Phase-I Study of Bortezomib in Combination with Irinotecan in Patients with Relapsed/Refractory High Risk Neuroblastoma

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ABBREVIATIONS KEY:

HR-NBL	High risk Neuroblastoma
TITE-CRM	Time To Event Continuous Reassessment Method
DL	Dose level
MTD	Maximum tolerated dose
DLT	Dose limited toxicity
OR	Objective responses
PR	Partial remission
SD	Stable disease
NBL	Neuroblastoma
UPP	Ubiquitin proteasome pathway
CT	Computed tomography
MRI	Magnetic resonance imaging
MIBG	Metaiodobenzylguanidine
BM	Bone marrow
INRC	International Neuroblastoma Response Criteria
DFS	Disease Free Survival

GCSF	Granulocyte colony stimulating factor
CR	Complete response
ORR	Overall response rate
VGPR	Very good partial response

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 Continuous Reassessment Method (TITE-CRM). Bortezomib (1.2 mg/m2/day) was administered on days 1, 4, 8 and 11 intravenously, irinotecan given IV on days 1-5 (35, 40, or 45 mg/m2/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 2 patients receiving > 40 courses of therapy. Two dose limiting toxicities (DLT) were reported, including a grade-4 thrombocytopenia (DL2) and a grade-3 irritability (DL3). MTD was estimated as DL3. 2/17 (12%) evaluable patients

showed objective responses (OR) lasting > 40 courses, including 1 partial remission (PR), 1 complete remission (CR). Four patients (23%) had prolonged stable disease (SD) lasting 6 or more courses with a total of 35% study patients demonstrating clinical benefit in 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.

INTRODUCTION

Despite recent advances in high risk Neuroblastoma (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 < 25%[4-7]. Better understanding the biology of neuroblastoma is critical in identifying new therapeutic targets and improving outcomes for this patient population.

Irinotecan is a camptothecin prodrug which 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] as well as in patients with refractory NBL in multiple clinical trials[11-15].

Bortezomib is a selective inhibitor of the ubiquitin proteasome pathway (UPP) 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 anti-cancer activity is unknown

[18]. NF-κB is a known regulator of neuroblastoma tumor cell survival[19,20] and bortezomib has shown ability to decrease NF-κB activity by 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 high risk 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 it has shown to be as safe as standard rolling six or 3+3 designs. 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 (PBTC)[26].

PATIENTS AND METHODS

Eligibility

Patients with histology proven high-risk NBL 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 4 cycles of multi-agent chemotherapy. Patients were required to have either measurable disease (>10mm in one dimension) on computed tomography (CT) or magnetic resonance imaging (MRI), or evaluable disease with uptake in at least 1 abnormal site by metaiodobenzylguanidine (MIBG) scintigraphy, with or without bone marrow (BM) involvement. However, patients whose only known site of disease was bone marrow involvement were not eligible for the study. Other eligibility criteria included Karnofsky or Lansky scores ≥ 60, recovery from acute toxic effects of prior therapies; negative pregnancy test for women of child-bearing potential; absolute neutrophil count ≥1,000/uL, platelet count ≥100,000/DL, and adequate renal and hepatic function as defined by serum creatinine ≤1.5x normal serum creatinine as adjusted for age, ALT ≤2.5x ULN for age, and bilirubin <1.5x ULN for age. Patients with BM involvement at study entry were eligible irrespective of hematologic parameters, but they were not eligible for evaluation for hematological 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 diarrhea (CTCv3.0). Patients had to complete 2 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.

Drug Administration

Irinotecan was administered intravenously (IV) over 60 minutes on Days 1-5. The starting dose of irinotecan was selected as a 35mg/m2/day, which is 70% of a single agent IV irinotecan MTD[15]. The study planned to examine 5 dose levels of irinotecan: DL 1-4 at 35, 40, 45 and 50mg/m2/day respectively, with a starting dose level of 35mg/m2/day and a de-escalation dose level -1 of 30mg/m2/day. No intra-patient dose escalation was allowed. Bortezomib (1.2 mg/m2/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 (8mg/kg/day with maximum daily dose of 400mg) or cefpodoxime (10mg/kg/day divided BID with a maximum daily dose of 800mg), 3 days prior and 2 days following chemotherapy completion. Patients were given instructions for use of loperamide to treat diarrhea occurring >24 hours after irinotecan. Filgrastim (granulocyte colony-stimulating factor) was only recommended to be administered during subsequent courses of therapy for patients who were hospitalized for fever and neutropenia or in whom re-initiation of prior cycles were delayed for > 14 days due to neutropenia.

Study Design

Study was conducted using a modification of the Time-to-Event Continual Reassessment Method (TITE-CRM). The TITE-CRM assumes a regression model for the probability of 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 dose level. Subjects were continuously recruited throughout the trial, without recruitment pauses. In this study, *a priori* DLT probabilities ("skeleton") for the five doses are, 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: 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 their 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 being supervised and approved by study statistician (LZ). Patients were allowed a maximum of 3 years provided that that they didn't develop disease progression, or other protocol related toxicity which necessitated cessation of protocol therapy.

Required observations included performance of a weekly history and physical exam, CBC with differential, comprehensive metabolic panel (including albumin, liver function, renal function and electrolytes) and spot urine VMA and 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 4 cycles on study thereafter. Bilateral bone marrow biopsy and aspirate were obtained with re-evaluation if positive at study entry.

Monitoring for Toxicity and Response Evaluation: Toxicities were graded according to Common Toxicity Criteria for Adverse Events (CTCAE) v 3.0[27]. For the purposes of the determination of dose limiting toxicities (DLT) and maximum dose tolerated dose (MTD), all toxicities observed during first 2 treatment cycles were included. We selected 2 cycles for MTD determination as median time for bortezomib induced neurotoxicity is around 2 month[28]. Hematological DLT's were defined as: platelets <10,000/μl on two consecutive blood draws, and/or more than one platelet transfusion required per week, grade 4 neutropenia (ANC < 500/μl) for > 7 days, neutropenia or thrombocytopenia which causes a delay of ≥ 14 days beyond the planned interval between treatment cycles. Patient with BM involvement at study entry were not eligible for evaluation of hematological DLT's.

Non-Hematological DLT's were defined as any grade 3-4 non-hematologic toxicity excluding: grade 3 nausea or vomiting; Grade 3 diarrhea lasting ≤72 hours; grade 3 fever or infection, with or without neutropenia; grade 3-4 stomatitis lasting ≤72

hours; 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 protocol therapy.

Response Evaluation

Patients who received at least two cycles of bortezomib and irinotecan were evaluable for response. RECIST criteria 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), examining the presence or absence of MIBG avidity in skeletal and soft-tissue sites. Overall response was defined using International Neuroblastoma Response Criteria (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.

RESULTS

Patient Characteristics: A total of 18 eligible patients with high risk NBL were enrolled between May 2008 to March 2012, at 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 DLTs 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), bone marrow involvement (n=14), and twelve were status post autologous BMT. Three patients had received prior therapeutic doses of I^{131}-MIBG therapy. Overall summary and clinical course of all study patients are described in **Table-2**.

Dose Limiting Toxicities (DLT): A total of 18 patients were treated, for a total of 142 courses of protocol therapy. Three patients were treated on DL1, 9 on DL2 and 6 were treated on DL3 **(Table-3).** Two DLT's were reported, including one at dose level 2 (irinotecan dose level 40mg/m2) and another at dose level-3 (irinotecan dose level 45mg/m2), with no DLT's reported at dose level-1 **(Table-3).** The first DLT was a grade 4 thrombocytopenia which required > 2 platelet transfusion for 2 weeks during cycle 2 but without any clinical bleeding. Though the patient was eligible to continue study therapy (with dose reduction), the patient was electively withdrawn from study. The second DLT occurred in a patient experiencing grade-3 irritability and inconsolable crying. The episode developed during the day 8 bortezimib dose during cycle 2, and continued for several hours and re-developed after the cycle 2, day 11 dose. Despite multiple consolation and supportive techniques including diphenhydramine and lorazepam, the symptoms only resolved 12 hours after the last treatment dose. The patient had no history of other injury. Follow up scans revealed stable disease with no disease progression in any bony sites or intracranial extension or evidence of any intracranial bleed. The patient exhibited otherwise unremarkable neurological examination including normal deep tendon reflexes,

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 2nd cycle. Five more patients were subsequently enrolled on dose level-3 without DLT. Based on the TITE-CRM design described above, we calculated the DLT rate at each dose level given DLT information in Table 3. We found that posterior probabilities of DLT at Dose level-3 and 4 are 0.17 and 0.26, respectively. Hence, dose level-3 (irinotecan 45mg/m2) was determined to be the MTD (the highest dose with DLT probability of no more than 0.25). Patients with bone marrow 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 hematological DLT definition, including 3 patients with grade 4 neutropenia for > 7 days and 2 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%) experienced grade 3 or 4 neutropenia, only 1 patient (6%) developed grade 3 febrile neutropenia without the use of granulocyte colony stimulating factor (GCSF). Three patients (18%) required multiple transfusions (1 PRBC and 2 platelet transfusions).

Even though both agents include diarrhea as a DLT in single agent clinical trials, only 3 (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 2 patients (11%) experiencing sensory neuropathy (1 Grade-2, 1 Grade-3).

Anti-Tumor Activity: Two objective responses (OR) were observed out of 17 eligible patients (12%), as defined by INRC NBL criteria[30]. One response was in 3-year-old patient with refractory NBL with *N-MYC* non-amplified disease, with measurable soft tissue lesions in in the liver, and maxillary sinus on the right. The patient demonstrated a partial response by CT and MIBG scans after 6 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 PR for 2 years post protocol therapy. A second response was seen in a 7-year-old boy with *N-MYC* non-amplified, evaluable disease in sacral bone by MIBG scan and iliac crest bone marrow by bone marrow evaluation. The patient had prior exposure to irinotecan with no objective response. However, after 2 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 post protocol therapy.

Four additional patients (23%) received 6 or more cycles of protocol therapy with minimal toxicity and prolonged stable disease (SD) (6, 6, 8 and 8 cycles respectively). Overall, 35% of these patients received clinical benefit from the study therapy, with prolonged objective responses (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).**

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 pro-apoptotic proteins like caspases and cleavage of DNA repair enzymes, 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-kB 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 neuroblastoma, favorable toxicity profile, with less myelosuppressive effects as compared to other cytotoxic agents. We really wanted to explore the effect of maximum inhibition of proteasome and its clinical effect when combined with escalating doses of irinotecan and because of this 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 2 DLT's in 18 patients and there were 6 patients who received between 6-48 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 non-hematologic DLT was irritability and inconsolable crying, a complaint not reported in other pediatric studies with these

agents. Though, 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 quality of life while on therapy with most continuing school or pre-school 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 as both agents are known to cause diarrhea. However, nausea and vomiting were minimal. Surprisingly, neurotoxicity was also minimal which has been a major dose limiting toxicity in older adults. Prior exposure to cisplatin and vincristine in pre-protocol 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 for using 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 be enrolled 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 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 and[24].

We enrolled 18 patients in approximately 3 years and 10 months, which took much longer than we expected. Generally, the TITE-CRM design doesn't have much advantage of shortening the trial duration in this situation when the patient recruitment is slow, but there were 4 patients enrolled within 2 weeks after the previously enrolled patient. It's 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. But 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 in selecting Dose level-3 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, Dose level-4 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 Dose level-4, we can't 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 1 patient with prior irinotecan exposure. This is similar to other standard therapy used in relapsed neuroblastoma [4,7]. Even though the OR were seen only in patients with evaluable disease, 35% of patients had prolonged SD or better from 6-48 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 4 patients without bone marrow involvement who were eligible for determination of hematological toxicities and so the determination of hematological DLT was inadequate. C) it had large inter-patient 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 neuroblastoma.

AUTHORS' DISCLOSURES OF POTENTIAL CONFICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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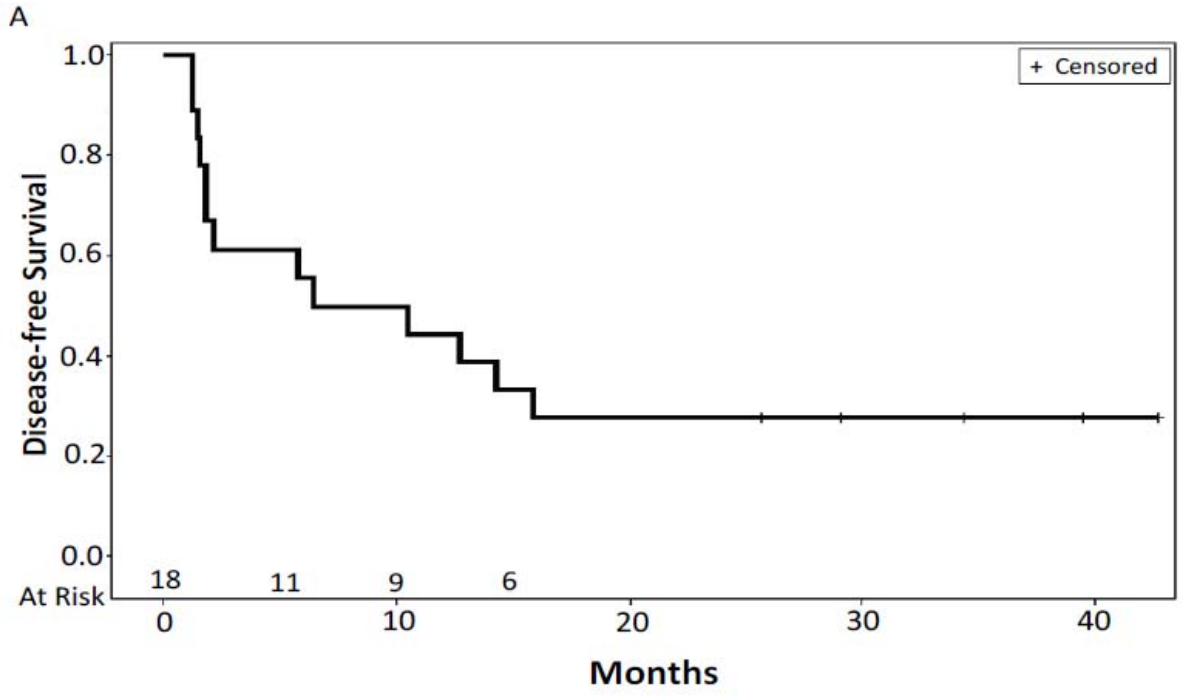
LEGENDS LIST:

Fig. 1: Kaplan-Meier Survival Curve of patients with relapsed or refractory NBL (A) Disease free survival. (B) Overall survival



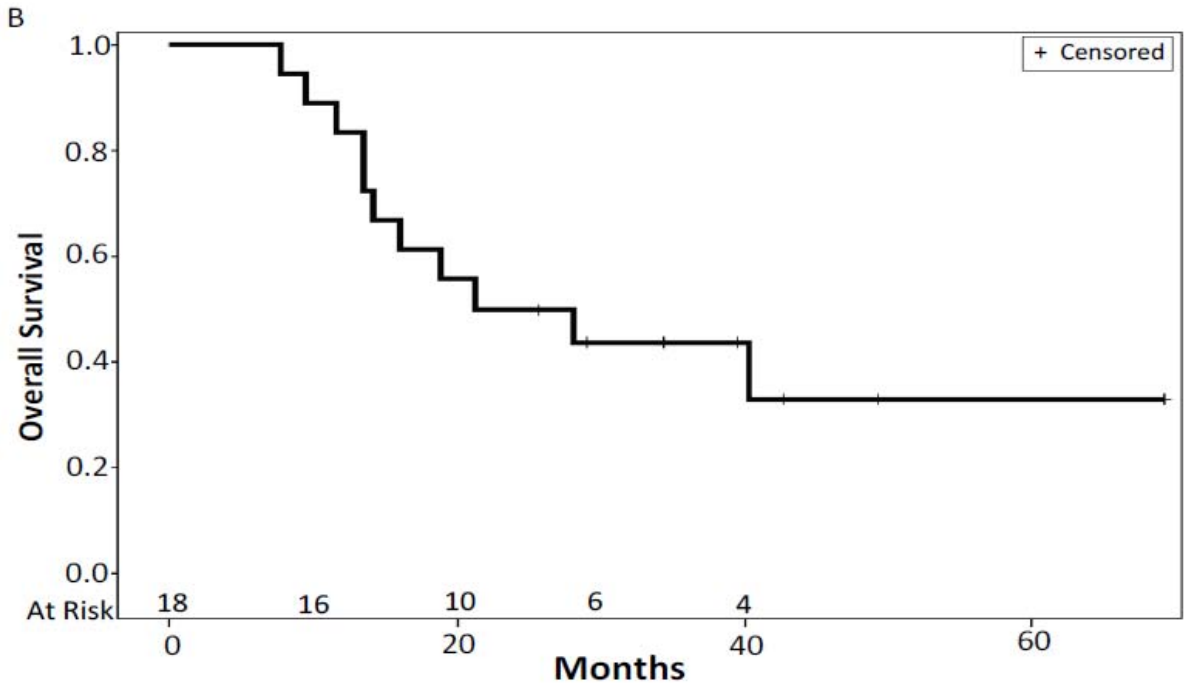


TABLE 1 Patient Demographics and Clinical Characteristics

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
Bone Marrow 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 Dose Level 1 (Bortezomib 1.2mg/m2/Day, Irinotecan 35mg/m2/D)	3
Patients on Dose Level 2 (Bortezomib 1.2mg/m2/Day, Irinotecan 40mg/m2/D)	9
Patients on Dose Level 3 (Bortezomib 1.2mg/m2/Day, Irinotecan 45mg/m2/D)	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%)
Abbreviations: BMT, Bone Marrow Transplant; MIBG, Metaiodobenzylguanidine; INRC, International Neuroblastoma Response Criteria	

TABLE 2 Summary of Study Patients

Patient ID	Age (Yr)	Gender	Stage at Diagnosis	N-Myc Amplification	Disease Status	Evaluable vs. Measurable Disease	BM Involvement	# of Previous Regimens	Prior Irinotecan Exposure	Prior BMT	Pri MIB
01	6	M	IV	Not amplified	Relapse	Evaluable	Yes	3	No	Yes	Ye
02	17	F	III	Amplified	Refractory	Evaluable	No	2	No	Yes	N
03	21	M	IV	Unknown	Refractory	Measurable	No	3	Yes	Yes	N
04	4	M	IV	Not Amplified	Refractory	Measurable	Yes	1	No	Yes	N
05	4	M	IV	Amplified	Relapse	Evaluable	No	2	No	Yes	N
06	5	F	IV	Unknown	Refractory	Evaluable	Yes	2	No	No	N
07	5	F	IV	Unknown	Relapse	Measurable	Yes	2	No	Yes	N
08	2	F	IV	Not Amplified	Relapse	Measurable	Yes	1	No	No	N
09	1	F	IV	Not Amplified	Refractory	Measurable	Yes	2	Yes	Yes	N
010	6	M	IV	Not Amplified	Relapse	Measurable	Yes	1	No	Yes	N
011	2	M	IV	Amplified	Relapse	Measurable	Yes	2	No	Yes	N
012	7	M	IV	Not Amplified	Refractory	Evaluable	Yes	4	Yes	Yes	Ye
013	8	F	IV	Amplified	Relapse	Measurable	Yes	3	Yes	No	N
014	2	F	IV	Unknown	Relapse	Measurable	Yes	2	No	Yes	N
015	3	F	IV	Not Amplified	Refractory	Evaluable	No	4	No	Yes	N
016	8	F	IV	Amplified	Relapse	Measurable	Yes	1	No	No	N
017	5	F	IV	Not Amplified	Relapse	Measurable	Yes	3	Yes	No	Ye
018	5	M	IV	Not Amplified	Refractory	Evaluable	Yes	1	No	No	N

TABLE 3 Irinotecan Dose Levels and Dose Limiting Toxicities (DLT)

Dose Level	Bortezomib Dose	Irinotecan Dose	# 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 3.0)*

Adverse Event	None	Grade 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

*** Common Terminology Criteria For Adverse Event Version 3.0**