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A phase II trial of nifurtimox combined with topotecan and cyclophosphamide for refractory or relapsed neuroblastoma and medulloblastoma

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Abstract

Children with relapsed/refractory (R/R) neuroblastoma (NB) and medulloblastoma (MB) have poor outcomes. We evaluated the efficacy of nifurtimox (Nfx) in a clinical trial for children with R/R NB and MB. Subjects were divided into three strata: first

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BBB, blood-brain barrier; CNS, central nervous system; CR, complete response; CT, computed tomography; FDA, Food and Drug Administration; HRNB, high-risk neuroblastoma; IRB, institutional review board; MB, medulloblastoma; MIBG, 123I-metaiodobenzylguanidine; MRI, magnetic resonance imaging; NB, neuroblastoma; Nfx, nifurtimox; OS, overall survival; PD, progressive disease; PET, positron emission tomography; PFS, progression-free survival; PNS, peripheral nervous system; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; REDCap, Research Electronic Data Capture; Response rate, CR + PR; ROS, reactive oxygen species; R/R, relapsed/refractory; SD, stable disease; Total benefit rate, CR + PR + SD; TOTEM, temozolomide and topotecan.

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relapse NB, multiply R/R NB, and R/R MB. All patients received Nfx (30 mg/kg/day divided TID daily), Topotecan (0.75 mg/m^2/dose, days 1-5) and Cyclophosphamide (250 mg/m^2/dose, days 1-5) every 3 weeks. Response was assessed after every two courses using International Neuroblastoma Response Criteria and Response Evaluation Criteria in Solid Tumors (RECIST) criteria. One hundred and twelve eligible patients were enrolled with 110 evaluable for safety and 76 evaluable for response. In stratum 1, there was a 53.9% response rate (CR + PR), and a 69.3% total benefit rate (CR + PR + SD), with an average time on therapy of 165.2 days. In stratum 2, there was a 16.3% response rate, and a 72.1% total benefit rate, and an average time on study of 158.4 days. In stratum 3, there was a 20% response rate and a 65% total benefit rate, an average time on therapy of 105.0 days. The most common side effects included bone marrow suppression and reversible neurologic complications. The combination of Nfx, topotecan and cyclophosphamide was tolerated, and the objective response rate plus SD of 69.8% in these heavily pretreated populations suggests that this combination is an effective option for patients with R/R NB and MB. Although few objective responses were observed, the high percentage of stabilization of disease and prolonged response rate in patients with multiply relapsed disease shows this combination therapy warrants further testing.

KEYWORDS
medulloblastoma, neuroblastoma, nifurtimox

What's new?

Outcomes remain poor for children with relapsed and refractory neuroblastoma and medulloblastoma. This single-arm, Phase II, open-label, multicenter clinical trial shows that the combination of the novel agent nifurtimox with topotecan and cyclophosphamide is tolerated in children with relapsed or refractory neuroblastoma and medulloblastoma. The observed response rate and the high percentage of children with disease stabilization and prolonged responses suggest that the drug combination may be an option for these heavily pretreated patients with multiply relapsed disease. The findings demonstrate the feasibility and tolerability of the drug combination, supporting the need for further testing.

1 | INTRODUCTION

Tumors of the central and peripheral nervous systems (CNS, PNS) are common in both children and adults, and patients with these tumors often suffer from poor outcomes, with significant late effects from therapy in the survivors. Neuroblastoma (NB) and medulloblastoma (MB) are common forms of CNS- and PNS-derived cancers that occur in children, and children with aggressive forms of these tumors have poor outcomes due to suboptimal treatment responses and frequent tumor recurrence.[1-3] While more recently employed treatment strategies, such as risk stratification using molecular subgrouping for MB and immunotherapy for NB treatment, have resulted in some improvement in patient outcomes, novel therapies continue to be sorely needed for the patients with these neural tumors.

Nifurtimox (Nfx) is a nitrofuran compound that has been employed for over 50 years as a primary treatment for Chagas' disease, a parasitic infection caused by *Trypanosoma cruzi*.[4,6] Nfx treatment leads to the production of superoxide and hydrogen peroxide, and *T. cruzi* parasites have limited ability to detoxify these reduced oxygen metabolites.[4,5] Prior studies have shown that Nfx inhibits NB and MB cell and tumor growth.[7,8] Induction of cell death has been shown to be an effective treatment strategy for neuroblastoma,[9] and Nfx induces the formation of reactive oxygen species (ROS) and increased oxidative stress, which have been previously identified as a mechanism to induce NB cell death by numerous agents.[10] Nfx also reduces *MYCN* expression in amplified NB cell lines,[11] and reduced *MYCN* expression has been associated with NB cell death.[12,13] Nfx has demonstrated significant antitumor activity in a range of preclinical models of pediatric cancers, including NB and MB.[8,14] In early phase clinical trials, children with relapsed NB tolerated Nfx treatment well, with the most commonly reported side effects including mild nausea and vomiting along with anorexia. Tumor responses and prolonged stable disease were observed in patients treated with Nfx both as a single agent and in combination with cyclophosphamide and topotecan.[15] This combination was continued in our study due to the positive

preliminary results and low side effect profile. We therefore hypothesized that Nfx would demonstrate tolerability and efficacy in children with R/R NB and MB.

2 | MATERIALS AND METHODS

2.1 | Study design and eligibility

This was a single arm, Phase II, open label, multicenter clinical trial for subjects with NB or MB who had completed therapy for refractory/relapsed disease with remaining disease. Subjects were enrolled onto the Beat Childhood Cancer Trial V0706 from January 2008 to May 2019 at 15 participating hospitals across the United States. Eligibility included histologic verification of NB or MB. Patients initially diagnosed below 21 years of age with R/R NB or MB after chemotherapy-containing treatment regimens were eligible. Eligible patients had measurable disease by either MRI, CT, MIBG or PET scans or positive on bone marrow biopsy or aspirate and had to demonstrate adequate organ function as well as recovery from the acute toxic effects of prior chemotherapy.

Nfx was supplied as 120 mg tablets by Bayer Pharma (Whippany, NJ). The drug was administered orally 3 times a day for 21 days, and doses were rounded to the nearest whole tablet. Patients with NB were treated with Nfx dosing at 30 mg/kg/day orally divided TID, while patients with MB were treated with 20 mg/kg/day divided TID.

The first 23 patients enrolled received Nfx monotherapy daily for 21 days for cycle 1, followed by 6 cycles of Nfx daily in combination with topotecan (0.75 mg/m^2 intravenous daily on days 1-5) and cyclophosphamide (250 mg/m^2 intravenous daily on days 1-5) for a 21-day cycle, following the design of the phase 1 trial.[16] Following amendment 4 of the trial, the therapy was changed to have Nfx, topotecan, and cyclophosphamide start concurrently in cycle 1. Patients enrolled prior to amendment 4 were included in the safety analysis of the trial but not in the efficacy analysis. Only subjects enrolled after amendment 4 were included in the efficacy analysis. Patients were allowed to continue on protocol therapy while tolerating medications until progression. After completion of 6 cycles of combination therapy, patients could remain on either combination therapy or Nfx as a single agent, as long they demonstrated benefit, as defined by CR/PR/SD.

During combination therapy, weekly monitoring for treatment-related toxicities included a physical examination including a complete neurologic exam, complete blood count, complete metabolic panel. In addition, urinary catecholamines (for NB patients) were collected before every 21-day cycle and at the end of combination therapy. After cycles 2 and 4 a neurologic structured interview as performed. Clinical and laboratory adverse events (AEs) were graded according to the National Cancer Institute Common Toxicity Criteria version 3. All Grade 3 (CTCAE Version 3.0) or higher AE's (except hematologic) were required to be captured. For patients who remained on treatment after 6 cycles of therapy, monitoring continued once every 21 days.

Dose modifications for toxicity occurred in any patients who demonstrated grade 3 or 4 CNS/PNS toxicity. At this point, Nfx was held and if symptoms resolved within 14 days, patients could resume Nfx at lower doses, with the first dose reduction down to 20 mg/kg/day for patients with NB and to 15 mg/kg/day for patients with MB. Further dose reduction to 15 mg/kg/day and 10 mg/kg/day, respectively, was allowed for recurrent toxicity, with no further dose reductions allowed. Any patients who demonstrated grade 2 CNS/PNS toxicity could have Nfx held at the discretion of the treating physician, during which an evaluation of the symptoms was conducted for a period up to 7 days maximum. Patients who developed any other grade 4 toxicity attributable to Nfx (other than hematologic or febrile neutropenia) had Nfx held and were followed until resolution of toxicity or until it was grade 2. If resolution occurred within 7 days, patients could resume Nfx at the lower dose level. Patients who developed any expected grade 3 or 4 toxicity (except hematologic or infectious) attributable to cyclophosphamide or topotecan could have that drug held but remain on study to evaluate Nfx toxicity. These patients were censored for response to treatment at that point.

For both NB and MB patients, reevaluation included scans (MIBG, PET, CT, or MRI of primary tumor) every 2 cycles. For NB patients, bone marrow aspirate and biopsy (if positive at study entry) and urinary catecholamines were performed at the end of every 2 cycles. Measurable tumor response was measured by Response Evaluation Criteria in Solid Tumors (RECIST). Overall response was measured using the International Neuroblastoma Response Group (INRG) 1993 criteria,[17] which takes into consideration measurable disease, MIBG response, bone marrow, and HVA/VMA. Time to event analyses were calculated and presented using the Kaplan-Meier method.[18] Tabulation of response and AE data are reported. Study data were collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at Atrium Health. REDCap is a secure, web-based software platform designed to support data capture for research studies.[19,20]

3 | RESULTS

3.1 | Patients

One hundred and twelve patients with R/R NB or MB were enrolled on our study between January 2008 and November 2018. Twenty-three patients enrolled prior to amendment 4, and 89 patients enrolled after amendment 4. One patient that enrolled prior to amendment 4 was a screen failure and did not start protocol therapy. Of the 89 patients enrolled after amendment 4, 13 were not evaluable for efficacy (11 discontinued therapy prior to evaluations, 1 died of infection, and 1 failed screening after enrollment), leaving 76 evaluable for efficacy (Figure 1). Of the patients evaluable for response, 13 were in stratum 1 (first relapse NB), 43 were in stratum 2 (multiply R/R NB), and 20 were in stratum 3 (R/R MB). Among all patients enrolled on study (N = 112) (Table 1), the mean age at diagnosis was 5.5 years, with a range of 1.1 to 21.5 among all three strata. 65.2% of patients enrolled on the trial were males, and 34.8% were females. The patients enrolled were of diverse ethnicities including: 65.2% white, 9.8% black/African American, 0.9% American Indian/Alaskan Native, 14.3% Hispanic, 3.6% Asian, 1.8% Hawaiian, 1.8% more than one, and 2.7% unknown. 100% of stratum 1 patients were stage 4 at diagnosis.

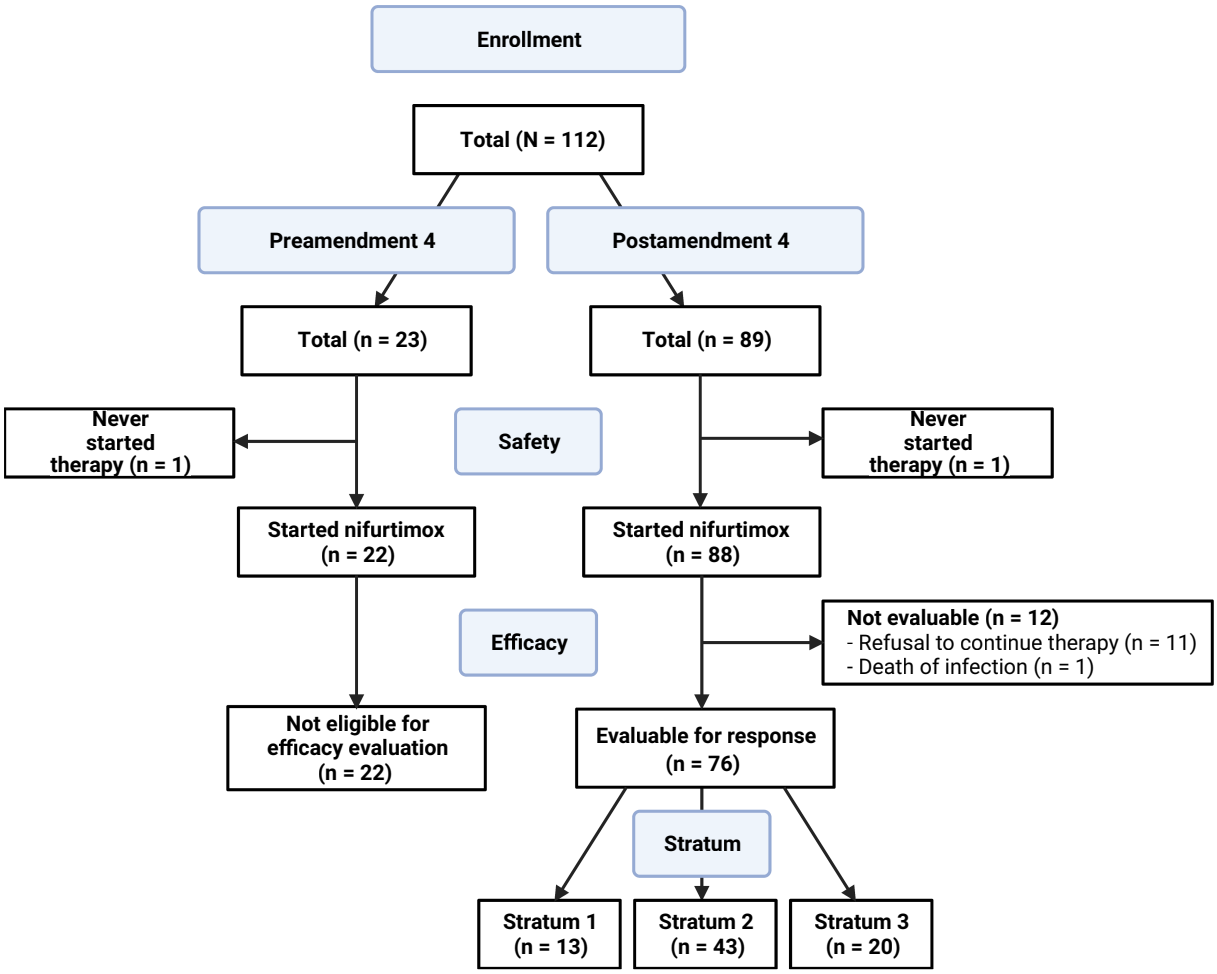

FIGURE 1 Consort diagram. [Color figure can be viewed at wileyonlinelibrary.com]

48.6% of stratum 2 patients were stage 4 at diagnosis with 2.9% stage 3 and 48.6% unknown. Stratum 3 was comprised of 16% M0, 4% M2, 4% M3, 8% M4, 4% M+, and 64% unknown stages at diagnosis. In stratum 1, 17.6% of patients had *MYCN* amplification with 58.8% nonamplified and 23.5% unknown. In stratum 2, 20% of patients had *MYCN* amplification with 32.9% nonamplified and 47.1% unknown. The overall mean time from diagnosis to Nfx therapy was 3.1 years with a range of 0.5 to 12.1 years.

3.2 | Toxicities

One hundred and ten patients received at least 1 dose of Nfx and were evaluable for toxicity. Nfx-related expected and unexpected AEs observed are shown in Table 2. Hematologic (27% of the total AEs) and neurologic (41% of the total AEs) toxicities were the most commonly noted. Thirty patients experienced hematologic toxicities, the most notable being 7 patients reported with neutropenia (grade 4) and 7 reported with thrombocytopenia (6 grade 4, 1 grade 3) found to be attributed to Nfx therapy. Four patients stopped therapy due to prolonged cytopenia. There were 47 patients with reported cases of neurologic toxicities related to Nfx, among the most notable being 5 patients with seizures (grade 3), 10 with ataxia (grade 3), 6 with confusion (grade 3), and 7 with motor neuropathy/weakness (6 grade 3, 1 grade 4). Forty-seven patients had nonhematologic/nonneurologic AEs related to Nfx. Of these, the most common included anorexia (7 grade 3), febrile neutropenia (5 grade 3), nausea (6 grade 3), and vomiting (6 grade 3). There was one death due to infection on day 10 of cycle 1.

3.3 | Patient responses

Eighty-nine patients were enrolled in the efficacy portion of the trial. Of these, 1 patient was a screen failure, 1 patient died of infection prior to first time point evaluation, and 11 patients discontinued therapy prior to the first evaluation time point. Seventy-six patients were ultimately eligible for evaluation, 13 in stratum 1, 43 in stratum 2, and 20 and stratum 3. 1 year event-free survival (EFS) rates were 38% for patients in stratum 1, 19% for patients in stratum 2, and 20% for patient in stratum 3 (Figure 2). Within stratum 1 (first relapse NB) 5 patients had a complete response (CR), 2 patients had partial

TABLE 1 Patient characteristics.

Nifurtimox patient characteristics	Stratum 1 (n = 17)	Stratum 2 (n = 70)	Stratum 3 (n = 25)	Total (N = 112)
Mean age at diagnosis, years (range)	4.7 (2.0, 11.7)	4.2 (1.1, 15.2)	7.7 (2.2, 21.5)	5.5 (1.1, 21.5)
Sex, n (%)	11 (64.7%)	44 (62.9%)	18 (72.0%)	73 (65.2%)
Male	6 (35.3%)	26 (37.1%)	7 (28.0%)	39 (34.8%)
Female				
Ethnicity, n (%)	9 (52.9%)	51 (72.9%)	13 (52.0%)	73 (65.2%)
White	3 (17.6%)	5 (7.1%)	3 (12.0%)	11 (9.8%)
Black/African American	1 (5.9%)	0 (0%)	0 (0%)	1 (0.9%)
American Indian/Alaskan Native	2 (11.8%)	9 (12.9%)	5 (20.0%)	16 (14.3%)
Hispanic	1 (5.9%)	3 (4.3%)	0 (0%)	4 (3.6%)
Asian	0 (0%)	0 (0%)	2 (8.0%)	2 (1.8%)
Hawaiian	0 (0%)	1 (1.4%)	1 (4.0%)	2 (1.8%)
More than one	1 (5.9%)	1 (1.4%)	1 (4.0%)	3 (2.7%)
Unknown				
Stage at diagnosis, n (%)	2:0 (0%)	2:0 (0%)	M0: 4 (16.0%)	
	3:0 (0%)	3:2 (2.9%)	M2: 1 (4.0%)	
	4:17 (100.0%)	4:34 (48.6%)	M3: 1 (4.0%)	
	UNK: 0 (0%)	UNK: 34 (48.6%)	M4: 2 (8.0%)	
			M+: 1 (4.0%)	
			UNK: 16 (64.0%)	
MYCN status, n (%)	3 (17.6%)	14 (20%)		
Amplified	10 (58.8%)	23 (32.9%)		
Nonamplified	4 (23.5%)	33 (47.1%)		
Unknown				
Mean time from diagnosis to NFX, years (range)	2.4 (1.4, 3.8)	2.9 (0.5, 9.2)	4.1 (0.5, 12.1)	3.1 (0.5, 12.1)

response (PR), 2 patients had prolonged SD, and 4 patients had progressive disease (PD). One patient with SD also showed clinical response. There was a 53.9% response rate (CR + PR), and 69.3% total benefit rate (CR + PR + SD). The average time on therapy for stratum 1 was 165.2 days. Within stratum 2 (multiply R/R NB), 1 patient had a CR, 6 patients had a PR, 24 had SD, and 12 had PD. Thirteen patients with prolonged SD also showed clinical response. There was a 16.3% response rate and a 72.1% total benefit rate. The average time on study for stratum 2 was 158.3 days. Within stratum 3 (R/R MB), 1 patient had a CR, 3 had PR, 9 had SD and 7 had PD. There was a 20% response rate and a 65% total benefit rate. The average time on study for stratum 3 was 105 days (Table 3).

4 | DISCUSSION

Oral Nfx has been used at lower doses for treatment of Chagas' disease. In 2020, Nfx was FDA approved in the United States for treatment in pediatric patients at doses of 8-20 mg/kg/day for 60 days.[21] Nfx is typically administered for 60 to 90 days to treat acute *T. cruzi* infections, but extended treatment can be employed for chronic *T. cruzi* infections.[22,23] In adults receiving Nfx for *T. cruzi* treatment, the most common side effects have included nausea, vomiting, diarrhea, anorexia, and weight loss. Additional common side effects include irritability, sleep disorders, and peripheral neuropathies.[22] Extended treatment is associated with additional side-effects such as tremors, muscle weakness, mild paresthesia, and polyneuritis.[24,25] In contrast to adult patients with Chagas' disease, infants and young children that are treated for Chagas' disease with Nfx generally experience only minor AEs, such as anorexia and diarrhea.[26,27] In children with NB that were treated with Nfx in a phase I study, the reported side effects included anorexia, nausea, stomach pain, neuropathies, and infrequent seizures.[15] The maximum tolerated dose in this patient population was determined to be 30 mg/kg/day.[15]

One key limitation of treatments for both children and adults with CNS tumors is the limited ability of many therapeutics agents to penetrate the blood-brain barrier (BBB). Nfx penetrates the BBB in preclinical models,[28,29] and Nfx treatment has been shown to be effective in patients with late-stage African trypanosomiasis (African sleeping sickness), defined by the presence of active CNS infection,[30,31] demonstrating the likely ability to penetrate the BBB, although the relative levels of BBB penetration by Nfx in patients with parasitic infections compared to those with CNS malignancies remains unknown. In addition to our reported results, Nfx has also demonstrated efficacy in orthotopic models of CNS tumors,[7,8] suggesting that Nfx can both

TABLE 2 Adverse events—Grade 3 or higher expected and unexpected adverse events attributed (possibly, probably or definitely) to nifurtimox.

Nifurtimox adverse events (n = 110)	Grade 3	Grade 4	Grade 5
Hematologic			
Anemia	7 (6%)	2 (2%)	
Leukopenia	3 (3%)	4 (4%)	
Neutropenia		7 (6%)	
Thrombocytopenia	1 (<1%)	6 (5.5%)	
Neurologic			
Aphasia	1 (<1%)		
Ataxia	10 (9%)		
Blurry vision/diplopia	1 (<1%)		
Cognitive disturbance	1 (<1%)		
Confusion	6 (5.5%)		
Dizziness	1 (<1%)		
Dysarthria	1 (<1%)		
Dysphagia	1 (<1%)		
Memory impairment	3 (3%)		
Mental status change	1 (<1%)		
Motor neuropathy/weakness	6 (5.5%)	1 (<1%)	
Nystagmus	1 (<1%)		
Peripheral sensory neuropathy	4 (4%)		
Pyramidal tract dysfunction	1 (<1%)		
Seizures	5 (5%)		
Toxic metabolic encephalopathy	1 (<1%)		
Tremors	2 (2%)		
Nonhematologic/Nonneurologic			
Abdominal pain	1 (<1%)		
ALT elevation	3 (3%)		
AST elevation	2 (2%)		
Anorexia	7 (6%)		
Dehydration	1 (<1%)		
Fatigue	2 (2%)		
Febrile neutropenia	5 (5%)		
Hypokalemia	1 (<1%)	1 (<1%)	
Hyponatremia	1 (<1%)		
Hypophosphatemia	1 (<1%)		
Infection, other			
Insomnia	1 (<1%)	1 (<1%)	
Malnutrition	1 (<1%)		
Mucositis	1 (<1%)		
Nausea	6 (5.5%)		
Pain	4 (4%)		
Vomiting	6 (5.5%)		
Weight loss	2 (2%)		

Note: Percentages calculated by # of patients with event divided by # of patients in group that received the drug.

penetrate the BBB and reach effective levels in patients with CNS tumors such as MB.

Despite the reported preclinical and clinical efficacy of Nfx against neural tumors, the mechanism(s) underlying Nfx efficacy is not well understood. The effects of Nfx against parasites is likely due to cellular reduction of the nitro heterocyclic Nfx molecule to nitro anion free radicals, hydrogen peroxide, and superoxide free radicals, leading to the generation of intracellular ROS.[32] In preclinical models of NB, Nfx induces apoptosis via ROS induction of ROS that are generated in the presence of catecholamines,[7] but the mechanisms of action of Nfx in MB tumor cells and in other tumors in the absence of catecholamines have not been determined.

While there have been improvements in outcomes for newly diagnosed MB, relapsed MB continues to have extremely poor overall survival. Therapy for relapsed MB has consisted of resection when possible, high-dose chemotherapy,[33] reirradiation,[34] and high-dose chemotherapy with stem cell rescue.[35] As these intense modalities have shown poor outcomes with a high level of morbidity, more recent therapies using low-dose metronomic therapies, such as temozolomide[36] or oral etoposide,[37] and antiangiogenic therapy with bevacizumab, irinotecan, and temozolomide[38] have been tried. However, most studies have shown limited improvements in event free survival and overall survival rates for patients with relapsed disease.

To date, the best outcomes reported have been through the European consortium who recently reported a multi-institutional phase 2 study utilizing temozolomide and topotecan (TOTEM).[39] There were 29 patients on study, 1 was a CR, 7 were PR and 9 patients had SD for an overall objective response rate of 28% (CR + PR) and an overall total benefit rate of 59% (CR + PR + SD). Our results are comparable with 1 CR, 3 PR, and 9 SD, equating to a 20% response rate and a 65% total benefit rate. Patients in the European trial had either 1 or 2 therapies prior to starting the TOTEM trial, with the majority (26/29) only having received 1 prior to starting treatment. On our trial, the range of prior therapies was 1-8, with a mean of 3 prior therapies before starting treatment.

With new treatment approaches, including targeted therapies and immunotherapy, the outcomes for children with high-risk neuroblastoma (HRNB) have significantly improved over the past several decades, but many of these children continue to suffer from poor responses to initial therapy or from disease relapse during or after the completion of treatment. Relapsed NB has been considered invariably fatal in the past,[40] and recent studies determined a median overall survival time for children with refractory NB of approximately 28 months and an overall survival time of 11 months for patients with relapsed disease.[41] Reviews of patient outcomes in children after the initial relapse of NB have identified a 5-year overall survival rate of 20%,[42,43] with outcomes dependent on both the time of relapse and the initial patient tumor stage,[42-45] emphasizing the need for additional treatment options for these children.

While there are no prior studies that have reported the efficacy of cyclophosphamide and topotecan in patients with R/R MB, there are a

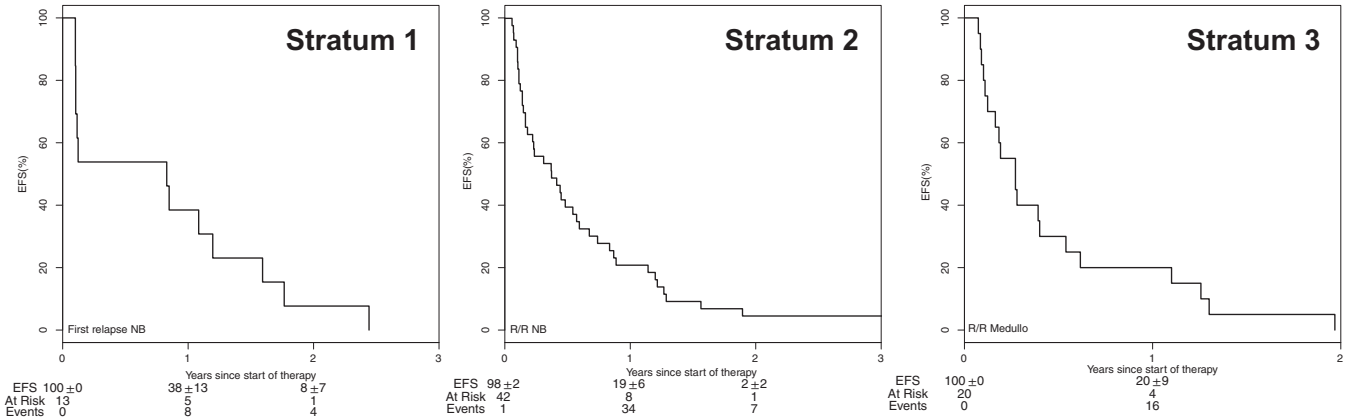

FIGURE 2 Kaplan-Meier curves on nifurtimox. Shown are the event-free survival curves for the different strata: Stratum 1: first relapse NB, Stratum 2: relapsed/refractory (R/R) NB, Stratum 3: relapsed/refractory (R/R) MB. The median survival time is 9.96, 4.44 and 3.24 months, respectively.

TABLE 3 Patient responses.

Nifurtimox patient responses	CR	PR	SD	PD	Total response rate (CR + PR)	Total benefit (SD + PR + CR)	Mean time on study
Stratum 1 (n = 13)	5	2	2	4	53.9%	69.3%	165 (38, 527)
Stratum 2 (n = 43)	1	6	24	12	16.3%	72.1%	158 (21, 1955)
Stratum 3 (n = 20)	1	3	9	7	20%	65%	105 (27, 354)
Total (n = 76)	7	11	35	23	23.7%	69.8%	145 (21, 1955)

Note: Responses displayed as n. Mean time on study displayed as days (range).

number of studies reported for children with R/R NB. Objective responses were observed in 6 of 13 patients in an initial phase II study using cyclophosphamide and topotecan in children with relapsed NB.[46] A subsequent clinical trial for children with recurrent NB who were treated with the combination of cyclophosphamide and topotecan demonstrated that the combination was associated with higher rates of response compared to isolated topotecan, although both treatments were found to have similar overall survival rates.[47,48] In a study that combined increased doses of cyclophosphamide and topotecan with vincristine, the overall response rate for children with primary refractory NB was 19%, with a 52% response rate for children that were treated at the time of their initial relapse.[49] Although we had a small cohort of patients with first relapse, the response rate of 54% and the total benefit rate of 69% are comparable to other studies of first relapse. In addition, we report on the outcomes of patients who have been heavily pretreated. In stratum 2, the response rate was only 16%, but the total benefit rate was 72%, and patients remained on study for an average of 158 days.

For our MB stratum, there are no previously published results of studies with the combination of cyclophosphamide and topotecan in R/R MB, making understanding the contribution of Nfx to the outcomes difficult. However, the results are quite favorable compared to other therapies previously published, of which most have been with high dose chemotherapy, which frequently has high morbidity, while patients on our study had relatively low morbidity while on treatment. At the start of this trial, the new subclasses for MB had not yet been reported, so this information was not collected on our patients, preventing benefit analysis for individual subgroups. A randomized study could help to evaluate the contribution of Nfx to these results. The low number of patients in stratum 1 was partially related to the concurrent trial with anti-GD2 antibody, temozolomide, and irinotecan,[50] which also enrolled NB patients with first relapse, and, since its publication, has become the new standard of care in NB patients at the time of their first relapse.

The dose of Nfx was chosen from the MTD of the phase 1 trial (30 mg/kg/day divided TID daily) and it is possible that an alternative dose or schedule could improve these results. In the three strata, we noted that a significant number of patients required a dose reduction due to side effects related to Nfx. Although not an aim of the study, we observed a significantly prolonged time on study for patients who had a dose reduction vs those that did not have a dose reduction. In stratum 1, 8 of 13 patients had a dose reduction with an average time on study of 206 days vs 99 days for those without a dose reduction. Stratum 2 patients had the greatest difference in time on study with those with a dose reduction of 251 days vs 76 days for those without a dose reduction (20 of 43 patients), while those in stratum 3 had the lowest difference in time on study of 120 days vs 86 days (11 of 20 patients). The patients with medulloblastoma (stratum 3) started at a lower dose of 20 mg/mg/day and had a smaller dose reduction when required which may account for the smaller change in time difference for those with a dose reduction vs no dose reduction, as the

dose reduction for this strata was proportionally less than for strata 1 and 2. These data suggest that a lower dose may be more beneficial than the starting dose derived from the phase 1 trial.

For relapsed cancers with poor OS, alternative measurements such as PFS, time on therapy, and morbidity associated with therapy should be considered as surrogate measures of benefit to the patient. In this trial, patients with both R/R NB and MB showed clinical benefit with significant high percentages of clinical response (CR + PR) and clinical benefit (CR + PR + SD) along with prolonged time on therapy. One drawback with the use of Nfx was the high percentage of patients with neurologic AEs (41%). However, these were all reversible upon holding Nfx, and, in most cases, therapy could resume with dose reduction of the drug, which allowed patients to continue on treatment. AEs were otherwise comparable to other treatment regimens. Additionally, patients who continued on Nfx alone after seeing clinical benefit were found to have very few AEs. One patient with NB remained on single agent therapy for greater than 1900 days with no identifiable long-term complications or progression of disease.

In our study, we describe the use of the novel agent Nfx in combination with cyclophosphamide and topotecan for the treatment of both R/R NB and MB in a phase II study. While both populations were heavily pretreated, patients showed benefit from the treatment. Patients with first relapse had good overall responses, while patients with multiple previous treatments showed clinical benefit from the treatment, with relatively low complications. The combination of chemotherapy with Nfx demonstrated feasibility and with good overall response rates and clinical benefit rates, Nfx shows promise for further clinical evaluation. While our results are not able to demonstrate the added value of Nfx over chemotherapy alone, they do demonstrate the feasibility and tolerability of this combination, and a further prospective randomized controlled trial would be needed to determine the added benefit.

AUTHOR CONTRIBUTIONS

Conceptualization: Giselle L. Saulnier Sholler, Genevieve Bergendahl, Don Eslin, Peter E. Zage, William Ferguson, William Roberts, Jacqueline Kraveka, Deanna Mitchell, Gina Hanna. *Data collection*: Giselle L. Saulnier Sholler, Genevieve Bergendahl, Don Eslin, Peter E. Zage, William Ferguson, William Roberts, Jacqueline Kraveka, Deanna Mitchell, Michael S. Isakoff, Jawhar Rawwas, Randal K. Wada, Mark Fluchel, Valerie I. Brown, Kevin Ginn. *Data analysis*: Giselle L. Saulnier Sholler, Genevieve Bergendahl, Don Eslin, Peter E. Zage, Elizabeth Lewis, Timothy Higgins, Abhinav BeeravallyNagulapally, Karl Dykema. *Supervision*: Giselle L. Saulnier Sholler, Genevieve Bergendahl, Jacqueline Kraveka. *Writing*: Giselle L. Saulnier Sholler, Don Eslin, Genevieve Bergendahl, Peter E. Zage, Elizabeth Lewis, Abhinav BeeravallyNagulapally. *Editing*: All authors. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of our study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This was a phase II, open-label, multicenter, study with Nfx in pediatric subjects with R/R NB and MB. Subjects were enrolled onto trial from January 2008 to November 2018. This trial was approved by the Western Institutional Review Board (WIRB) as well as by local IRBs. Prior to study entry, written informed consent for study participation was obtained from the subjects or legal representative (if age 18 or over) or from a parent and/or legal guardian (if under age 18). All methods were performed in accordance with relevant guidelines and regulations to the principles of the Declaration of Helsinki, the International Council for Harmonization guideline for Good Clinical Practice, and all applicable local regulatory requirements. ClinicalTrials.gov Identifiers: NCT00601003.

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