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SHORT REPORT

A subset analysis of a phase II trial evaluating the use of DFMO as maintenance therapy for high-risk neuroblastoma

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Abstract

Neuroblastoma is a sympathetic nervous system tumor, primarily presenting in children under 6 years of age. The long-term prognosis for patients with high-risk neuroblastoma (HRNB) remains poor despite aggressive multimodal therapy. This report provides an update to a phase II trial evaluating DFMO as maintenance therapy in HRNB. Event-free survival (EFS) and overall survival (OS) of 81 subjects with HRNB treated with standard COG induction, consolidation and immunotherapy followed by 2 years of DFMO on the NMTRC003/003b Phase II trial were compared to a historical cohort of 76 HRNB patients treated at Beat Childhood Cancer Research Consortium (BCC) hospitals who were disease-free after completion of standard upfront therapy and did not receive DFMO. The 2- and 5-year EFS were 86.4% [95% confidence interval (CI) 79.3%-94.2%] and 85.2% [77.8%-93.3%] for the

NMTRC003/003b subset vs 78.3% [69.5%-88.3%] and 65.6% [55.5%-77.5%] for the historical control group. The 2- and 5-year OS were 98.8% [96.4-100%] and 95.1% [90.5%-99.9%] vs 94.4% [89.3%-99.9%] and 81.6% [73.0%-91.2%], respectively. DFMO maintenance for HRNB after completion of standard of care therapy was associated with improved EFS and OS relative to historical controls treated at the same institutions. These results support additional investigations into the potential role of DFMO in preventing relapse in HRNB.

KEYWORDS

DFMO, high-risk neuroblastoma, maintenance

1 | INTRODUCTION

Neuroblastoma (NB) is a tumor of the autonomic nervous system, typically originating from the adrenal medulla. This cancer occurs in 1/7000 children, with 800 cases annually in the US. Risk stratification of NB is determined by a variety of factors including age, stage, histology and cytology, with *MYCN* gene amplification serving as the primary prognostic indicator. Patients with low- and intermediate-risk disease often achieve long-term remission with moderate courses of chemotherapy and/or surgical resection. In contrast, patients with high-risk NB (HRNB), which represents approximately 50% of cases, demonstrate poorer long-term survival despite aggressive, multimodal frontline therapy including 5-8 cycles of multiagent induction chemotherapy followed by surgical resection, consolidation therapy with high-dose chemotherapy plus autologous stem cell support, radiation therapy and maintenance immunotherapy with dinutuximab plus *cis*-retinoic acid.[1-6] Data from the Children's Oncology Group's (COG) pivotal ANBL0032 study show a 2-year event-free survival (EFS) from the start of immunotherapy of 66% ± 5%,[5] decreasing to 59% ± 5% at 4 years,[7] thus demonstrating the need for further improvements in post-immunotherapy maintenance therapy. While more recent data have reported an improved 3-year EFS and overall survival (OS) for patients receiving tandem courses of high-dose chemotherapy with stem cell support prior to immunotherapy (73.3% [95% confidence interval (CI), 65.2%-81.3%] and 84.0% [77.3%-90.7%], respectively), approximately 25% of patients will still relapse.[8] While patients with relapsed/refractory disease after upfront treatment may respond transiently to salvage therapies, the rate of subsequent relapse remains extremely high at 80%-90% within 2 years.[9-11] Thus, interventions designed to prevent relapse after upfront therapy may provide an important strategy to improve the long-term survival of HRNB patients.

The antineoplastic mechanism of difluoromethylornithine (DFMO) is proposed to involve irreversible inhibition of the rate-limiting enzyme of polyamine biosynthesis, ornithine decarboxylase (*ODC*).[12] Elevated polyamine levels, due to overexpression of *ODC*, are thought to contribute to neoplastic transformation and have been identified in NB and other tumors. Furthermore, laboratory studies have demonstrated that a reduction of polyamine levels through

What's new?

The long-term prognosis for patients with high-risk neuroblastoma (HRNB) remains poor despite aggressive multimodal therapy. This phase II clinical trial update is the first study to demonstrate statistically and clinically significant improvement in event-free and overall survival in HRNB patients as a result of DFMO administration following standard upfront therapy relative to a closely matched historical control. The findings support DFMO maintenance as a novel strategy to prevent relapse in a high-risk population for relapse following frontline treatment.

DFMOs inhibition of *ODC* prevents progression through the stages of carcinogenesis both in vitro and in xenograft models.[13-16] DFMO also possesses downstream activity in the LIN28/Let7 axis and has been shown to reverse cancer stem cell pathways. In vitro and xenograft models have demonstrated decreased neurosphere and tumor formation as a result of decreased LIN28 and increased Let7 after DFMO treatment.[17,18]

DFMO has been clinically studied through the Beat Childhood Cancer Research Consortium (BCC) since 2010. An initial Phase I trial established the safety of DFMO in multiply relapsed patients at dose levels up to 1500 mg/m^2 by mouth twice a day without reaching a maximum tolerated dose; three patients enrolled in our study remain alive >9 years poststudy without further therapy. A subsequent Phase II study evaluated the EFS and OS for patients receiving DFMO as maintenance therapy after completion of upfront (Stratum I) or relapsed/refractory therapy (Stratum II) for HRNB. Those who received DFMO after completion of standard therapy (Stratum I) demonstrated a 2-year EFS of 84% and OS of 97% while those who received DFMO after therapy for relapsed/refractory disease had a 2-year EFS of 51% and OS of 84%.[19] The majority of patients enrolled on Stratum I received initial treatment as per the standard of care used by our enrolling hospitals (COG HRNB upfront therapy). BCC conducted a retrospective study (BCC001) to identify a historical control population from the same hospitals as a statistical comparator.

The following report updates the results of the subset of patients on NMTRC003/003b who received standard COG upfront therapy and compares them to the outcomes of patients identified by the BCC001 retrospective study.

2 | MATERIALS AND METHODS

2.1 | NMTRC003/003b study design, subjects and treatment

This Phase II clinical trial evaluated the use of DFMO for patients with HRNB as maintenance therapy after standard upfront therapy (Stratum I) or relapsed/refractory therapy (Stratum II). Subjects were enrolled from June 2012 to February 2016, and results have been previously reported.[19] This trial was approved by the Western Institutional Review Board (WIRB) as well as by all local Institutional Review Boards (IRB) at 22 enrolling hospitals across the United States. Consent for study participation was obtained on all subjects according to institutional guidelines. All methods were performed in accordance with relevant guidelines and

regulations. ClinicalTrials.gov identifiers include: NCT01586260, Unique ID: NMTRC003, released April 24, 2012 and NCT02395666, Unique ID: NMTRC003B, released on March 5, 2015.

Patients with HRNB who completed standard upfront therapy without progression were eligible for enrollment. DFMO was initiated within 120 days of the completion of therapy at a dose of 750 ± 250 mg/m^2/dose, twice daily. For the purpose of this report, we focus on a subset (n = 81) of the 100 enrolled patients who had been treated with the standard of care according to the COG guidelines for HRNB (induction, surgical resection, consolidation, radiation therapy and immunotherapy maintenance with dinutuximab) followed by maintenance DFMO and compare their outcomes to a retrospective cohort of patients treated at participating BCC hospitals with the same upfront therapies but without DFMO.

2.2 | BCC001 study design, subjects and treatment

The historical control was generated through a retrospective study, BCC001 "Retrospective Chart Review of HRNB Patients", that was

TABLE 1 Subject characteristics

Subject characteristics	NMTRC003/003b (n = 81)	BCC001 (n = 76)	P value
Mean age at diagnosis, years	4.5	3.4	.0076
Sex, n (%)			.4862
Male	48 (59.3%)	48 (63.2%)	
Female	33 (40.7%)	27 (35.5%)	
Unknown	0 (0%)	1 (1.3%)	
Stage at diagnosis, n (%)			.3610
1	0 (0%)	0 (0%)	
2A	0 (0%)	0 (0%)	
2B	2 (2.5%)	0 (0%)	
3	7 (8.6%)	5 (6.6%)	
4	72 (88.9%)	70 (92.1%)	
4S	0 (0%)	1 (1.3%)	
Unknown	0 (0%)	0 (0%)	
MYCN, n (%)			.3288
Amplified	37 (45.7%%)	30 (39.5%)	
Nonamplified	41 (50.6%)	39 (51.3%)	
Unknown	3 (3.7%)	7 (9.2%)	
Histology, n (%)			.0004
Unfavorable	43 (53.1%)	59 (77.6%)	
Favorable	6 (7.4%)	8 (10.5%)	
Unknown	32 (39.5%)	9 (11.8%)	
Response to induction therapy, n (%)			.0461
CR	39 (48.1%)	23 (30.3%)	
VGPR	10 (12.3%)	11 (14.5%)	
PR	25 (30.9%)	37 (48.7%)	
SD	5 (6.2%)	1 (1.3%)	
Unknown	2 (2.5%)	4 (5.3%)	

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; VGPR, very good partial response.
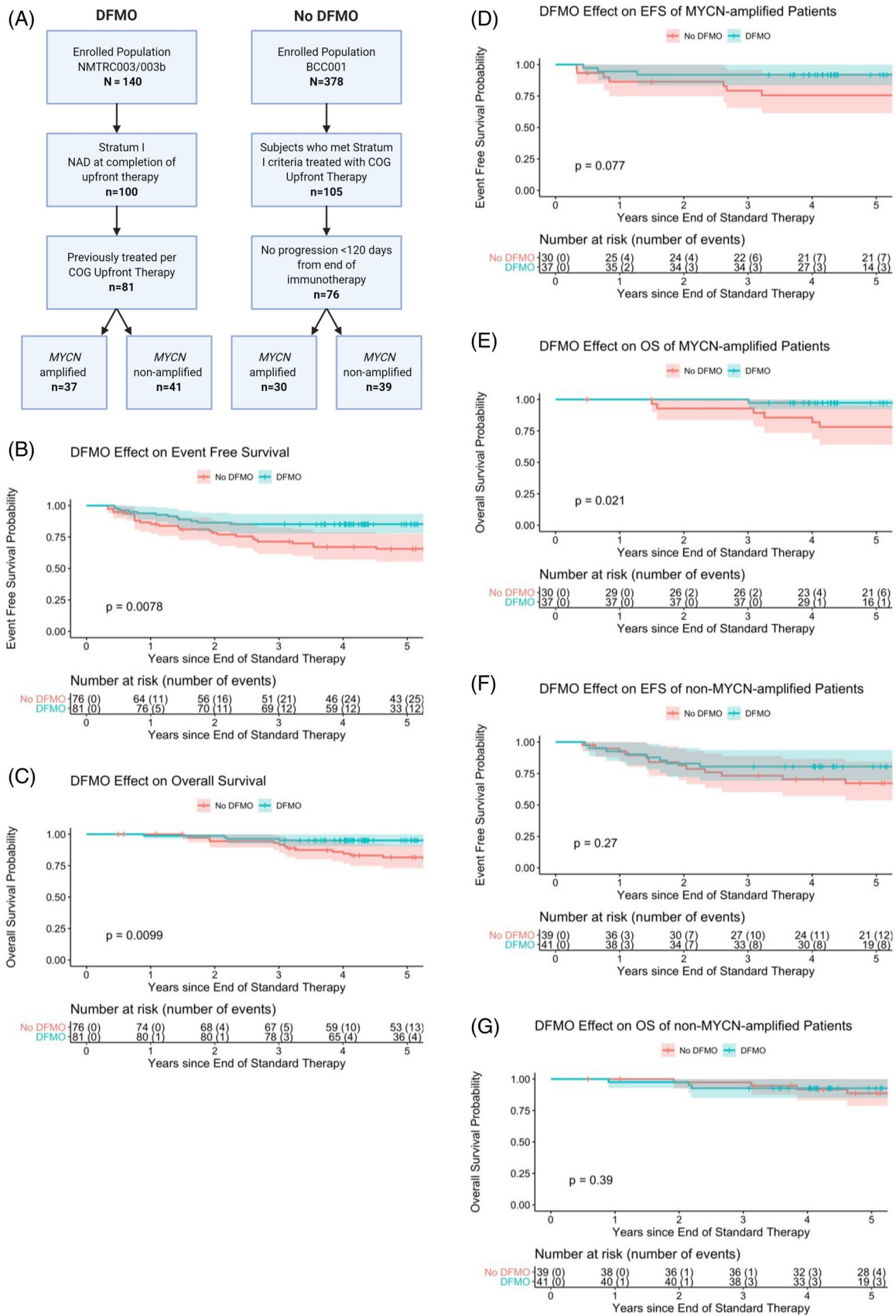
FIGURE 1 Legend on next page.

TABLE 2 EFS and OS Outcomes for NMTRC003/003b vs BCC001 groups

Study					
EFS	2-year	5-year	*P* value	HR	*P* value
NMTRC003/003b (DFMO)	0.864 (0.793, 0.942)	0.852 (0.778, 0.933)	.0078*	0.404 (0.203, 0.805)	.0100*
BCC001 (No DFMO)	0.783 (0.695, 0.883)	0.656 (0.555, 0.775)			
OS					
NMTRC003/003b (DMFO)	0.988 (0.964, 1.000)	0.951 (0.905, 0.999)	.0099*	0.257 (0.085, 0.779)	.0163*
BCC001 (No DFMO)	0.944 (0.893, 0.999)	0.816 (0.730, 0.912)			
EFS					
MYCN-A					
DFMO	0.919 (0.835, 1.000)	0.919 (0.835, 1.000)	.0766	0.315 (0.081, 1.217)	.0938
No DFMO	0.864 (0.749, 0.997)	0.756 (0.614, 0.931)			
OS					
MYCN-A					
DFMO	1.000 (1.000, 1.000)	0.973 (0.922, 1.000)	.0207*	0.123 (0.015, 1.023)	.0525
No DFMO	0.929 (0.838, 1.000)	0.781 (0.641, 0.953)			
EFS					
Non-*MYCN*-A					
DFMO	0.829 (0.722, 0.953)	0.805 (0.692, 0.936)	.2697	0.607 (0.248, 1.486)	.2750
No DFMO	0.813 (0.697, 0.948)	0.673 (0.537, 0.844)			
OS					
Non-*MYCN*-A					
DFMO	0.976 (0.930, 1.000)	0.927 (0.850, 1.000)	.3948	0.553 (0.139, 2.201)	.4010
No DFMO	0.973 (0.922, 1.000)	0.887 (0.788, 0.998)			

approved by the WIRB as well as all local IRBs of participating BCC sites. Fifteen BCC hospitals performed a retrospective review of all neuroblastoma cases from 2003 to 2018. Clinical case histories were collected including diagnostic and prognostic indicators, treatments, and outcomes. A total of 378 patients were screened. Inclusion criteria for evaluation were defined as completion of COG standard of care therapy for HRNB, meeting the eligibility criteria for enrollment on Stratum I of NMTRC003/003b (including event-free survival 120 days from completion of antibody), and no treatment with DFMO. Therefore, this control population was matched to the subset of NMTRC003/003b patients in all aspects except DFMO administration. These subjects were utilized for statistical comparison of DFMO treatment effect on EFS and OS.

2.3 | Statistical analysis

For each covariate in Table 1, we reported a *P*-value of the differences in that covariate between the subset of Stratum 1 NMTRC003/003b group and the control group. For continuous covariates (age), we tested the differences between these groups using a *t* test. For discrete covariates (sex, stage, *MYCN*, histology and response to induction), we tested the differences between treatment groups using Chi-squared tests. The EFS/OS survival curves were compared between the subset of NMTRC003/003b Stratum I and the control group, with additional subset analysis based on *MYCN* amplification. Time "0" was defined as the time from the end of standard therapy, as this was a consistent time point between the two groups. The R packages survival and survminer were used to create and plot survival curves from the Kaplan-Meier estimates, including the corresponding 95% pointwise confidence interval bands obtained using Greenwood's method. The *P* values were provided for the associated log-rank test. We additionally reported the hazard ratio estimated using a Cox proportional-hazards regression model, the 95% confidence interval of the hazard ratio and the resulting *P*-value. The Cox regression was estimated controlling for the treatment variable only.

FIGURE 1 A, Consort diagram of comparison groups between NMTRC003/003b (DFMO) and BCC001 (No DFMO). B, EFS of NMTRC003/003b vs BCC001 subjects with comparison at 2 and 5 years of DFMO (86.4%, 85.2%) vs no DFMO (78.3%, 65.6%), respectively. C, OS of NMTRC003/003b vs BCC001 subjects with comparison at 2 and 5 years of DFMO (98.8%, 95.1%) and vs no DFMO (94.4%, 81.6%), respectively. D, EFS of *MYCN* amplified subjects, with comparison at 2 and 5 years DFMO (91.9%, 91.9%) vs no DFMO (86.4%, 75.6%). E, OS of *MYCN* amplified subjects, with comparison at 2 and 5 years DFMO (100%, 97.3%) vs no DFMO (92.9%, 78.1%). F, EFS of *MYCN* non-amplified subjects, with comparison at 2 and 5 years DMFO (82.9%, 80.5%) vs no DFMO (81.3%, 67.3%). G, OS of *MYCN* nonamplified subjects, with comparison at 2 and 5 years DFMO (97.6%, 92.7%) vs no DFMO (97.3%, 88.7%)

3 | RESULTS

3.1 | Subject characteristics

Of the 100 subjects eligible for enrollment on Stratum I of NMTRC003/003b, 81 subjects had been treated per COG standard of care (including completion of dinutuximab after enrollment on the postrandomization extension of COG ANBL0032) and started DFMO within 120 days of completion of immunotherapy. The BCC001 retrospective chart review identified 76 subjects treated at the same centers as NMTRC003/003b who met inclusion criteria for Stratum I of NMTRC003/003b (ie, treated per standard COG therapy with dinutuximab on ANBL0032 and remaining event-free at least 120 days from completion of immunotherapy) and did not receive DFMO as maintenance therapy. See consort diagram for an outline of the flow of inclusion (Figure 1A). Subject characteristics for both groups have been outlined in Table 1. High-risk features of the subject populations for both NMTRC003/003b and BCC001 groups are similar to those previously reported for HRNB population studies regarding age, stage, MYCN, histology, ploidy and induction response.[5,20] The groups are statistically similar with the exception of histology, age, and induction response. The large number of unknown histology found in the NMTRC003/003b group can account for this difference; when "unknown" is removed as a variable, the percentage of patients with unfavorable histology in each group is virtually identical with NMTRC003/003b at 87.8% and BCC001 at 88.1%. The BCC001 group showed a statistically significant difference in median age (3.4-4.5 years). However, these patients are within the same risk group for children >18 months. The response to induction therapy was on the cusp of statistical significance, with more CR reported in the NMTRC003/003b group and more PR in the BCC001 group.

3.2 | Outcomes

The comparison between the NMTRC003/003b subset and the BCC001 historical control, outlined in Table 2 and Figure 1B,C, demonstrates statistically significant differences for both EFS and OS. The 2- and 5-year EFS probabilities for NMTRC003/003b were 86.4% [95% CI, 79.3%-94.2%] and 85.2% [95% CI, 77.8%-93.3%], respectively. The 2- and 5-year EFS for the control group were 78.3% [95% CI, 69.5%-88.3%] and 65.6% [95% CI, 55.5%-77.5%]. The 2- and 5-year OS probabilities for NMTRC003/003b were 98.8% [95% CI, 96.4%-100%] and 95.1% [95% CI, 90.5%-99.9%]. The 2- and 5-year OS for the control group were 94.4% [95% CI, 89.3%-99.9%] and 81.6% [95% CI, 73.0%-91.2%]. The hazard ratio from a Cox proportional hazards model conditioning on treatment only for EFS is 0.404 with a 95% confidence interval of (0.203, 0.805). For OS, the hazard ratio from the Cox model conditioning on treatment only is 0.257, with a confidence interval (0.085, 0.779).

Analysis of the effect in MYCN amplified and nonamplified tumor subgroups (Figure 1D-G) shows a trend toward improved EFS and OS in both groups at 5 years, although most comparisons did not reach statistical significance. The OS difference in MYCN amplified tumors who received DFMO did reach statistical significance (P = .0207) with an increase in OS from 78.1% to 97.3% at 5 years. This analysis was limited by the small sample size of these subgroups and needs further investigation.

4 | DISCUSSION

In this report, we provide a comparison of the EFS and OS of a subset of subjects from Stratum I of the NMTRC003/003b study who received DFMO after treatment as per the contemporaneous COG standard of care with a historical control group of similar patients treated at BCC centers who did not receive DFMO. Our results show an improvement in 2- and 5-year EFS and OS in subjects who received DFMO for 2 years vs the historical control. Also, in contrast to the historical control, survival after DFMO treatment is maintained and demonstrates little decrease in EFS and OS over time through 5 years. These results suggest that there is a clinically significant benefit to maintenance DFMO, justifying further study of this drug as maintenance therapy.

To evaluate comparable subjects, we used a subset of enrolled subjects (n = 81 of those on Stratum I) who were treated per COG standard of care (used at all BCC hospitals) and received dinutuximab immediately prior to enrollment on NMTRC003/003b. For comparison, a cohort of subjects treated at the same institutions using similar COG treatment regimens for HRNB, including dinutuximab but without DFMO, was obtained via a retrospective chart review. The investigators recognize that historical control group comparisons have inherent limitations including sampling variability, drift and selection bias.[21] However, all patients evaluated in our study received similar upfront treatment at the same cohort of hospitals, regardless of whether they received DFMO, thereby mitigating the effect of drift. To decrease biases in the historical control group, only patients who met all NMTRC003/003b Stratum I inclusion criteria, including being event-free 120 days after completion of immunotherapy (latest possible date to start therapy on NMTRC003/003b), were included in this evaluation. This excluded any subjects from the control group who would have been screen failures for entry onto NMTRC003/003b secondary to disease progression after immunotherapy. This approach has the potential to favor the control group in terms of EFS as all patients were required to be event-free 120 days after completion of immunotherapy, whereas enrollment on NMTRC003/003b typically occurred less than 120 days from completion of immunotherapy. The patient characteristics in each group were well matched, although the minor difference in induction response (0.046) should be acknowledged as a potential limitation influencing outcome. The differences in histology were due to the unknown group as known categories were similar. The age difference is within the same risk group and unlikely to influence outcome.

In the COG ANBL0032 clinical trial, the 2-year EFS, from start of immunotherapy, was 66 ± 5% for those in the experimental arm who received immunotherapy with dinutuximab.[5] The European

experience with dinutuxmab antibody in HRNB patients who were treated with a similar overall therapy strategy had a 3-year EFS of 46.5% ± 4.1% and OS of 86.5% ± 3.9%.[3] More recent results of the SIOPEN HR-NBL1 trial[22] in which 406 patients were randomized to receive dinutuximab beta with or without subcutaneous interleukin-2 (IL-2) reported a 3-year EFS, from start of immunotherapy, of 56% (95% CI, 49%-63%) with dinutuximab beta and 60% (95% CI, 53%-66%) with dinutuximab beta plus IL-2. Three- and five-year OS were 68% (95% CI, 62%-75%) and 63% (95% CI, 55%-69%) with dinutuximab beta vs 70% (95% CI, 63%-75%) and 62% (95% CI, 55%-69%) with dinutuximab beta plus IL-2. These studies, along with their predecessor therapies for HRNB, have all shown progressive, refractory and relapsed disease occurring during upfront therapy. Therefore, initiation of DFMO at an earlier timepoint, such as during immunotherapy, may play a role in prevention of early relapse.

The proposed mechanism of action of DFMO, studied in HRNB, is inhibition of *ODC* resulting in a decrease in *MYCN*.[16,17,23] *MYCN* amplification is present in 30% to 40% of HRNB patients and is associated with a poorer prognosis. Therefore, treatment with DFMO, with subsequent inhibition of *MYCN*, would be expected to play a more important role in this subset of patients.[16,17,23] However, subjects on NMTRC003/003b study showed improvement in EFS and OS relative to historical controls regardless of *MYCN* status, although this subset analysis was not powered for significance due to small sample size. The observation that the baseline EFS for *MYCN* amplified (75.6%) is greater than that for *MYCN* nonamplified (67.3%) patients, though not statistically significant, is unexpected and may be due to either the effect of previous antibody therapy or to small sample size. The 5-year EFS/OS improved from 75.6%/78.1% to 91.9%/97.3% for *MYCN* amplified tumors and from 67.3%/88.7% to 80.5%/92.7% for *MYCN* nonamplified tumors at 5 years. These results suggest that DFMOs targeting of *ODC* and *MYCN* may provide a greater benefit in terms of improved survival for the *MYCN* amplified group, which has historically had a worse outcome. However, because DFMO also appeared to improve survival in the *MYCN* nonamplified group, it may be of interest to study c-*MYC* as a potential target as it has been noted that c-*MYC* is found in up to 20% of neuroblastoma.[24] In colon cancer, DFMO inhibition of c-*MYC* has been shown to be important in tumor response.[25] The outcome differences between *MYCN* amplified and nonamplified patients should be further studied prospectively to elucidate the mechanisms and differences in these subgroups.

In conclusion, comparison of children with HRNB from BCC hospitals who received DFMO after standard therapy showed a significant increase in EFS and OS relative to those that did not receive DFMO. It is possible that earlier addition of DFMO within maintenance therapy in combination with immunotherapy and *cis*-retinoic acid may further improve these outcomes. This concept is being studied in a randomized clinical trial. In addition, further study of DFMO involving dose escalation as maintenance therapy is underway.

CONFLICT OF INTEREST

Dr Donald Berry is a co-owner and statistical consultant with Berry Consultants, LLC. The remaining authors declare no competing interests.

DATA ACCESSIBILITY

The datasets generated during the NMTRC003/003b study are available in the clinicaltrials.gov repository: https://clinicaltrials.gov/ct2/show/NCT02395666. The data obtained via the BCC001 retrospective review that support the findings of our study are available from the corresponding author upon reasonable request.

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