF and PCSQoL:DT questionnaire as well as the PCSQoL:MTD questionnaire (as outlined in section 11.0).

9.2. *Prior* to study entry (within 30 days of start of therapy)- **must** be done after last previous chemo dose:
- CT or MRI of measurable disease sites
- MIBG scan (not required if subject's disease is previously determined to be not MIBG avid or for subjects with medulloblastoma)
- Bone marrow aspirate and biopsy (required for neuroblastoma subjects, optional for medulloblastoma. (Should be strongly considered for medulloblastoma subjects that have low platelet and ANC counts, and slow recoveries of counts). Extra bone marrow samples to be sent (if subject agrees to biology portion of study) per section 18.2.
- CSF cell count and cytology (medulloblastoma subjects only)

9.3. Days 1 and 4 of Cycle 1: Pharmacokinetic studies are to be drawn prior to the subject taking their 1st dose of Nifurtimox dose and again 3 hours following the observed morning dose. See section 10.0 for specific PK collection and handling information.

9.4. Each week (+/-3 day window) for first 21 days:

- Physical exam including neurological exam
- CBC with differential
- Electrolytes, BUN, creatinine
- LDH, AST, ALT, bilirubin

9.5. *Prior* to every 21 day treatment cycle (+/- 3 day window for cycle start) within 7 days of start of next cycle:

- Physical Exam including neurological exam
- CBC with differential
- Electrolytes, BUN, creatinine
- LDH, AST, ALT, bilirubin
- Urine catecholamines (for neuroblastoma subjects).

Treating physician may obtain additional studies at their discretion.

9.6. Additional studies at the end of Cycles 2 and 4:

- MRI/CT re-evaluation of disease sites
- MIBG scan (for MIBG avid neuroblastoma subjects only)
- Bone marrow biopsy and aspirate (if positive at study entry)
- CSF for cell count and cytology (medulloblastoma only and if positive at study entry)
- Neurological structured interview (see Appendix 1), Clinical Total Neuropathy Scale (TNSc), and measures on the BRIEF and PCSQoL:DT questionnaire (as outlined in section 11.0 of protocol).
- Additional imaging or studies may be done if clinically indicated by symptoms, exam, or tumor markers

9.7. At the End of Protocol Therapy (Cycle 6) or at Early Withdrawal:

- Physical Exam including neurological exam and Clinical Total Neuropathy Scale (TNSc)
- CBC with differential
- Electrolytes, BUN, creatinine
- LDH, AST, ALT, Bilirubin
- Urine catecholamines (for neuroblastoma subjects)Neurological structured interview (see Appendix 1)
- Measures on the BRIEF and PCSQoL:DT questionnaire (as outlined in section 11.0 of protocol).
- MRI/CT re-evaluation of disease sites
- MIBG scan (for MIBG avid neuroblastoma subjects only)
- Bone marrow biopsy and aspirate (if positive at study entry)
- CSF for cell count and cytology (medulloblastoma only and if positive at study entry)

- If the subject withdraws early, scans need not be repeated if recently performed unless clinically indicated. Additional imaging or studies may be done if clinically indicated by symptoms, exam, or tumor markers. Treating physician may obtain additional studies at their discretion.

9.8. Follow up for subjects remaining on Nifurtimox but off protocol therapy (OPTF):

9.8.1. Within 7 days of start of each cycle (+/- 3 day window for cycle start):
- Physical Exam including neurological exam
- CBC with differential
- Electrolytes, BUN, creatinine
- LDH, AST, ALT, bilirubin
- Urine catecholamines (for neuroblastoma subjects).
- Treating physician may obtain additional studies at their discretion.

9.8.2. Minimum of every three cycles (or sooner if indicated):
- MRI/CT re-evaluation of disease sites
- MIBG scan (for MIBG avid neuroblastoma subjects only)
- Bone marrow biopsy and aspirate (if positive at study entry)
- CSF for cell count and cytology (medulloblastoma only and if positive at study entry)
- Treating physician may obtain additional studies at their discretion.

9.9. Long term follow up for subjects that are off study (have stopped taking nifurtimox):

9.9.1. Subjects will be followed at 30 days (+7 days) past last dose of study drug as follows:
- Physical exam including neurological exam
- CBC with differential
- Electrolytes, BUN, creatinine
- LDH, AST, ALT, bilirubin
- Urine catecholamines (for neuroblastoma subjects)
- Neurologic structured interview (see appendix I)

9.9.2. 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):
- 2 months
- 3 months
- 5 months
- 7 months
- 9 months
- 1 year

9.10. Scan Submission: All required study scans (CT's, MRI's, and MIBG's) will be reviewed by central radiology at the University of Vermont and Fletcher Allen Health Care according to section 13.0. All study required scans should be sent on disc to:

Alyssa VanderWerff
Clinical Program Coordinator
NMTRC
100 Michigan Avenue NE MC 272
Grand Rapids, MI 49503
Tel: (616) 267-0327
Fax: (616) 391-2785
E-Mail: Alyssa.VanderWerff@ helendevoschildrens.org

10.0 PHARMACOKINETIC STUDIES

10.1. On Days 1 and 4 of cycle 1 PK specimens will be collected.

10.1.1. The subject will hold (not take) their 1st dose of nifurtimox on days one and four until arriving to the clinic and pre-dose blood specimen is collected.

10.1.2. Pre-dosing PK sample will be collected and processed as per Section 10.3 and 10.4

10.1.3. Once the pre-nifurtimox specimen is collected the subject will take their prescribed morning dose of nifurtimox. If gel capsules are being used, the nifurtimox should NOT be placed in the Gel Capsules for this dose.

10.1.4. Three hours following (+/- 15 minutes) the subject's morning dose of nifurtimox, the next PK sample will be collected.

10.1.5. The date and time of all specimen collections and of the dose of nifurtimox will be noted in the subject's chart.

10.2. **PK Sample requirements:**

One 2cc whole blood specimen will be collected in a serum separator tube (SST).

10.3. **Specimen Handling:**

10.3.1. Specimen will be collected with aluminum foil wrapped around the tube in order to protect the sample from light deterioration.

10.3.2. Following collection the specimen will to be allowed to clot at room temperature for no less than twenty to thirty minutes.

10.3.3. Specimen will be spun in a centrifuge at 1,000- 1,300g for 10-15 minutes

10.3.4. Following centrifugation the serum from the SST will be aliquoted into a plastic screw capped cryovial.

10.3.5. Specimens must be labeled with the subject's initials, unique study identifier, and date and time of specimen collection.

10.3.6. Specimen will then be stored in a -70° C. freezer until ready to be shipped.

10.4. **Specimen shipping:**

10.4.1. All specimens will be shipped on dry ice following the federal IATA hazardous Specimen Shipping Guidelines

10.4.2. Specimens should be batch shipped (after approximately 5 subjects) on a Monday-Thursday (lab does not accept Saturday deliveries).

10.4.3. Specimens will be shipped to the following address:

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

11.0 MONITORING OF POTENTIAL ADVERSE NEUROLOGICAL EFFECTS

Previously described neuropharmacological and toxicological properties of nifurtimox mandate close monitoring for potential adverse neurologic effects. Nifurtimox is known to cross the blood-brain barrier, raising the possibility of adverse CNS effects as well as peripheral neurotoxicity. The symptoms to be monitored are guided both by prior reported adverse behavioral/subjective effects (which might be neurologically mediated), as well as potential side effects resulting from the catecholaminergic neurotoxicity of the study drug. Potential catecholaminergic targets include sympathetic adrenergic neurons of the adrenal medulla, neurons of the locus ceruleus in the medulla, and dopaminergic neurons of the basal ganglia. Collectively, these neurons modulate autonomic (sympathetic) cardiovascular function, motor control, attention, reward systems of the brain, and neuro-endocrine function (e.g., prolactin release).

Although some drug information summaries mention peripheral neuropathy as a possible side effect of nifurtimox, these references appear to reflect *plausible* neuropathic symptoms (numb or weak hands, feet) rather than specific clinical or laboratory evidence of peripheral neuropathy (depressed tendon jerks, abnormal electrophysiological findings). A review of the literature did not reveal any prior definitive evidence of nifurtimox-induced neuropathy. However, toxicity may become evident at the higher drug doses to be studied in this protocol; therefore, interval neurological examinations and questions regarding toxicity will be directed to detecting nifurtimox-related neuropathy. Because one potential site of CNS neurotoxicity is the locus ceruleus—a brainstem nucleus associated with regulation of attention and arousal—executive function will be assessed (e.g., "behavior regulation" and "metacognition") with the *Behavior Rating Index of Executive Function (BRIEF).*

The *Behavior Rating Inventory of Executive Function* (BRIEF) is designed to assess executive functioning in children and adolescents ages 5-18. It consists of two possible rating forms - a parent and a teacher questionnaire - which require 10-15 minutes to administer and 15-20 minutes to score. We will only be using the parent completed questionnaire in this study. The BRIEF is suited for both home and school settings. The BRIEF is useful in evaluating children with a wide spectrum of developmental and acquired neurological conditions such as learning disabilities, Attention-Deficit/Hyperactivity Disorder (ADHD), Traumatic Brain Injury (TBI), Low Birth Weight, Tourette's Syndrome, and Pervasive Developmental Disorders/Autism.

Each BRIEF questionnaire contains 86 items in 8 nonoverlappping clinical scales and 2 validity scales. These theoretically and statistically derived scales form two broader indexes:

1) Behavioral Regulation: Inhibit, Shift, Emotional Control
2) Metacognition: Initiate, Working Memory, Plan/Organize, Organization of Materials, Monitor

Assessing Quality of Life (QoL)
The quality of life (QoL) of children/families in treatment is an important consideration in clinical drug trials. For the child, social-educational behavior can be affected by cognitive abilities and neurotoxicities and the other side-effects of treatment drugs. How QoL is affected by a specific study drug is also of interest given that QoL is used by parents, physicians and subjects as a measure of drug efficacy. Two questionnaires will be used to measure the QoL effects of the study drug(s) on subjects and their families.

Two separate questionnaires will be used to examine two aspects of QoL: (1) How subjects' QoL is affected by nifurtimox (and with combinations of additional agents/therapies), and (2), how the parents of pediatric cancer patients perceive the importance of various QoL effects while making treatment decisions for their child. For the former, the *Pediatric Cancer Survey: Parent Assessment of Children's Quality of Life During Treatment* (PCSQoL:DT). The second aspect of QoL will be assessed with *Pediatric Cancer Survey: For Parents Making Decisions* (PCSQoL:MTD).

The proposed plan and schedule for monitoring of adverse neurotoxic effects includes (1) serial neurologic examination, (2) iterative structured interviews (See Appendix 1 for "Structured Interview for Adverse Neurologic Events" form) and (3) BRIEF assessments and two QoL questionnaires (Appendix 3).

	Neurologic Exam	Structured Interview	BRIEF	PCSQoL:DT	PCSQoL:MTD
Baseline	√	√	√^	√^	√^
End of Week 1	√				
End of Week 2	√				
End of every Cycle	√				
End of Cycles 2 & 4		√	√^	√^	
End of Protocol Therapy/Early Withdrawal	√	√	√^	√^	
3 month follow up					
6 month follow up					
9 month follow up					
1 Year follow up					

Note. BRIEF: *Behavior Rating Index of Executive Function (BRIEF);* PCSQoL:DT: *Parent Assessment of Children's Quality of Life During Treatment*; PCSQoL:MTD: *For Parents Making Treatment Decisions*.
^ The BRIEF and QOL questionnaires will be completed by the parents

The neurologic exam and Clinical Total **Neuropathy** Scale (TNSc) will be performed by a pediatric neurologist or oncologist who is experienced at performing neurological exams on children in the anticipated age group. Some specific features of the exam to be recorded include deep tendon reflexes, pupil size, length-dependent sensory changes, and tremor/other abnormal spontaneous movements.

An outline of the structured interview to be conducted is included in Appendix I of this protocol.

12.0 SUBJECT WITHDRAWAL AND TRIAL DISCONTINUATION

12.1. Criteria for Subject Off-Therapy

Subjects will be removed from the study therapy for the following reasons:

- Progressive neoplastic disease
- Subject or guardian withdraws consent to continue in the trial
- 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
- Death

12.2. Criteria for Subject Off-Study

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

- Completion of all study requirements
- Subject or guardian withdraws consent to continue in the trial (if this occurs, no further study visits or data may be collected)
- Subject is lost to follow-up (defined as the inability to contact the subject on 3 separate occasions over a period of 2 weeks)
- Death

13.0 EVALUATION/RESPONSE CRITERIA.

13.1. **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 statuses described below.

13.2. **Response Criteria For Patients with Solid Tumors**- This study will use the (RECIST) Response Evaluation Criteria in Solid Tumor from the NCI.

13.3. **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.

13.3.1. Measurable Disease Response will be assessed at end of Cycle 2 end of cycle 4, and end of protocol therapy.

13.3.2. For patients with Measurable Disease: This study will use the (RECIST) Response Evaluation Criteria in Solid Tumor from the NCI.

13.3.3. Serial measurements of lesions are to be done with CT or MRI. The same method of assessment is to be used to characterize each identified and reported lesion at baseline and during follow-up.

13.3.4. Quantification of Disease Burden The sum of the longest diameter (LD) for all target lesions will be calculated and reported as the

disease measurement. For this evaluation, all scans will undergo central review at the University of Vermont.

Complete Response (CR): Disappearance of all target lesions.
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 entry.
Stable Disease (SD): Neither sufficient decrease to qualify for PR or sufficient increase to qualify for PD from study entry.
Progressive Disease (PD): At least a 20% increase in the sum of the longest diameters of all target lesions compared to study entry, the appearance of unequivocal new lesions, or laboratory evidence of clinical progression (e.g., spread to bone marrow or increasing catecholamines).

13.4. Response Criteria for Patients with Bone Marrow Disease:

13.4.1. Those patients 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 subsequent bilateral bone marrow aspirates and biopsies done at least three weeks apart after study entry.
Progressive disease: Tumor seen on morphology on two consecutive bone marrows done at least three weeks apart in patients who had NO tumor in bone marrow at study entry. (Note: Patient 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 an amount of tumor in the bone marrow by morphology that does not meet criteria for either complete response or progressive disease.

13.5. Response Criteria for Patients with MIBG Positive Lesions

13.5.1. Patients who have a positive MIBG scan at the start of therapy will be evaluable for MIBG response. The use of 123I 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 patient is strongly encouraged. All MIBG's will be performed at the research institution and centrally reviewed at the University of Vermont.

The following criteria will be used to report MIBG response by the research institution on the end of course report forms:

Complete response = complete resolution of all MIBG positive lesions

Partial response = resolution of at least one MIBG positive lesion, with persistence of other MIBG positive lesions.

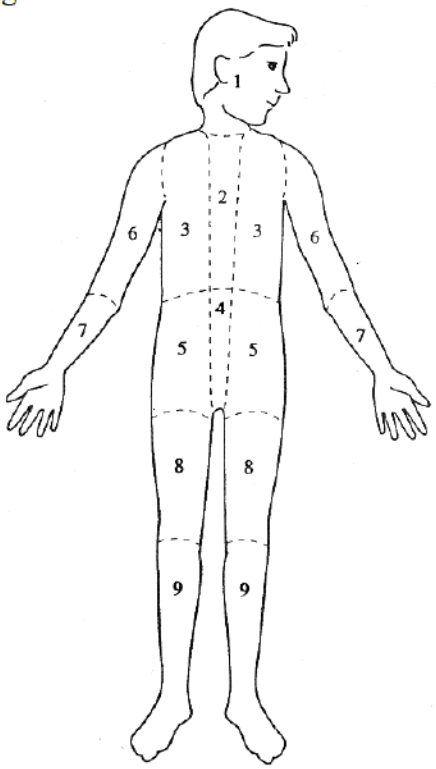
Stable disease = no change in MIBG scan in number of positive lesions (includes patients who have same number of positive lesions but decreased intensity)

Progressive disease = Development of new MIBG positive lesions

13.6. Central Review

13.6.1. The response of MIBG lesions will be assessed on central review using the Curie scale as outlined below. Central review responses will be used to assess efficacy for study endpoint. NOTE: This scoring is NOT required to be done by the research institution for end of course response assessments.

13.6.2. The body is divided into 9 anatomic sectors for osteomedullary lesions, with a 10th general sector allocated for any extra-osseous lesions. In each region, the lesions are scored as follows. The **absolute extension score** is graded as: 0 = no site per segment, 1 = 1 site per segment, 2 = more than one site per segment, 3 = massive involvement (>50% of the segment). The absolute score is obtained by adding the score of all the segments. See diagram of sectors below:



13.6.3. **The relative score** is calculated by dividing the absolute score at each time by the corresponding pretreatment overall score. The relative score of each patient is calculated at each response assessment and classified as below**:**

Complete response: all areas of uptake on MIBG scan completely resolved.
Partial response: Relative score < 0.2 (lesions almost disappeared) to < 0.5 (lesions strongly reduced).
Stable disease: Relative score > 0.5 (lesions weakly but significantly reduced) to > 0.9 (lesions not reduced).
Progressive Disease: New Lesions on MIBG scan.

13.7. Definition of Overall Response for Each Patient (Central Review)

13.7.1. This will be utilized as a basis to integrate response at all sites defined as measurable in this study, including CT/MRI lesions which meet RECIST criteria, MIBG positive lesions, and bone marrow disease. These criteria will be used to define the overall response for the patient in both strata in the statistical analysis**.**

13.7.2. **Complete Response (CR)**

Disappearance of all target lesions. No evidence of tumor at any site (chest, abdomen, liver, bone, bone marrow, nodes, etc.), and HVA/VMA normal.

13.7.3. **Partial Response (PR)**

At least a 30% decrease in the disease measurement for CT/MRI target lesions, taking as reference the disease measurement done to confirm measurable disease in target lesions at study entry. Bone marrow with CR. MIBG with either PR/CR in bone lesions; MIBG may be SD or CR in soft tissue lesions corresponding to lesions on CT/MRI. HVA/VMA may still be elevated.

13.7.4. **Progressive Disease (PD)**

Any one of the following:
a) At least a 20% increase in the disease measurement for CT/MRI target lesions, taking as reference the smallest disease measurement recorded since the start of treatment.
b) Appearance of one or more new lesions or new sites of tumor.
c) PD as defined above for either bone marrow or MIBG lesions.

13.7.5. **Stable disease (SD)**

The patient will be classified as stable disease if there are no new lesions; no new sites of disease, and they do not fit the criteria for PD/PR/CR as above.

14.0 SAFETY MONITORING

14.1. Toxicity data must be submitted to the Study Chair at the end of each cycle of therapy.

14.2. Gina Hanna, PharmD will serve as the Safety Officer for this protocol. The Safety Officer will review all toxicity data at each DSMB meeting or sooner if there is a safety concern.

14.3. The NMTRC V0706 monitoring Plan will be followed for this protocol.

15.0 ADVERSE EVENT REPORTING

15.1. Nifurtimox is supplied through an IND and therefore responsibility to the FDA includes:

15.1.1. Reporting any unexpected fatal or life-threatening adverse experience associated with use of the drug by telephone, fax, or overnight mail, no later than 7 calendar days after initial receipt of information.

15.1.2. Reporting any adverse experience associated with use of the drug that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of information.

15.1.3. Submitting annual progress reports.

15.1.4. Febrile neutropenia is an expected result of topotecan/cyclophosphamide and so does *not* need to be reported.

15.2. A "Data Form for Recording Adverse Events" will be provided to record non-serious Adverse Events. All Grade 3 (CTCAE Version 3.0) or higher AE's (except hematologic) will need to be captured.

15.3. The DSMB chaired by the safety officer will regularly meet per NMTRC policy and DSMB charter to review all safety reports and determine whether protocol modifications are warranted.

16.0 SERIOUS ADVERSE EVENT REPORTING

16.1. All Serious Adverse Events (SAE's) that occur at participating sites must be reported to the coordinating site within 24 hours of discovery and coordinating centers must disseminate this information to the other sites as soon as possible.

All serious, unexpected events and Grade 4 and Grade 5 toxicities, both expected and unexpected, must be reported to the Study Chair (who will then report it to the Safety Officer) within 24 hours of discovery. The participating site will then be responsible for submitting all required Medwatches that occur at their sites to the FDA (and providing a copy to the coordinating site study chair), and submitting paperwork to all their own local IRB requirements.

16.2. **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.

17.0 AGENT INFORMATION

17.1. **Nifurtimox- See attached product brochure.**

17.1.1. **Formulation and Stability: The drug is stable at room temperature, and is light sensitive.**

17.1.2. **Guidelines for Administration: See sections under 5.1.**

17.1.3. **Supplier: The drug will be provided to each site by Bayer in pill form.**

Risks and side effects related to nifurtimox:
Those indicating need for medical attention:

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
While taking nifurtimox: Usually disappear spontaneously after withdrawal of the medication.		**Skin Rash, headache or vertigo (dizziness)**	Central nervous system toxicity including disorientation (confusion), disturbances of equilibrium such as ataxia (clumsiness or unsteadiness), and nystagmus (uncontrolled back-and-forth and/or rolling eye movements); excitation, forgetfulness, insomnia (trouble in sleeping), irritability, psychosis (mood or mental changes), seizures (convulsions), muscle weakness, peripheral neuropathy (numbness, tingling, pain, or weakness in hands or feet) and tremors; a high eosinophil count, fever, impotence (decreased sexual drive or ability), or a low white blood cell count with a risk of infection can occur with high doses
Late: Any time after completion of treatment	There are no known late side effects of Nifurtimox at this time.		

Risks and side effects related to nifurtimox
Those indicating need for medical attention only if they continue or are bothersome:

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
While taking nifurtimox:	Gastrointestinal disturbances, such as anorexia with weight loss (loss of appetite), epigastric pain (abdominal or stomach pain), nausea and/or vomiting occur in 20-80% of patients.	Headache or vertigo (dizziness) occur in 10-12% of patients.	

17.2. **Cyclophosphamide NSC #026271**

17.2.1. **Source and Pharmacology**: An alkylating agent, related to nitrogen mustard, which is biochemically inert until it is metabolized to its active components by the liver phosphamidases. It is non-phase-specific. The drug and its metabolites are excreted exclusively by the kidney after parenteral administration. The plasma half-life ranges from 4 to 6.5 hours.

17.2.2. **Formulation and Stability**: See package insert.

17.2.3. **Guidelines for Administration**: The cyclophosphamide will be 250 mg/m2/dose prepared per institutional standards in normal saline and administered intravenously over 30minutes. See sections under 5.2. **Toxicity**:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Anorexia (L), nausea (L), vomiting(L)	Metallic taste (L), Inappropriate ADH1	Transient blurred vision[1] cardiac toxicity with arrhythmias[1] myocardial necrosis [2](L)
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (L), alopecia (L)	Hemorrhagic cystitis (L)	
Delayed: Any time later during therapy, excluding the above conditions	Immunosuppression, gonadal dysfunction /sterility (L)		Pulmonary fibrosis3(L)
Late: Any time after completion of treatment			Secondary malignancy, bladder fibrosis

1 Less common with lower doses. 3 Risk increased with chest radiation.
2Only with very high doses. (L) Toxicity may also occur later.

17.2.5. **Supplier**: Commercially available. See package insert for further information.

17.3. **Topotecan NSC #609699**

17.3.1. **Source and Pharmacology**: Topotecan AS is a semisynthetic analogue of camptothecin, an alkaloid derived from the camptothecin tree which grows widely throughout Asia. The drug is a specific Topoisomerase-I inhibitor. Topotecan interferes with the repair activity of topoisomerase I by stabilizing the formation of a covalently bonded DNA-Topoisomerase I complex. Thus, the 5'-phosphoryl terminus of the enzyme catalyzed single strand DNA

break remains bound to the tyrosine of the enzyme thereby interfering with replication. The drug exists as two species in equilibrium in aqueous solutions. One is a more active closed-ring lactone and the other a less active open ring form. Acidic conditions favor the closed ring lactone form while basic and physiologic pH favors the open ring form. In human plasma the lactone form is about 21% bound to plasma protein. The drug is excreted 39% in urine and the remainder in the stool, the latter is presumably via biliaryexcretion. In rats, over 90% of the radioactivity from [14C]topotecan was recovered in stool and urine in the first 96 hours. The half life for the lactone form is 180 minutes.

17.3.2. **Formulation and Stability:** See package insert.

17.3.3. **Guidelines for Administration**: Topotecan at a dose of 0.75 mg/m^2 will be prepared per institutional standards in normal saline and administered intravenously, infused over 30 minutes. See sections under 5.2.

17.3.4. **Toxicity**:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug		Nausea, vomiting, diarrhea ***(L)***, mucositis (L), flu-like symptoms (L), headache, rash (L), elevated transaminases, elevated alkaline phosphatase, elevated bilirubin	Abdominal pain, rigors
Prompt: Within 2-3 weeks, prior to next course	Myelosuppression, alopecia		
Delayed: Anytime later		Asthenia	Microscopic hematuria

during therapy, excluding the above conditions			
Late: Anytime after completion of therapy			

(L) Toxicity may also occur later.

17.3.5. **Supplier**: Commercially available. See package insert for further information.

17.4. **Filgrastim NSC # 614629**

17.4.1. **Source and Pharmacology**: Filgrastim is a human graulocyte colony stimulating factor (G-CSF), produced by recombinant DNA technology. Filgrastrim is a 175 amino acid protein with a molecular weight of 18,800 daltons manufactured by recombinant DNA technology utilizing E. coli bacteria into which has been inserted the human granulocyte colony stimulating factor gene. It differs from the natural protein in that the N-amino acid in methionine and the protein is not glycosylated. G-CSF is a lineage specific colony-stimulating factor which regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation and selected end-cell functional activation(including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigen). The elimination half-life is similar for subcutaneous and intravenous administration, approximately 3.5 hours. The time to peak concentration with administered subcutaneously is 2 to 8 hours.

17.4.2. **Formulation and Stability**: See package insert.

17.4.3. **Guidelines for Administration**: Filgrastim or pegfilgrastim should begin 24-48 hours after the completion of day 5 chemotherapy if needed, and continue until neutrophil recovery. Pegfilgrastim may be substituted at the discretion of the treating physician.

17.4.4. **Toxicity**:

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

Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
• Aching or pain in the bones	• Local irritation at the site of the injection • Headache • Higher than normal levels of liver enzymes and uric acid in the blood • A low number of platelets in the blood which may cause you to bruise and bleed	• Allergic reactions which can be life threatening with shortness of breath, low blood pressure, rapid heart rate, hives and facial swelling. This reaction is very rare and has been associated mainly with intravenous

	more easily • Low fever • Enlargement of the spleen which may cause pain in the abdomen or left shoulder • Worsening of skin rashes • Higher than normal white blood count	administration. • If you are known to have sickle cell disease, filgrastim may cause a sickle cell crisis • Rupture of the spleen • Difficulty breathing and lung damage that may be due to the white blood cells that are stimulated by filgrastim traveling to the lungs when they are inflamed or infected.

17.4.5. **Supplier:** Commercially available. See package insert for further information**.**

17.5. **Pegfilgrastim (pegylated filgrastim, SD/01, Neulasta®) (072006**

17.5.1. **Source and Pharmacology**:. Pegfilgrastim is the pegylated form of recombinant methionyl human G-CSF(filgrastim). Pegfilgrastim is produced by covalently binding a 20-kilodalton (kD) monomethoxypolyethylene glycol molecule to the N-terminal methionyl residue of filgrastim. The molecular weight of pegfilgrastim is 39 kD. G-CSF is a lineage specific colony-stimulating factor which regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens). After subcutaneous injection the elimination half-life of pegfilgrastim ranges from 15 to 80 hours and the time to peak concentration ranges from 24 to 72 hours. Serum levels are sustained in most patients during the neutropenic period postchemotherapy, and begin to decline after the start of neutrophil recovery, consistent with neutrophil-dependent elimination.

17.5.2. **Formulation and Stability**: See package insert.

17.5.3. **Guidelines for Administration**: Filgrastim or pegfilgrastim should begin 24-48 hours after the completion of day 5 chemotherapy if needed, and continue until neutrophil recovery. Pegfilgrastim may be substituted at the discretion of the treating physician.

17.5.4. **Toxicity**:

Risks and side effects related to pegfilgrastim include those which:

Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
• Aching or pain in the bones	• Local irritation at the site of the injection • Headache • Higher than normal levels of liver enzymes and uric acid in the blood • A low number of platelets in the blood which may cause you to bruise and bleed more easily • Low fever • Enlargement of the spleen which may cause pain in the abdomen or left shoulder • Worsening of skin rashes • Higher than normal white blood count	• Allergic reactions which can be life threatening with shortness of breath, low blood pressure, rapid heart rate, hives and facial swelling. This reaction is very rare and has been associated mainly with intravenous administration. • If you are known to have sickle cell disease, filgrastim may cause a sickle cell crisis • Rupture of the spleen • Difficulty breathing and lung damage that may be due to the white blood cells that are stimulated by filgrastim traveling to the lungs when they are inflamed or infected.

17.5.5. Supplier: **Commercially available. See package insert for further information.**

18.0 BIOLOGICAL STUDIES (Optional)

18.1. Tumor collection and establishment of primary neuroblastoma and medulloblastoma cell lines in culture (to be performed at the the Neuroblastoma Translational Research Lab).

18.1.1. Viable, fresh tumor >0.2grams from tumor biopsy or 2 cc from bone marrow aspirate should be placed in RPMI tissue culture media using sterile technique for cell line generation. Cells should be labeled with subjects initials and study number.

*If there is not adequate tissue available then cells will be obtained from the cytogenetics laboratory after completion of their testing.

18.1.2. If excess tissue is available, then snap frozen tumor tissue approximately 5mm in size should be wrapped in foil, snap frozen in liquid nitrogen and stored at -20ºC. Tissue samples will be coded. Fixed tissue remaining from diagnostic evaluation may be used.

18.1.3. Tumor cells will be grown to 70% confluency and transferred to Neurobasal media with EGF and FGF.

18.1.4. Cell lines will be maintained in culture for biology studies. These will include determining the growth curves and responsiveness of cells derived

from these tumors to a variety of growth factors, cytokines, neurotrophic factors, and cytotoxic agents (such as nifurtimox). We will study the genes expressed by these cells using reverse-transcriptase polymerase chain reaction (RT-PCR), Northern blot analysis, real time PCR, and DNA and mRNA micro arrays before and after treatment with nifurtimox and other agents. Further study of the proteins expressed by these cells will be done using immunocytochemistry and Western blotting. Flow cytometry will be used to detect residual neuroblastoma in bone marrow samples.

18.2. **Bone Marrow Sample Instructions (for all participating sites):**

18.2.1. If subject agrees to optional biology portion of study, bone marrow samples should be sent at the following time points:

All patients- Enrollment

For Bone Marrow Positive patients only; also send samples at: End of Cycle 2, End of Cycle 4, and End of Protocol Therapy (end of cycle 6).

18.2.2. **Sample Collection-**

Send green top (sodium heparin) tube(s) to each of the following sites with a minimum of 2cc and preferably 5cc of bone marrow aspirate in them. Both can be shipped room temperature- but need 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 notify each site (at e-mail address listed below of pending shipment arrivals). Please enclose "Bone Marrow Shipment Transmission Form" with each shipment- one for each site.

18.2.3. **Sample Shipment Addresses:**

One sample will go to:
Ping Zhao
Spectrum Health, Cooper's Landing1345 Monroe Ave; Suite 121
Grand Rapids, MI 49505
Ping.zhao@helendevoschildrens.org

The other sample will go to:
Spectrum Health Flow Cytometry Lab

Lemmen-Holton Cancer Pavilion
145 Michigan Street NE Suite 6201
Grand Rapids, MI 49503
phone: 616-486-6233

18.3. **Biomarker Development**

Biomarkers are objectively measured and evaluated indicators of normal biological processes, pathogenic processes, or pharmacologic responses to

a therapeutic intervention. In oncology, there is particular interest in the molecular changes underlying the oncogenic processes that may identify cancer subtypes and the stage of disease, assess the extent of tumor growth, provide surrogate measures of disease progression or metastasis, or predict responses to investigational products. These investigations may be useful in developing markers to identify disease subtypes, guide therapy and/or predict disease progression. In addition to testing the safety and effectiveness of the investigational product in this study, we may attempt to develop blood and/or tumor test(s) designed to identify subjects most likely to benefit from the investigational product. A 5cc blood sample will be drawn for biomarker development at screening, cycle 2, cycle 4, and at end of study, and at the safety follow-up visits.

To study the effects of human genomic (phenotypic) variation on drug response, we plan to conduct exploratory pharmacogenomic studies as part of this study. Molecular analyses on biological samples obtained from subjects on this study may be used to identify response patterns to nifurtimox, identify cancer subtypes, stage disease, assess the amount of tumor growth or predict disease progression or metastasis. These molecular analyses will not include exploratory pharmacogenetic analysis.

All subjects will be asked to provide paraffin tumor blocks (or a minimum of 20 unstained slides, if available) from the primary and/or metastatic tumor that are archived. In addition, all subjects will be asked to provide existing snap frozen tumor samples.

Corresponding pathology reports should also be provided. As an optional part of this study, subjects may be asked to undergo tissue sampling by core biopsy of a locally advanced or metastatic lesion for exploratory biomarker analyses prior to the start of study treatment. The tumor tissue biopsies must be accessible with minimal morbidity to the subject by a percutaneous core needle biopsy either with or without the aid of a CT scan or ultrasound guidance. Biopsy specimens will be obtained using standard sterile surgical techniques. All tumor biopsy samples should have a portion of the biopsy stained with hematoxylin and eosin and reviewed by a pathologist to confirm the presence and estimate the percentage of tumor tissue. The purpose of this analysis is to develop an approach for the identification and validation of transcripts that may predict which subjects are likely to respond to nifurtimox.

18.4. **Pharmacogenetic Studies**

DNA will be collected and extracted from blood and tumor samples to be utilized for exploratory pharmacogenetic analysis. These pharmacogenetics analyses are different from the blood and tumor analysis for biomarker development as described above because they are focused on evaluating different inherited or disease-induced gene forms in tumor DNA that may influence the different responses subjects have to the same drug. The goals of these exploratory studies are to identify potential genetic markers that may help in the investigation of cancer and/or

subjects who may have the best possible response to nifurtimox. The current plan is to conduct exploratory pharmacogenetic studies on tumor tissue to evaluate for mutations of genes (e.g. MYCN) in an attempt to determine if the presence of any of these mutations/rearrangements correlates with response.

Additional gene categories for analyses may include tumor suppressor genes (e.g. p53, PTEN), oncogenes (e.g., N-myc, MET), cell adhesion, invasion and metastasis genes (e.g., αvβ3, CDH1, MMP's), genes involved in regulating cell growth and the cell cycle (e.g., cyclin D1, GHRH), immune function-related and tumor antigen genes (e.g., TNF, SCYA2, KLK3), drug metabolism and transport genes (e.g., CYP3A4/5, PXR, GSTM1, ABCB1), and genes involved in angiogenesis (e.g., HIF1α VEGF, FGFR3). Those relevant to study drug action include apoptosis genes (e.g., BCL2, IAPs, TNFSF6) and the putative drug targets/pathways.

18.5. **Sample Storage and Destruction**

Blood and tumor samples collected for biomarker development and pharmacogenetic studies and any other components from the cells may be stored for up to 20 years to research scientific questions related to cancer and/or nifurtimox. The subject retains the right to have the sample material destroyed at any time by contacting the principal investigator. The PI/sponsor will be the exclusive owner of any data, discoveries, or derivative materials from the sample materials and is responsible for the destruction of the sample(s) at the request of the research subject through the principal investigator or at the end of the storage period. If a commercial product is developed from this research project, the PI/sponsor will own the commercial product. The subject will have no commercial rights to such product and will have no commercial rights to the data, information, discoveries, or derivative materials gained or produced from the sample.

19.0 REFERENCES

1. Sholler, GS, Kalkunte, S, Greenlaw, C, McCarten, K, Forman, E, Anti-Tumor activity of Nifurtimox observed in a patient with Neuroblastoma. J Pediatr Hematol Oncol. 2006 Oct;28(10):693-695

2. Solari A, Ortiz S, Soto A, Arancibia C, Campillay R, Contreras M, Salinas P, Rojas A, Schenone H, Treatment of Trypanosoma cruzi-infected children with Nifurtimox: a 3 year follow-up by PCR. Journal of Antimicrobial Chemotherapy. 2001;48:515-519

3. Ramakrishna Rao, DN, Mason, R, Generation of Nitro Anions of Some 5-Nitrofurans, 2- and 5-Nitroimidazoles by Norepinephrine, Dopamine, and Serotonin. J. Biol. Chem. 1987;262(24):11731-11736

Appendix I: Structured Interview for Adverse Neurologic Events

Study ID #:: _____ Initials: _____

Date: _____ Cycle #: _____

Provider completing form Signature: _____

Please complete with the family.

Toxicity	Grade				
	None	1	2	3	4
Headache?					
Light Sensitivity?					
Stomach upset?					
Vomiting?					
Imbalance?					
Dizziness/Lightheadedness?					
Pain in limbs?					
Cold hands/feet?					
Speech hard to understand?					
Disorientation/confusion?					
Excessive sleepiness?					
Difficulty or change in sleep routine?					
Unusual movements?					
Tremor?					
Unusual crying, irritability?					
Change in his/her interest in toys, play?					
Change in appetite?					
Change in how his/her eyes move?					
Change in ability to chew or swallow?					
Seizure or other sudden change in level of awareness?					

In each case of a positive, parents will be asked to grade the severity of the sign or symptom in a manner that will allow us to categorize the sign/symptom as class 1-5* according to the classification system as follows:

Grade	Parent's Qualitative Description of the adverse sign/symptom
Grade 1	Present only as a sign, not a problem in any sense
Grade 2	Child complains, but still the symptom is not interfering with child's usual activities
Grade 3	Interfering with child's usual activities
Grade 4	Significantly and pervasively effecting the child's daily life

[*A grade of 5 in the classification corresponds to Death and so will clearly not be the classification resulting from parent interview.]

Appendix II: REQUIRED OBSERVATIONS

Time Point:	Prestudy	Cycle 1,wk 2	Cycle 1, wk 3	Prior to each cycle‡	End of cycles 2 and 4¶	End of Protocol Therapy or Early Withdrawal	30 Day Follow-up
Physical Exam	X	X	X	X		X	X
Neuro exam	X	X	X	X		X	X
Neuro interview/TNSc	X				X	X	X
BRIEF assessment	X				X	X	
PCSQoL:MTD	X						
PCSQoL:DT	X				X	X	
Urine pregnancy test †	X						
Cbc/diff	X	X	X	X		X	X
Lytes, BUN, Creat	X	X	X	X		X	X
LDH, AST, ALT, Bili	X	X	X	X		X	X
Ferritin	X						
HVA/VMA (NB only)	X			X		X	X
CT or MRI	X				X	X	
MIBG*	X				X	X	
Bone marrow	X				X#	X#	
CSF cell count/cytology	X§				X§#	X§#	

* =MIBG not required if subject's neuroblastoma was previously determined to be not MIBG avid or for subjects with medulloblastoma
# = Repeat only if positive at study entry
† = Only for female subjects of child bearing potential (≥13 years of age)
‡ = testing to occur within 7 days of the start of each cycle (+/- 3 days)
¶ = testing to occur within 7 days of completing cycle (+/- 3 days)
§ = Medulloblastoma subjects only

ID#_____

PEDIATRIC CANCER SURVEY

Parent Assessment of Children's Quality of Life During Treatment

GENERAL DIRECTIONS

When a child is undergoing cancer treatment, it usually has major effects on the child's quality of life and the lifestyle of the family. We are interested in learning how these effects change as a result of being in certain cancer treatments/trials. On the next pages there are questions and statements describing a range of effects your child/family might experience during treatment.

PLEASE PROVIDE THE FOLLOWING INFORMATION:

Your name: _____

Your relationship to child: _____

Child's name: _____

Child's Diagnosis: _____

Child's current age: _____ (years)

Today's Date: _____

1. Does your child complain of pain? Yes___ No___
If yes, how often does he/she complain per day? _____

2. Is your child currently taking pain medications? Yes___ No___
If yes, please check medication(s) being used and indicate dose and frequency.

Medication	Dose/Type (e.g., oral, IV, patch, PCA)	How often per day?
___dilaudid		
___morphine		
___fentanyl		
___methadone		
___Tylenol		
___Tylenol with codeine		
___ibuprofen/Motrin		
___decadron		
___other pain medicine		

3. Do these medications control your child's pain? Yes___ No___

4. Besides medicines, do you use other methods to control pain? Yes___ No___
If yes, please describe: _____

5. Does your child require TPN or NG/G-button nutrition? Yes___ No___

6. Does your child take an appetite stimulant (e.g., Megace)? Yes___ No___

7. Does your child attend school? Yes___ No___
If no, does your child have a tutor/home teacher? Yes___ No___

If yes, how many days have been missed in the last 2 weeks? _____

Reasons for missing school:
___low blood counts
___pain control
___nausea
___unable to walk
___other – please describe: _____

8. Has your child had a seizure in the last 2 weeks? Yes___ No___
If yes, (1) date of last seizure _____
(2) number of seizures in last two weeks _____

9. Is your child currently on anti-seizure medication? Yes___ No___
If yes, (1) how long ago did he/she begin using the medication? _____
(2) have there been seizures since starting the medication? Yes___ No___

10. Has your child been hospitalized in the past two weeks? Yes___ No___
If yes, (1) what was the length of the hospitalization? _____
(2) describe the reason(s) for the hospitalization:
_____
_____

DIRECTIONS: Below are statements describing a range of effects your child/family might experience during treatment. For each statement, please circle the response (only one) that best describes the frequency of what has happened to your child/family during the last 2 months to the present time.	Very Frequently	Frequently	Occasionally	Rarely	Very Rarely	Never
1. My child experiences reflux.	5	4	3	2	1	0
2. My child takes antiemetic medications (for nausea) such as Zofran, Vistaril or Kytril.	5	4	3	2	1	0
3. My child experiences nausea for long periods of time.	5	4	3	2	1	0
4. My child requires weekly red blood cell transfusions.	5	4	3	2	1	0
5. My child requires weekly platelet transfusions.	5	4	3	2	1	0
6. My child requires social isolation due to low white cell counts (low immunity).	5	4	3	2	1	0
7. My child requires 7-10 days of sub-cutaneous injections of a drug that will help his/her white blood cells recover.	5	4	3	2	1	0
8. My child has to wear a mask on his/her face in crowds due to low immunity.	5	4	3	2	1	0
9. I have noticed my child's hands trembling (tremors).	5	4	3	2	1	0
10. My child experiences "neuropathic" pain – chronic pain associated with damaged nerves that is difficult to treat.	5	4	3	2	1	0
11. My child's attention span seems shorter than it used to.	5	4	3	2	1	0
12. My child talks in his/her sleep.	5	4	3	2	1	0
13. My child has nightmares.	5	4	3	2	1	0
14. I have noticed that my child is forgetful.	5	4	3	2	1	0
15. My child gets infections.	5	4	3	2	1	0
16. My child has sore bumps on his/her head.	5	4	3	2	1	0
17. My child needs steroids for pain and swelling (e.g. decadron).	5	4	3	2	1	0
18. My child has no appetite.	5	4	3	2	1	0
19. My child has mouth sores.	5	4	3	2	1	0
20. My child has trouble swallowing.	5	4	3	2	1	0

21. My child cries.	5	4	3	2	1	0
22. My child has diarrhea.	5	4	3	2	1	0
23. My child is constipated.	5	4	3	2	1	0
24. My child is not able to walk on his/her own.	5	4	3	2	1	0
25. My child takes antibiotics (e.g., vancomycin) for extended periods of time (oral/IV).	5	4	3	2	1	0
26. My child vomits.	5	4	3	2	1	0
27. My child is lethargic.	5	4	3	2	1	0
28. My child has trouble urinating.	5	4	3	2	1	0
29. My child sleeps a lot.	5	4	3	2	1	0
30. My child has a difficult time taking pills orally.	5	4	3	2	1	0
31. My child cannot concentrate.	5	4	3	2	1	0
32. My child complains of pain.	5	4	3	2	1	0
33. My child seems unhappy.	5	4	3	2	1	0
34. My child is not able to play and do things he/she wants to do.	5	4	3	2	1	0
35. My child does not smile/laugh much.	5	4	3	2	1	0
36. I believe my child has a poor quality of life.	5	4	3	2	1	0
37. My child cannot ride his/her bike/tricycle.	5	4	3	2	1	0
38. My child is unable to do things he/she wants to do on his/her own.	5	4	3	2	1	0
39. My child sleeps poorly.	5	4	3	2	1	0
40. My child is sad because we have to travel/be separated from our other family members.	5	4	3	2	1	0
41. My child cannot run.	5	4	3	2	1	0

ID#_____

PEDIATRIC CANCER SURVEY

For Parents Making Treatment Decisions

GENERAL DIRECTIONS

When a child is undergoing cancer treatment, it usually has major effects on the child's quality of life and the lifestyle of the family. Parents weigh these effects as treatment decisions are made. We are interested in learning what factors are important to you as you make these treatment decisions for your child.

On the next pages there are statements about what could happen to your child during treatment. For each statement, please circle the response (only one) that best describes the importance of each one as you consider treatment options for your child.

PLEASE PROVIDE THE FOLLOWING INFORMATION:

Your name: _____

Your relationship to child: _____

Child's name: _____

Child's current age: _____

Child's diagnosis (circle one): Medullo blastoma | First Relapse Neuroblastoma | Relapsed Neuroblastoma (includes refractory NB)

Date of first relapse: _____ (years)

Has Hospice been Recommended to you? (circle one) yes no

Today's Date: _____

DIRECTIONS: Below are statements about possible effects of treatment on your child and family during cancer treatment. Circle the number (only one) that shows how important this effect is to you as you make treatment decisions for your child.	Very Important	Important	Moderately Important	Of Little Importance	Unimportant
1. Your child's hair is likely to fall out.	4	3	2	1	0
2. Your child is likely to become nauseous and require antiemetic medications such as Zofran, Vistaril or Kytril.	4	3	2	1	0
3. Your child is likely to feel nausea for long periods of time.	4	3	2	1	0
4. Your child will likely require frequent red blood cell transfusions.	4	3	2	1	0
5. Your child will likely require weekly platelet transfusions.	4	3	2	1	0
6. Your child will be able to receive treatment drugs in clinic.	4	3	2	1	0
7. Your child will likely require social isolation following treatment drugs due to low white cell counts (low immunity).	4	3	2	1	0
8. Your child will require 7-10 days of injections of a drug that will help his/her white blood cells recover.	4	3	2	1	0
9. Your child may have to wear a mask on his/her face in crowds due to low immunity following treatment.	4	3	2	1	0
10. Your child may have hearing loss following the infusion of some treatment drugs.	4	3	2	1	0
11. Your child may require narcotic pain medicines (e.g., morphine, dilaudid, methadone).	4	3	2	1	0
12. Following treatment with some drugs, your child may experience "neuropathic" pain – chronic pain associated with damaged nerves that is difficult to treat.	4	3	2	1	0
13. Your child may miss many days of school due to low immunity or hospitalization.	4	3	2	1	0
14. Your child's permanent teeth may be adversely affected by some treatment drugs.	4	3	2	1	0
15. Your child's port will most likely be accessed and re-accessed frequently.	4	3	2	1	0
16. Your child may require a new port.	4	3	2	1	0
17. Your child may require a PIC line or femoral line as a temporary measure in the event of an infected port.	4	3	2	1	0
18. Your child may acquire an infection in his port.	4	3	2	1	0

19. You may have to relocate indefinitely to another city to receive treatment.	4	3	2	1	0
20. Your child may need steroids for pain and swelling (e.g., decadron).	4	3	2	1	0
21. Your child will most likely lose weight and suffer anorexia (stop eating).	4	3	2	1	0
22. Your child may have to travel to another city/state to do scans, biopsies and other tests on the treatment protocol.	4	3	2	1	0
23. Your child will most likely need an NG/G-button for nutrition.	4	3	2	1	0
24. Your child will most likely be able to receive continued treatment on this clinical trial at your home institution.	4	3	2	1	0
25. You may have to travel to another city/state to receive treatment.	4	3	2	1	0
26. Your child will most likely require nutrition through her port (TPN)	4	3	2	1	0
27. Your child may develop mouth sores during or after therapy.	4	3	2	1	0
28. Your child is likely to develop diarrhea during therapy.	4	3	2	1	0
29. Your child is likely to be on antibiotics for extended periods of time (oral/IV).	4	3	2	1	0
30. Your child is likely to vomit during therapy.	4	3	2	1	0
31. Your child may frequently feel lethargic and sleepy.	4	3	2	1	0
32. Therapeutic agents used on a clinical trial may be non-FDA approved for use in children or of your child's cancer.	4	3	2	1	0
33. Your child may experience reflux and other upper GI problems (esophageal strictures) as a result of therapy.	4	3	2	1	0
34. Your insurance company may be less cooperative for some clinical trial drugs and experimental therapies.	4	3	2	1	0
35. It may be necessary for you to acquire temporary housing near your treating institution.	4	3	2	1	0
36. Your child may require IV medication in the clinic.	4	3	2	1	0
37. Your child may need to take anti-seizure medication	4	3	2	1	0

38. Your child could receive combination therapy (multiple agents used at the same time) instead of single agent therapy.	4	3	2	1	0
39. Your child may require hospital admission for IV medications.	4	3	2	1	0
40. Your child may have to learn to take pills orally.	4	3	2	1	0