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Phase I study of bortezomib in combination with irinotecan in patients with relapsed/refractory high-risk neuroblastoma

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Abstract

Purpose: Prognosis for relapsed/refractory high-risk neuroblastoma (HR-NBL) remains poor. Bortezomib, a proteasome inhibitor, has shown preclinical activity against NBL as a single agent and in combination with cytotoxic chemotherapy including irinotecan.

Patients and Methods: Eighteen HR-NBL patients with primary refractory (n = 8) or relapsed (n = 10) disease were enrolled in a Phase I study using modified Time To Event Continual Reassessment Method. Bortezomib (1.2 mg/m^2/day) was administered on days 1, 4, 8, and 11 intravenously (IV) and irinotecan was given IV on days 1–5 (35, 40, or 45 mg/m^2/day, on dose levels [DL] 1–3, respectively). The maximum tolerated dose (MTD), dose-limiting toxicity (DLT), and response rate were examined.

Results: Eighteen NBL patients were evaluable for toxicity; 17 were evaluable for response assessment. A total of 142 courses were delivered (mean 8.2, median 2, range 1–48), with two patients receiving more than 40 courses of therapy. Two DLTs were reported, including a grade 4 thrombocytopenia (DL2) and a grade 3 irritability (DL3). MTD was estimated as DL3. Two of 17 (12%) evaluable patients showed objective responses (ORs) lasting more than 40 courses, including 1 partial remission and 1 complete remission. Four patients (23%) had prolonged stable disease (SD) lasting six or more courses, with a total of 35% study patients demonstrating clinical benefit in the form of prolonged OR or SD.

Conclusion: The combination of bortezomib and irinotecan was well tolerated by patients with relapsed/refractory NBL with favorable toxicity profile. It also showed modest but promising clinical activity and merits further testing in Phase II studies.

KEYWORDS
irinotecan, proteasome inhibitor, relapsed/refractory high-risk neuroblastoma

1 | INTRODUCTION

Despite recent advances in high-risk neuroblastoma (HR-NBL) therapy, long-term survival for patients with metastatic or high-risk disease remains less than 40%.[1–3] Further, patients who experience a relapse of their disease or fail to achieve complete remission have an extremely poor prognosis with 2-year survival rates of less than 25%.[4–7] Better understanding of the biology of NBL is critical in identifying new therapeutic targets and improving outcomes for this patient population.

Irinotecan is a camptothecin prodrug that induces cytotoxicity through its active metabolite (SN-38) by inhibiting the nuclear enzyme topoisomerase I. It has shown single-agent activity against NBL in both *in vivo* mouse models of NBL[8–10] and in patients with refractory NBL in multiple clinical trials.[11–15]

Bortezomib is a selective inhibitor of the ubiquitin proteasome pathway, which is essential for the degradation of most intracellular proteins involved in critical cellular processes, including cell cycle regulation, apoptosis, and transcription factor activation.[16,17] Bortezomib selectively sensitizes malignant cells to apoptosis, although the precise mechanism of its anticancer activity is unknown.[18] nuclear NF-κB is a known regulator of NBL tumor cell survival[19,20] and bortezomib

Abbreviations: BM, bone marrow; CR, complete response; CT, computed tomography; CTCAE, Common Toxicity Criteria for Adverse Events; DFS, disease-free survival; DL, dose level; GCSF, granulocyte colony-stimulating factor; HR-NBL, high-risk neuroblastoma; INRC, International Neuroblastoma Response Criteria; IV, intravenously; MIBG, metaiodobenzylguanidine; MRI, magnetic resonance imaging; MTD, maximum tolerated dose; OR, objective response; SD, stable disease; DLT, dose-limiting toxicity; TITE-CRM, Time To Event Continual Reassessment Method

has shown the ability to decrease NF-κB activity by the inhibition of I-κB degradation in NBL cell lines as well as other systems.[21] In addition, bortezomib has been shown to be synergistic with a variety of chemotherapy agents including irinotecan in both *in vitro* and *in vivo* xenograft models of disease.[17,22] Bortezomib has already been shown to be safe in children with refractory solid tumors in a Phase I study and is well tolerated.[23] In view of the potential synergy between irinotecan and bortezomib, we designed a Phase I clinical trial to study the safety, maximum tolerated dose (MTD), and clinical activity of this combination in patients with relapsed/refractory HR-NBL. The traditional 3 + 3 design has been the standard for conducting pediatric Phase I clinical trials. However, it has several disadvantages, including intermittent suspension for toxicity evaluation between dose escalations. In a single-institution Phase I trial for a rare disease, where an eligible patient can come anytime, these stoppages can lead to unnecessary delays and many eligible patients would not be able to participate if they present during trial closure. To avoid this, we deployed a novel, more adaptive, simulation model-based dose escalation design, known as the Time To Event Continual Reassessment Method (TITE-CRM).[24] This design has been tested in adult Phase I studies and has shown to be as safe as standard rolling six or 3 + 3 design. Importantly, TITE-CRM trials have the advantage of being the most efficient trial design as far as timely completion of trial is concerned.[25] More recently, modified Phase I CRM design was also shown to be more accurate in predicting MTD, exposing fewer patients to potentially toxic doses in Pediatric Brain Tumor Consortium trial.[26]

2 | PATIENTS AND METHODS

2.1 | Eligibility

Patients with histologically proven HR-NBL who were 30 years or younger at diagnosis were eligible if they had either recurrent/progressive disease or refractory disease after front-line therapy with a minimum of four cycles of multiagent chemotherapy. Patients were required to have either measurable disease (>10 mm in one dimension) on computed tomography (CT) or magnetic resonance imaging (MRI) or evaluable disease with uptake in at least one abnormal site by metaiodobenzylguanidine (MIBG) scintigraphy, with or without bone marrow (BM) involvement. However, those patients whose only known site of disease was BM involvement were not eligible for the study. Other eligibility criteria included Karnofsky or Lansky scores of 60 or more, recovery from acute toxic effects of prior therapies, negative pregnancy test for women of child-bearing potential, absolute neutrophil count 1,000 per μl, platelet count 100,000 or more per DL, and adequate renal and hepatic function as defined by serum creatinine less than or equal to 1.5× normal serum creatinine as adjusted for age, alanine amino transferase (ALT) less than or equal to 2.5× upper limit of normal (ULN) for age, and bilirubin less than 1.5× upper limit of normal for age. Patients with BM involvement at the study entry were eligible irrespective of hematologic parameters, but they were not eligible for evaluation for hematologic toxicities. More than 3, 6, or 8 weeks must have elapsed from prior cytotoxic chemotherapy, high-dose MIBG therapy, or autologous stem cell transplantation, respectively. Exclusion criteria included use of enzyme-inducing anticonvulsants, active infection, or grade 2 or higher diarrhea (Common Toxicity Criteria for Adverse Events [CTCAE] v3.0). Patients had to complete two cycles of chemotherapy in order to be eligible for response evaluation. Clinical protocol and informed consent documents were approved by local institutional review boards prior to patient enrollment.

2.2 | Drug administration

Irinotecan was administered intravenously (IV) over 60 min on days 1–5. The starting dose of irinotecan was selected as a 35 mg/m^2/day, which is 70% of a single-agent IV irinotecan MTD.[15] The study planned to examine five dose levels (DLs) of irinotecan: DL1–4 at 35, 40, 45, and 50 mg/m^2/day, respectively, with a starting DL of 35 mg/m^2/day and a de-escalation DL1 of 30 mg/m^2/day. No intrapatient dose escalation was allowed. Bortezomib (1.2 mg/m^2/day, IV bolus infusion) was administered following irinotecan on days 1, 4, 8, and 11 of each 21-day cycle, with dosing based upon a prior pediatric Phase I trial for refractory solid tumors.[23]

Supportive care: Supportive care measures included daily oral cefixime (8 mg/kg/day, with maximum daily dose of 400 mg) or cefpodoxime (10 mg/kg/day divided BID, with a maximum daily dose of 800 mg) 3 days prior and 2 days following chemotherapy completion. Patients were given instructions for use of loperamide to treat diarrhea occurring more than 24 hr after irinotecan. Filgrastim (granulocyte colony-stimulating factor [GCSF]) was only recommended to be administered during subsequent courses of therapy for patients who were hospitalized for fever and neutropenia or in whom reinitiation of prior cycles was delayed for more than 14 days due to neutropenia.

2.3 | Study design

Study was conducted using a modification of the TITE-CRM. The TITE-CRM assumes a regression model for the probability of dose-limiting toxicity (DLT) as a function of dose and allows information from all patients enrolled in the trial to be employed when allocating a new patient to a DL. Subjects were continuously recruited throughout the trial, without recruitment pauses. In this study, *a priori* DLT probabilities ("skeleton") for the five doses are as follows, in dose order: 0.05, 0.10, 0.15, 0.25, and 0.35. The power model (containing a single parameter β) was used to model the DLT rate of each dose. We placed a normal distribution with mean 0 and standard deviation 0.3 on the parameter β. To incorporate patients with partial follow-up for DLT, uniformly distributed DLT times were assumed. The MTD was defined as the highest dose with DLT probability of no more than 0.25. Furthermore, we have adopted a number of steps that limit escalation and obtain a safety profile. These steps include the following: (1) the dose assigned to each patient has an estimated DLT rate closest to, but not greater than, the target probability; (2) dose escalation is restricted to one level between adjacent patients; (3) escalation from the current dose is not allowed until at least one patient assigned to the current dose completes his or her follow-up; and (4) discontinue the trial when the probability of DLT at the lowest dose is larger than 25%. Details of TITE-CRM study design and simulation studies of the proposed design

have been summarized in detail in an earlier report.[24] All dose escalation decisions were supervised and approved by the study statistician (LZ). Patients were allowed a maximum of 3 years, provided they did not develop disease progression or other protocol-related toxicity that necessitates cessation of protocol therapy.

Required observations included performance of a weekly history and physical examination, complete blood count (CBC) with differential, comprehensive metabolic panel (including albumin, liver function, renal function, and electrolytes), and spot urine vanillylmandelic acid (VMA) and homovanillic acid (HVA) repeated at the beginning of each cycle. Re-evaluation was performed using CT/MRI chest/abdomen/pelvis, I^{123}-MIBG scan, after cycles 2, 4, 8, and 12 and every four cycles on study thereafter. Bilateral BM biopsy and aspirate were obtained with re-evaluation if positive at study entry.

Monitoring for toxicity and response evaluation: Toxicities were graded according to CTCAE v3.0.[27] For the purposes of the determination of DLTs and MTD, all toxicities observed during first two treatment cycles were included. We selected two cycles for MTD determination, as the median time for bortezomib-induced neurotoxicity is around 2 months.[28] Hematologic DLTs were defined as platelets less than 10,000 per μl on two consecutive blood draws and/or more than one platelet transfusion required per week, grade 4 neutropenia (absolute neutrophil count < 500 per μl) for more than 7 days, and neutropenia or thrombocytopenia, which causes a delay of 14 days or more beyond the planned interval between treatment cycles. Patients with BM involvement at study entry were not eligible for the evaluation of hematologic DLTs.

Nonhematologic DLTs were defined as any grade 3–4 nonhematologic toxicity excluding grade 3 nausea or vomiting; grade 3 diarrhea lasting 72 hr or less; grade 3 fever or infection, with or without neutropenia; grade 3–4 stomatitis lasting 72 hr or less; or grade 3 transaminase elevation resolving to original eligibility criteria prior to next cycle of chemotherapy. Recommended dose modifications were specified in the study. Any patient experiencing DLT after an initial dose modification of irinotecan or bortezomib was removed from the protocol therapy.

2.4 | Response evaluation

Patients who received at least two cycles of bortezomib and irinotecan were evaluable for response. Response Evaluation Criteria in Solid Tumors (RECIST) were used for response assessment in patients with measurable disease.[29] For patients with MIBG-avid lesions only, response was based upon International Neuroblastoma Response Criteria (INRC), which examines the presence or absence of MIBG avidity in skeletal and soft-tissue sites. Overall response was defined using INRC.[30] Survival curves were constructed using the Kaplan–Meier method.[31] Disease-free survival (DFS) and overall survival were calculated as a secondary endpoint of the study.

3 | RESULTS

Patient characteristics: A total of 18 eligible patients with HR-NBL were enrolled between May 2008 and March 2012 at the C.S. Mott Children's Hospital/University of Michigan Medical Center, with all 18 patients eligible for toxicity evaluation, while 17 patients were eligible for response evaluation. One patient was taken off protocol therapy after one cycle of therapy due to poor compliance with study dosing. The patient did not experience any DLT during study therapy. Demographics and clinical characteristics of the study patients are shown in Table 1. Patients were enrolled on the study at the time of declaration of primary refractory disease (n = 8) or at the time of presenting with relapsed/progressive disease (n = 10). The majority of patients were heavily pretreated (median prior regimen = 2), with measurable disease (n = 11), BM involvement (n = 14), and 12 were status post-autologous BM transplant. Three patients received prior therapeutic doses of I^{131}-MIBG. Overall summary and clinical course of all study patients are described in Table 2.

DLTs: A total of 18 patients were treated for a total of 142 courses of protocol therapy. Three patients were treated on DL1, nine on DL2, and six on DL3 **(Table 3)**. Two DLTs were reported, including one at DL2 (irinotecan DL 40 mg/m^2) and another at DL3 (irinotecan DL 45 mg/m^2), with no DLTs reported at DL1 (Table 3). The first DLT was a grade 4 thrombocytopenia, which required more than two platelet transfusion for 2 weeks during cycle 2 but without any clinical bleeding. Although eligible to continue study therapy (with dose reduction), the patient was electively withdrawn from the study. The second DLT occurred in a patient experiencing grade 3 irritability and inconsolable crying. The episode developed during day 8 of bortezimib dose during cycle 2 and continued for several hours and redeveloped after cycle 2, day 11 dose. Despite multiple consolation and supportive techniques including diphenhydramine and lorazepam, the symptoms only resolved 12 hr after the last treatment dose. The patient had no history of other injury. Follow-up scans revealed stable disease (SD) with no disease progression in any bony sites or intracranial extension or evidence of any intracranial bleed. The patient exhibited otherwise unremarkable neurologic examination including normal deep tendon reflexes and sensory and motor examinations. The true cause of irritability was unclear; however, after full evaluation to rule out other etiology, it is our clinical impression that it was related to neuropathic pain, a well-known toxicity of bortezomib in adults.[32] Irritability has not been reported as a side effect of bortezomib before and the patient made a full recovery from this toxicity. The family elected to pursue another investigational study following the second cycle. Five more patients were subsequently enrolled on DL 3 without DLT. Based on the TITE-CRM design described above, we calculated the DLT rate at each DL from the given DLT information in Table 3. We found that posterior probabilities of DLT at DL3 and DL4 are 0.17 and 0.26, respectively. Hence, DL3 (irinotecan 45 mg/m^2) was determined to be the MTD (the highest dose with DLT probability of no more than 0.25). Patients with BM involvement were not eligible for heme DLT assessment. However, if we were to apply the heme DLT definition to all 14 patients with BM involvement, 5/14 (36%) of patients would have met hematologic DLT definition, including three patients with grade 4 neutropenia for more than 7 days and two with thrombocytopenia requiring multiple transfusions in a week.

Other toxicities: Overall toxicity experience during the entire course of therapy is described in Table 4. Even though 10 patients (56%)

TABLE 1 Patient demographics and clinical characteristics

Clinical and biological feature	Numbers
Age, median Years (range)	5 (1–21)
Gender (male/female)	8/10
N-Myc amplification, yes/no/unknown	5/9/4
Disease status, primary refractory/relapsed	8/10
Measurable disease, yes/no	11/7
BM involvement, yes/no	14/4
Prior therapies, median (range)	2 (1–4)
Prior irinotecan exposure (yes/no)	5/13
Autologus BMT (yes/no)	12/6
High-dose MIBG (yes/no)	3/15
Patients on DL 1 (bortezomib 1.2 mg/m^2/day, irinotecan 35 mg/m^2/day)	3
Patients on DL 2 (bortezomib 1.2 mg/m^2/day, irinotecan 40 mg/m^2/day)	9
Patients on DL 3 (bortezomib 1.2 mg/m^2/day, irinotecan 45 mg/m^2/day)	6
Number of courses of protocol therapy received, median (range)	2 (1–48)
Number of patient eligible for toxicity evaluation	18
Number of patients eligible for response evaluation	17
Overall responses by INRC (percentages)	2 (12%)

BMT, bone marrow transplant.

experienced grade 3 or 4 neutropenia, only 1 patient (6%) developed grade 3 febrile neutropenia without the use of GCSF. Three patients (18%) required multiple transfusions (one packed red blood cell and two platelet transfusions).

Even though both agents include diarrhea as a DLT in single-agent clinical trials, only three (17%) reported grade 3–4 diarrhea without any DLT with very little moderate to severe nausea or vomiting. The incidence of peripheral neurotoxicity was relatively low, with two patients (11%) experiencing sensory neuropathy (one grade 2, one grade 3).

Antitumor activity: Two objective responses (ORs) were observed out of 17 eligible patients (12%), as defined by the INRC NBL criteria.[30] One response was in 3-year-old patient with refractory NBL with *N-MYC* nonamplified disease, with measurable soft-tissue lesions in the liver and maxillary sinus on the right. The patient demonstrated a partial response by CT and MIBG scans after six cycles and maintained the response for a total of 48 cycles (Table 2). At that point, the patient came off study to enroll in another investigational study, remaining in partial remission for 2 years after protocol therapy. A second response was seen in a 7-year-old boy with *N-MYC* nonamplified, evaluable disease in sacral bone by MIBG scan and iliac crest BM by BM evaluation. The patient had prior exposure to irinotecan, with no OR. However, after two cycles of protocol therapy, he had complete response (CR), with complete resolution of MIBG avidity and clearance of marrow disease. He maintained in CR for a total of 44 cycles before coming off study to enroll on a vaccination study at an outside institution. The patient remains in CR 30 months after protocol therapy.

Four additional patients (23%) received six or more cycles of protocol therapy with minimal toxicity and prolonged SD (six, six, eight, and eight cycles, respectively). Overall, 35% of these patients received clinical benefit from the study therapy, with prolonged ORs (n = 2) or prolonged disease stabilization (n = 4). Three-year DFS and OS rates for this study cohort were 28% (±11%) and 44% (±12%), respectively (Figs. 1A and 1B).

4 | DISCUSSION

Bortezomib is a first in class boronic proteasome inhibitor that reversibly inhibits activity of the 20S proteasome, but its true mechanism of action is still unknown. However, inhibition of NF-κB, activation of proapoptotic proteins like caspases and cleavage of DNA repair enzymes, and increasing the susceptibility of cancer cells to classes of DNA-damaging agents are some of the proposed mechanisms.[21,33,34] Based on the role of NF-κB in NBL pathogenesis, our group and others explored the role of bortezomib *in vivo* and *in vitro* against NBL and have shown a synergy between bortezomib and irinotecan and other cytotoxic agents[22,35,36] forming the basis of this trial. To our knowledge, this is the first clinical trial of bortezomib against NBL. As most of the patients with refractory/relapsed NBL have already seen very dose-intense cytotoxic chemotherapy, we chose irinotecan given its known activity against NBL, favorable toxicity profile, and less myelosuppressive effects as compared to other cytotoxic agents. Since we really wanted to explore the effect of maximum inhibition of proteasome and its clinical effect when combined with escalating doses of irinotecan, we elected to keep a constant dose of bortezomib with maximum proteasome suppression.

The combination of bortezomib and irinotecan was very well tolerated, with only two DLTs in 18 patients; there were 6 patients who

TABLE 2 Summary of Study Patients

Patient ID	Age (years)	Gender	Stage at diagnosis	N-Myc amplification	Disease status	Evaluable vs. measurable disease	BM involvement	No. of previous regimens	Prior irinotecan exposure	Prior BMT	Prior MIBG	DL	Total no. of cycles on therapy	DLT	Best Best overall response
01	6	Male	IV	Not amplified	Relapse	Evaluable	Yes	3	No	Yes	Yes	1	6		SD
02	17	Female	III	Amplified	Refractory	Evaluable	No	2	No	Yes	No	1	2		PD
03	21	Male	IV	Unknown	Refractory	Measurable	No	3	Yes	Yes	No	1	1		Not evaluable for response
04	4	Male	IV	Not Amplified	Refractory	Measurable	Yes	1	No	Yes	No	2	2		PD
05	4	Male	IV	Amplified	Relapse	Evaluable	No	2	No	Yes	No	2	2	Grade 4 thrombo-cytopenia	SD
06	5	Female	IV	Unknown	Refractory	Evaluable	Yes	2	No	No	No	2	2		SD
07	5	Female	IV	Unknown	Relapse	Measurable	Yes	2	No	Yes	No	2	8		SD
08	2	Female	IV	Not Amplified	Relapse	Measurable	Yes	1	No	No	No	2	2		PD
09	1	Female	IV	Not Amplified	Refractory	Measurable	Yes	2	Yes	Yes	No	2	6		SD
010	6	Male	IV	Not Amplified	Relapse	Measurable	Yes	1	No	Yes	No	2	2		PD
011	2	Male	IV	Amplified	Relapse	Measurable	Yes	2	No	Yes	No	3	2	Grade-3 Irritability, incon-solable crying	PD
012	7	Male	IV	Not Amplified	Refractory	Evaluable	Yes	4	Yes	Yes	Yes	2	44		CR
013	8	Female	IV	Amplified	Relapse	Measurable	Yes	3	Yes	No	No	2	2		SD
014	2	Female	IV	Unknown	Relapse	Measurable	Yes	2	No	Yes	No	3	2		SD
015	3	Female	IV	Not Amplified	Refractory	Evaluable	No	4	No	Yes	No	3	48		PR
016	8	Female	IV	Amplified	Relapse	Measurable	Yes	1	No	No	No	3	3		SD
017	5	Female	IV	Not Amplified	Relapse	Measurable	Yes	3	Yes	No	Yes	3	4		SD
018	5	Male	IV	Not Amplified	Refractory	Evaluable	Yes	1	No	No	No	3	8		SD

BMT, bone marrow transplant; PR, partial remission.

TABLE 3 Irinotecan DLs and DLTs

DL	Bortezomib dose	Irinotecan dose	No. of patients	DLT
1	1.2 mg/m^2	35 mg/m^2/day	3	–
2	1.2 mg/m^2	40 mg/m^2/day	9	1 (thrombocytopenia)
3	1.2 mg/m^2	45 mg/m^2/day	6	1 (irritability, inconsolable crying)

TABLE 4 Highest grade of overall toxicities (CTCAE v3.0)*

Adverse event	None	Grades 1–2	Grade 3	Grade 4
Leukopenia		12 (66%)	3 (17%)	3 (17%)
Neutropenia		8 (45%)	6 (33%)	4 (22%)
Anemia		17 (94%)	1 (6%)	0
Thrombocytopenia		13 (72%)	2 (11%)	3 (17%)
Nausea		17 (94%)	1 (6%)	0
Vomiting		17 (94%)	1 (6%)	0
Diarrhea	3 (17%)	12 (66%)	2 (11%)	1 (6%)
Peripheral sensory neuropathy/irritability	16 (88%)	1 (6%)	1 (6%)	0
Febrile neutropenia	17 (94%)		1 (6%)	0

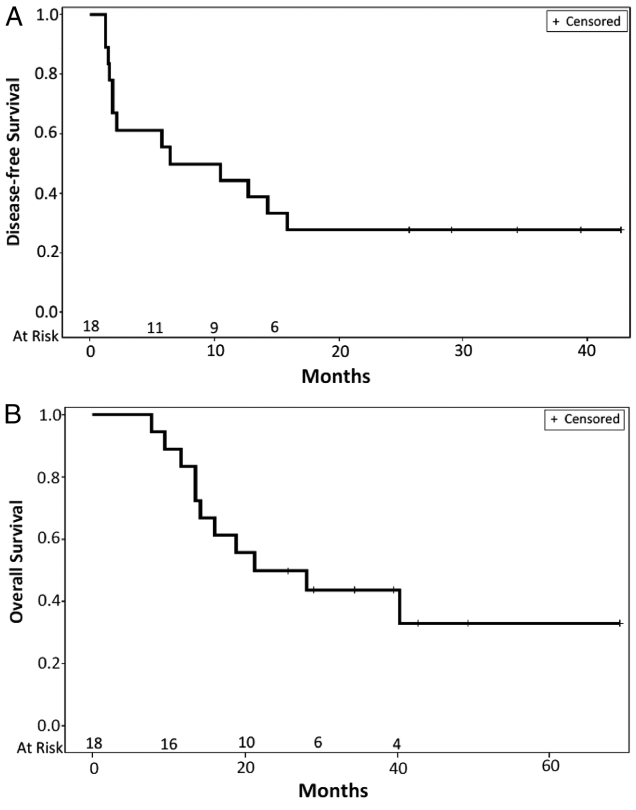

FIGURE 1 Kaplan–Meier survival curve of patients with relapsed or refractory NBL: (A) disease free survival and (B) overall survival

received between 6 and 48 cycles of chemotherapy. Thrombocytopenia has been reported in many other single-agent bortezomib and irinotecan studies.[14,23] In the current trial, only 4 of 18 patients were eligible for assessment of hematologic toxicity, thus limiting our ability to assess thrombocytopenia. One nonhematologic DLT was irritability and inconsolable crying, a complaint not reported in other pediatric studies with these agents. However, the etiology of the event remains unclear, it is possible that it was a manifestation of neurotoxicity in a toddler. In terms of other toxicities seen in our trial, in general, they were mild. Overall, patients had no adverse impact on the quality of life while on therapy, with most continuing school or preschool activities while on protocol therapy. The infectious complications were minimal, with only one episode of febrile neutropenia without any septic episodes. Gastrointestinal side effects were notable for diarrhea, which was expected because both agents are known to cause diarrhea. However, nausea and vomiting were minimal. Surprisingly, neurotoxicity was also minimal, which has been a major DLT in older adults. Prior exposure to cisplatin and vincristine in preprotocol therapy for primary NBL treatment did not increase the incidence of neurotoxicity secondary to bortezomib in our study. This is similar to what has been described in adults where increasing age was the only predictive variable associated with neurotoxicity in response to bortezomib not prior exposure to any neurotoxic agents.[37] The favorable toxicity profile makes this an ideal combination to be used as a backbone for adding additional investigational agents especially in children who cannot tolerate temozolomide orally.

Another novel aspect of our trial was the modified TITE-CRM Phase I study design. There are several potential advantages of this approach, including treating all eligible patients[24] and keeping a trial open continuously in between doses. During the entire time period when this trial was open, all eligible patients who were interested in participating in the study were able to enroll on our study. This could be of tremendous value in improving efficiency of pediatric oncology Phase I clinical trials, which are known for intermittent closures and lack of available spots when an eligible patient is identified. Based on extensive simulation studies we reported earlier, we were able to show that the TITE-CRM design was able to (1) identify the MTD more accurately without increasing the probability of exposing patients to toxic doses and (2) treat more patients at or close to MTD dosing when compared with

more traditional designs,[24] making a very compelling case for TITE-CRM to be a novel alternative to traditional Phase I designs for pediatric Phase I trials.[24]

We enrolled 18 patients in approximately 3 years and 10 months, which took much longer than we expected. Generally, the TITE-CRM design does not have much advantage of shortening the trial duration in this situation when patient recruitment is slow, but four patients were enrolled within 2 weeks after the previously enrolled patient. It is likely that these four patients would either have their entry delayed or would be treated off protocol with some other agent if the trial was of traditional design. Given the small sample size in Phase I trials, the selected MTD has a large uncertainty. The model-based design using all patients' data is likely to be more efficient than the rule-based designs. However, the TITE-CRM design relies on certain assumptions (such as the dose–toxicity function and the a priori DLT probabilities). Although it has been shown that results are quite robust to the choice of dose–toxicity model,[38,39] we need to carefully evaluate these assumptions for every trial design.

In this study, we have made a conservative choice of selecting DL3 as the MTD. The DLT probability at DL3 is 17%, which is the highest dose with DLT probability of no more than 0.25. However, DL4 has an estimated DLT probability of 26%, which is only slightly larger than 25%. Because of the limited sample size and no data (no patient was treated) at DL4, we cannot rule out the possibility of DL4 being the MTD. Thus, we suggest to consider both DL3 and DL4 in future trials.

Finally, the combination of bortezomib and irinotecan showed a modest hint of clinical efficacy within the confines of this Phase I trial, with an overall 12% OR rate, including in one patient with prior irinotecan exposure. This is similar to other standard therapy used in relapsed NBL.[4,7] Even though the ORs were seen only in patients with evaluable disease, 35% of patients had prolonged SD or better from 6 to 48 courses with minimal toxicity, suggesting a very favorable long-term toxicity profile. These findings will need to be confirmed in a larger Phase II trial to determine the true efficacy of this combination in refractory NBL. Going forward, there are other treatment strategies to take advantage of these agents in efforts to improve the clinical response in refractory NBL. As examples, irinotecan and temolozomide have shown to be active in combination against refractory NBL with minimal toxicity and are considered an ideal backbone for the addition of novel biologic agents for testing. It is intriguing to consider bortezomib in such a treatment combination.[4,7] In addition, both irinotecan and bortezomib have shown to be radio-sensitizers and could be considered in combination with either therapeutic doses of I^{131}-MIBG or conventional radiation therapy.[40–44] Finally, oral irinotecan and subcutaneous bortezomib have been shown to be equally efficacious in patient-friendly formulations, as both agents can be delivered in an ambulatory/home setting.[7,45]

Our study has several limitations: (a) it is a small, single-institution study; (b) it had only four patients without BM involvement who were eligible for determination of hematologic toxicities and so the determination of hematologic DLT was inadequate; and (c) it had large interpatient study entry time leading to a close to 4-year completion time and mitigated many benefits of TITE-CRM design.

In summary, the combination of bortezomib and irinotecan was very well tolerated, with favorable short- and long-term toxicity profile, and showed modest clinical activity. The clinical activity of this novel combination merits testing in a larger Phase II clinical trial against refractory/relapsed NBL.

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CONFICT OF INTEREST

The authors declare that there is no conflict of interest.

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