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Phase 1 Study of Vorinostat as a Radiation Sensitizer with ^{131}I-Metaiodobenzylguanidine (^{131}I-MIBG) for Patients with Relapsed or Refractory Neuroblastoma

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Abstract

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Purpose—^{131}I-metaiodobenzylguanidine (MIBG) is a radiopharmaceutical with activity in neuroblastoma. Vorinostat is a histone deacetylase inhibitor that has radiosensitizing properties. The goal of this phase 1 study was to determine the maximum tolerated doses of vorinostat and MIBG in combination.

Experimental Design—Patients ≤ 30 years with relapsed/refractory MIBG-avid neuroblastoma were eligible. Patients received oral vorinostat (dose levels 180 and 230 mg/m^2) daily Days 1–14. MIBG (dose levels 8, 12, 15, and 18 mCi/kg) was given on Day 3 and peripheral blood stem cells on Day 17. Alternating dose escalation of vorinostat and MIBG was performed using a 3+3 design.

Results—27 patients enrolled to 6 dose levels, with 23 evaluable for dose escalation. No dose-limiting toxicities (DLT) were seen in the first three dose levels. At dose level 4 (15 mCi/kg MIBG/230 mg/m^2 vorinostat), 1 of 6 patients had DLT with grade 4 hypokalemia. At dose level 5 (18 mCi/kg MIBG/230 mg/m^2 vorinostat), two patients had dose-limiting bleeding (one grade 3 and one grade 5). At dose level 5a (18 mCi/kg MIBG/180 mg/m^2 vorinostat), 0 of 6 patients had DLT. The most common toxicities were neutropenia and thrombocytopenia. The response rate was 12% across all dose levels and 17% at dose level 5a. Histone acetylation increased from baseline in peripheral blood mononuclear cells collected on Days 3 and 12–14.

Conclusions—Vorinostat at 180 mg/m^2/dose is tolerable with 18 mCi/kg MIBG. A phase 2 trial comparing this regimen to single-agent MIBG is ongoing.

Keywords

^{131}I-MIBG; Vorinostat; Radiation Sensitizer; Neuroblastoma; Relapse; Refractory

Introduction

Neuroblastoma is characterized by the presence of widespread metastatic disease in approximately 50% of patients (1). For patients with a poor response to initial therapy or with recurrent disease after a previous remission, the probability of long-term survival is low (2). Active treatment options for such patients are limited.

Neuroblastoma is known to be a radiosensitive tumor (3). ^{131}I-metaiodobenzylguanidine (MIBG) has multiple advantages as a form of targeted radiotherapy for patients with metastatic neuroblastoma. This systemic radiopharmaceutical is distributed to metastatic sites throughout the body (4). Approximately 90% of neuroblastoma tumors accumulate MIBG via the norepinephrine transporter (NET), making MIBG a targeted option for the majority of patients (5–6). MIBG at its usual maximum feasible dose of 18 mCi/kg has been shown to be among the most active agents for children with relapsed or refractory neuroblastoma (7–9). Non-hematologic toxicity is generally mild and hematologic toxicity can be abrogated with autologous stem cell support (10).

Our group has focused on improving the activity of MIBG by combining it with systemic radiation sensitizers. Previous clinical trials of MIBG together with cisplatin, topotecan, or irinotecan have demonstrated the feasibility of this approach (11–13). Several points support evaluation of MIBG together with vorinostat as a novel radiation sensitizer in

neuroblastoma. First, vorinostat, a histone deacetylase inhibitor (HDACi), has been shown to sensitize neuroblastoma cells to ionizing radiation and decrease neuroblastoma tumor growth in a metastatic neuroblastoma xenograft model (14). Second, vorinostat also increases the expression of NET by neuroblastoma cells, resulting in increased MIBG uptake *in vitro* and *in vivo* within 24 hours of vorinostat exposure (15). Third, vorinostat also has modest single-agent activity in preclinical models of neuroblastoma (16) and recent work suggests a role for HDACi as a strategy to target *MYCN* (17). Fourth, vorinostat has been evaluated as a single-agent in children, with a toxicity profile that largely does not overlap with the toxicity profile for MIBG (18). Common adverse events in patients treated with vorinostat include modest myelosuppression, fatigue, gastrointestinal toxicities, hypokalemia, and increased serum creatinine. Lastly, vorinostat in combination with external beam radiotherapy was tolerable in adults treated for colorectal cancer or for brain metastases (19–20), though no prior studies of vorinostat with a radiopharmaceutical have been reported.

Based upon this rationale, we conducted a phase 1 multicenter clinical trial conducted through the New Approaches to Neuroblastoma Therapy (NANT) consortium with the primary objective to determine the maximum tolerated doses (MTD) of vorinostat and MIBG when used in combination. Secondary objectives included assessment of antitumor activity of the combination and evaluation of vorinostat pharmacodynamic effects at the doses evaluated.

Materials and Methods

Patients

Patients were eligible if they were 2–30 years of age at time of enrollment, had relapsed or refractory high-risk neuroblastoma, and had MIBG-avid bone and/or soft tissue disease based upon MIBG diagnostic scan obtained within 4 weeks of study enrollment. All patients were required to have ≥ 2.0×10^6 CD34+ autologous hematopoietic stem cells (PBSCs)/kg available. Patients were required to have adequate performance score (Lansky or Karnofsky score ≥ 50) and life expectancy ≥ 6 weeks. Patients were required to be a minimum of two weeks from last systemic therapy, 12 weeks from prior stem cell transplant, two weeks from prior small port radiation, and three months from large field radiation. Patients previously treated with ^{131}I-MIBG, vorinostat, other HDACi, whole abdominal or total body radiation, or allogeneic transplant were excluded.

Patients were required to meet standard laboratory criteria prior to enrollment: absolute neutrophil count (ANC) ≥ 750/mm^3; unsupported platelet count ≥ 50,000/mm^3; hemoglobin ≥ 8 g/dL; creatinine ≤ 1.5 times the upper limit of age-adjusted normal value or estimated creatinine clearance ≥ 60 mL/min/1.73 m^2; total bilirubin ≤ 1.5 times upper limit of normal (ULN); and ALT and AST < 3 times ULN. Patients were also required to have adequate cardiac and pulmonary function as follows: cardiac ejection fraction ≥ 55% or shortening fraction ≥ 27%; corrected QT interval ≤ 450 msec; and lack of dyspnea at rest, exercise intolerance, pleural effusion, or oxygen requirement. With the finding of one patient at dose level 5 with grade 5 CNS hemorrhage in the setting of expected thrombocytopenia and unexpected prolonged prothrombin and partial thromboplastin times (PT and PTT), the

protocol was amended to also require baseline International Normalized Ratio (INR) ≤ 1.5 and PTT ≤ 1.5 ULN for the remaining 11 patients.

Patients were excluded if they were pregnant, breastfeeding, unable to tolerate radiation isolation, and/or receiving selected drugs known to prolong the QT interval. Patients with other serious concomitant medical illness or with a history of non-catheter related deep venous thrombosis were also excluded.

Each site's institutional review board (IRB) approved the study. Patients and/or legal guardians provided written informed consent, with assent obtained per local IRB guidelines.

Protocol Therapy

Patients received vorinostat orally once daily on Days 1–14 according to assigned dose level. To reduce dose deviations due to rounding for capsule sizes, vorinostat was administered as an extemporaneous oral suspension as previously described (18). On Day 3, vorinostat was administered one hour prior to MIBG.

MIBG (Jubilant DraxImage, Inc; Kirkland, Quebec, Canada) was administered intravenously over 90–120 minutes on Day 3 according to assigned dose level (maximum absolute dose of 1200 mCi). MIBG had a specific activity ≥ 29.7 mCi/mg unlabeled MIBG and a maximum free iodine content < 5%. Red cell transfusions were given prior to the infusion for hemoglobin < 10 g/dL. Hydration, use of bladder catheters, radiation isolation, and thyroid blockade were as previously described (11), except potassium perchlorate became unavailable during the trial and potassium iodide monotherapy was then used. Whole body radiation dose was estimated as previously described (21).

As the first study to combine vorinostat with a targeted radiopharmaceutical, the protocol included two additional safety provisions. First, creatinine was reassessed on Day 3 prior to MIBG infusion to ensure no decrease in renal function prior to MIBG infusion. Second, whole body radiation dose was calculated in real time and if the estimated whole body radiation dose exceeded 500 cGy (a value not typically exceeded with the planned doses of MIBG), vorinostat was to be discontinued early.

All patients received a minimum of 2.0×10^6 CD34+ cells/kg on Day 17. The use of filgrastim was according to institutional standard practice after autologous stem cell infusion.

After the first two dose levels proceeded without a dose limiting toxicity (DLT), subsequent patients were eligible to receive a second course of therapy after Day 56 if they had recovered to baseline criteria, did not have first course DLT, had PBSCs available to support a subsequent course, and had at least stable disease.

Toxicity and Response Evaluation

Toxicity was graded according to the Common Terminology Criteria for Adverse Events, version 3.0. DLT definitions included only toxicities deemed at least possibly related to therapy. Hematologic engraftment DLT was defined as: ANC < 500/mm^3 28 days after

PBSC infusion; platelets < 20,000/mm^3 56 days after PBSC infusion; or need for a second PBSC infusion prior to count recovery. Other hematologic DLTs were grade 4 hemolysis; life-threatening anemia; refractoriness to platelet transfusions with life-threatening bleeding; grade 4 thrombocytopenia on Days 1–7 or on Days 8–14 with platelet transfusion refractoriness; grade 4 neutropenia on Days 1–7 or on Days 8–14 with serious bacterial or fungal infection. Non-hematologic DLT was defined as grade ≥ 3 toxicity with the exception of the following grade 3 toxicities: nausea; vomiting; anorexia; weight loss; dehydration; fatigue; fever; infection; febrile neutropenia; electrolyte abnormality requiring < 24 hours of inpatient management; hepatic enzyme elevation returning to ≤ grade 1 by day 56; fever; infection; and febrile neutropenia. Grade 3 or 4 serum amylase elevation was also excluded as DLT if it resolved to grade 2 within 14 days and was not accompanied by lipase elevation or grade > 3 salivary gland toxicity (dry mouth; parotid pain).

Patients underwent disease staging at baseline and then at approximately Day 56 of each course. CNS imaging was not required. Response was graded according to the NANT Response Criteria version 1.0 as previously described, with an MIBG scan response requiring at least a 50% reduction in Curie score from baseline (11). Overall responses of CR or PR based on central review of radiologic scans and of bone marrow biopsy slides and reports were considered objective responses.

Correlative Pharmacodynamic Studies

The protocol included two optional correlative pharmacodynamics studies. In the first study, the extent of histone acetylation in peripheral blood mononuclear cells (PBMCs) was assessed at baseline, on Day 3 prior to vorinostat, on Day 3 one hour after vorinostat, and then on Day 12, 13, or 14 prior to vorinostat. Peripheral blood was collected into sodium heparin tubes and shipped overnight on ice packs to the reference laboratory at UCSF. PBMCs were isolated on a ficoll gradient and frozen at −70° C until ready for batch analysis. At the time of analysis, thawed PBMCs were washed with cold phosphate buffered saline, then cell lysis buffer was added, and lysate was sonicated on ice. The sample was microcentrifuged for 10 minutes at 4° C and supernatant (cell lysate) was transferred to a new tube. Acetylated histone H3 level was quantified using PathScan® Acetylated Histone H3 Sandwich ELISA kit (kit #7232S; Cell Signaling, Danvers, MA). Fifty micrograms cell lysate were incubated overnight at 4° C in histone H3 antibody-coated microplates. After washing, the samples were incubated with acetylated-lysine mouse monoclonal antibody at 37° C for 1 hour and then incubated with horseradish peroxidase (HRP)-linked anti-mouse IgG at 37° C for 30 minutes. After incubating the samples with the HRP substrate tetramethylbenzidine at 37° C for 10 minutes, the reaction was stopped by adding the stop solution. The optical density at 450 nm was measured within 30 minutes of termination. The experiment was performed in triplicate.

In the second study, the expression of *NET* mRNA in PBMCs was assessed at the same time points as used in the histone acetylation study. Peripheral blood was collected into PAXGene Blood RNA tubes (Qiagen) and shipped overnight on ice packs to the reference laboratory at UCSF where they were frozen at −70° C until ready for batch analysis. At the time of analysis, samples were thawed and RNA extracted using the PAXGene Blood RNA

Kit (Qiagen) following manufacturer instructions. Five hundred ng of total RNA from each sample was reverse transcribed into cDNA using SuperScript VILO cDNA Synthesis kit (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. Quantitative real-time PCR was carried out in 384-well reaction plates using 2X Taqman Fast Universal Master Mix (Applied Biosystems, Foster City, CA), 20X Taqman specific gene expression probes and 10 ng of the cDNA template.

Statistical Methods

Evaluation of dose levels followed the standard 3+3 dose escalation design. Only DLTs in the first course of therapy influenced dose escalation decisions. Patients were evaluable for DLT either if they had a DLT during the first course or met all of the following criteria: received at least 12 of 14 doses of vorinostat; received MIBG; and were followed through at least Day 42 or hematologic recovery, whichever occurred last. The recommended phase 2 dose was the highest dose level tested at which ≤ 1/6 patients had first course DLT. Standard descriptive statistics were used to summarize the clinical results of the trial. To analyze the extent of histone acetylation in PBMCs, a general linear regression model was used which contained fixed effects terms for vorinostat dose, timing of blood draw, and a dose*time interaction term to test whether changes in histone acetylation over time followed the same pattern for the two doses. Patients (nested within dose) were a random effect in the model. The mean of triplicate $\log_e$-transformed optical densities for each patient at each time point was used in all the analyses.

Results

Patient Characteristics

Twenty-seven patients enrolled and all were eligible. Characteristics of these 27 patients are shown in Table 1. Of these, 4 patients were inevaluable for dose escalation consideration (not followed for full DLT evaluation period, n = 2; received only one dose of vorinostat and no MIBG, n = 1; declined required stem cell infusion and had delayed engraftment, n = 1). Two patients were inevaluable for response (declined end of course disease evaluation, n = 1; received only one dose of vorinostat and no MIBG, n = 1).

Dose Escalation and Toxicity

A summary of the dose escalation is shown in Table 2. Three evaluable patients each were treated at dose levels 1–3 and none experienced DLT. One patient at dose level 4 developed dose-limiting grade 4 hypokalemia. This dose level was expanded and no additional DLTs were seen. At dose level 5, two patients had dose-limiting bleeding. One patient had grade 3 oral bleeding in the setting of grade 4 thrombocytopenia and evidence of platelet allosensitization. This patient also met criteria for platelet engraftment DLT. Another patient had grade 5 CNS hemorrhage on day 49 of the first course of therapy. This event occurred in the setting of ongoing thrombocytopenia and coagulopathy. The patient had no known intraparenchymal CNS metastatic disease, though an autopsy was not performed to determine if this event might have been associated with occult CNS progressive disease. The patient also had grade 3 QT prolongation that met DLT definition. With 2/2 patients with DLT at dose level 5, the dose was de-escalated to dose level 5a. Three evaluable patients

were initially treated without DLT. Dose level 5a was expanded to treat three additional evaluable patients, none with DLT. With 0/6 patients with DLT, dose level 5a is the MTD and recommended phase 2 dose.

First course hematologic toxicity by dose level is shown in Table 3. Of 12 patients treated with ≤ 12 mCi/kg MIBG, only 3 (25%) developed grade 4 neutropenia compared to 10/15 (67%) treated with 15 or 18 mCi/kg. All patients who developed grade 4 neutropenia engrafted within 28 days of stem cell infusion. Six of 12 (50%) patients treated with ≤ 12 mCi/kg MIBG had platelet nadir < 20,000/mm^3 compared to 12/15 (80%) treated with 15 or 18 mCi/kg. One patient (described above) had delayed platelet engraftment 56 days from stem cell infusion. Four patients were not evaluable for platelet engraftment (died prior to engraftment in 2; declined stem cells in 1; started myelosuppressive chemotherapy prior to engraftment in 1). All other patients with platelets < 20,000/mm^3 engrafted prior to 56 days from stem cell infusion.

Grade ≥ 3 first course non-hematologic toxicity was uncommon (Table 3). In addition to the DLTs described above and second malignancies described below, other grade 3 adverse events were mainly infectious and laboratory related (including hypokalemia and hyperamylasemia).

Only three patients received a second course, all at dose level 5a. The toxicity profile appeared similar during the second course of therapy and there were no second course DLTs.

No patients had estimated whole body radiation dose ≥ 500 cGy. The median first course whole body dose of radiation was 189 cGy (range 99 – 497 cGy).

Two patients developed myelodysplastic syndrome/acute myeloid leukemia 7 and 30 months after MIBG infusion on this study. Both patients were heavily pretreated with other agents (including induction chemotherapy that included cyclophosphamide, doxorubicin, and etoposide; high-dose chemotherapy with carboplatin, etoposide, and melphalan).

Responses

Responses according to dose level and site of disease involvement are shown in Table 4. The overall objective response rate and MIBG response rate across all dose levels were 12% and 28%, respectively. Responses in soft tissue disease (CT or MRI response) were seen in 22% of patients and by bone marrow biopsy in 13% of patients. At dose level 5a, the recommended phase 2 dose, the overall objective response rate and MIBG response rate were 17% and 67%.

Histone Acetylation Levels and NET Expression Levels

Fifteen patients provided a baseline sample and provided at least one follow-up sample for assessment of histone acetylation in PBMCs. Of the 37 follow-up samples, 31 (84%) showed increases in histone acetylation from baseline (Figure 1). There were no differences in the magnitude of the changes between the two vorinostat dose levels (p=0.84). Overall, the magnitude of the changes increased over time (p=0.028). The mean percent change

(relative to baseline) was +4.5% (95% confidence interval: −1.5%, 10.8%) on Day 3 pre-dose, +11.3% (95% confidence interval: 4.5%, 18.4%) on Day 3 post-dose, and +18.5% (95% confidence interval: 11.3%, 26.2) on Day 12–14 pre-dose. The 6 follow-up samples that showed decreases in histone acetylation from baseline were from 4 patients, two of whom had objective responses by MIBG scan.

Quantification of *NET* transcript in PBMCs yielded detectable transcript on Day 1 prior to vorinostat and/or Day 3 from 7 of 23 patients who submitted samples. *NET* mRNA was not abundant in any sample (all PCR cycle counts 30–40), making it difficult to detect a reliable change in *NET* mRNA in response to vorinostat therapy (Supplemental Figure).

Discussion

We have completed the first study of vorinostat together with a targeted radiopharmaceutical and conclude that this strategy is tolerable. Two potential dose levels could be considered for further development. Dose level 4 provides vorinostat at the full dose identified in the single-agent pediatric study of this agent (230 mg/m^2) with MIBG 15 mCi/kg, which is below the usual maximum feasible dose of 18 mCi/kg. Dose level 5a provides MIBG at its usual maximum feasible dose along with a lower dose of vorinostat (180 mg/m^2). MIBG is the main active agent in this combination and vorinostat's role is as a radiation sensitizer. Given this along with the favorable response rate at dose level 5a, we recommend dose level 5a for further study.

This study utilized a schedule in which vorinostat exposure preceded and followed the MIBG infusion. This schedule was chosen based upon preclinical studies suggesting that the degree of radiation sensitization with HDACi's is greater with both pre- and post-radiation exposure (22). After initial biological clearance of MIBG, the radiation exposure from this agent follows the physical half-life of ^{131}I of 8 days (4). We chose to administer vorinostat for 12 days during and after MIBG infusion to provide overlap during the majority of the radiation exposure following MIBG (1 ½ half-lives). Shorter administration schedules could be considered and, based upon experience using much shorter vorinostat exposures (23–24), it is possible that higher doses of vorinostat might be tolerable together with 18 mCi/kg. However, we note that two adult trials that combined vorinostat with external beam radiation identified the recommended phase 2 dose to be less than its usual adult dose of 400 mg/day (19–20).

The occurrence of two patients with bleeding as DLT at dose level 5 was unanticipated. Thrombocytopenia is a known toxicity of both vorinostat and MIBG when used as single agents (10-18-25), though bleeding is unusual with either agent. Moreover, the coagulopathy seen in one patient and the platelet allosensitization seen in the other patient are not expected adverse events with either agent. In fact, thrombosis has been reported as a rare adverse event in patients treated with vorinostat (20–26). Unusual bleeding was not observed in prior studies of vorinostat with external beam radiation (19–20). Whether this finding reflects an unanticipated interaction between vorinostat and MIBG (or radiation more generally) or chance will require further study.

Our overall response across all dose levels was modest. The response rate was lower than reported with single-agent MIBG (8–9) or in our previous dose escalation study of MIBG together with vincristine/irinotecan (11). We note that response rates (both overall and by MIBG scan) were more encouraging at the recommended dose level 5a of this regimen. Given the small sample size, it is possible that our modest response rate is due solely to chance rather than reflecting an unanticipated antagonistic effect of this combination. The promising MIBG scan response rate argues against the latter possibility. We will evaluate the clinical activity of this dose level more fully as part of an ongoing randomized phase 2 trial comparing MIBG as a single agent to MIBG/vorinostat to MIBG/vincristine/irinotecan (NCT02035137).

We observed evidence of expected pharmacodynamic effect of vorinostat at both vorinostat dose levels evaluated. Almost all patients assessed for changes in histone acetylation after vorinostat showed increases in histone acetylation, with similar modulation at both dose levels. Evaluation of *NET* mRNA levels was limited by the majority of samples showing undetectable transcript in PBMCs and only low levels of transcript in those samples with detectable transcript. Therefore, we were not able to determine whether vorinostat increased NET expression in PBMCs as a surrogate tissue and were not able to perform serial biopsies to assess change in NET expression in tumor tissue.

In conclusion, we have conducted the first clinical trial of vorinostat in combination with a targeted radiopharmaceutical. Our findings may have implications for other tumors treated with MIBG (eg. pheochromocytoma) and may provide insight into potential strategies to improve the clinical activity of other targeted radiopharmaceuticals used to treat other malignancies. Our results have already been incorporated into a subsequent randomized phase 2 trial that includes this regimen at the recommended phase 2 dose of vorinostat 180 mg/m^2 and MIBG 18 mCi/kg derived from this study. This randomized trial will assess whether the addition of a radiation sensitizer improves response rates compared with single agent MIBG alone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of Translational Relevance

Vorinostat has been shown to sensitize a range of cancer cells to the effects of ionizing radiation. ^{131}I-metaiodobenzylguanidine (MIBG) is a systemic radiopharmaceutical that provides targeted radiation to sites of neuroblastoma throughout the body. In this first evaluation of vorinostat together with a systemic radiopharmaceutical, we evaluated the tolerability of the combination of vorinostat and MIBG in patients with advanced neuroblastoma. Vorinostat was tolerable together with MIBG administered at its usual feasible maximum dose of 18 mCi/kg. These findings have implications for the study of other radiopharmaceuticals and add to a growing body of literature indicating that MIBG can be used in combination with a range of radiation sensitizers. As a next step in developing MIBG together with radiation sensitizers, we have initiated a randomized phase 2 trial (NCT02035137) comparing MIBG as a single agent to MIBG together with one of two different radiation sensitizers (vorinostat or irinotecan).

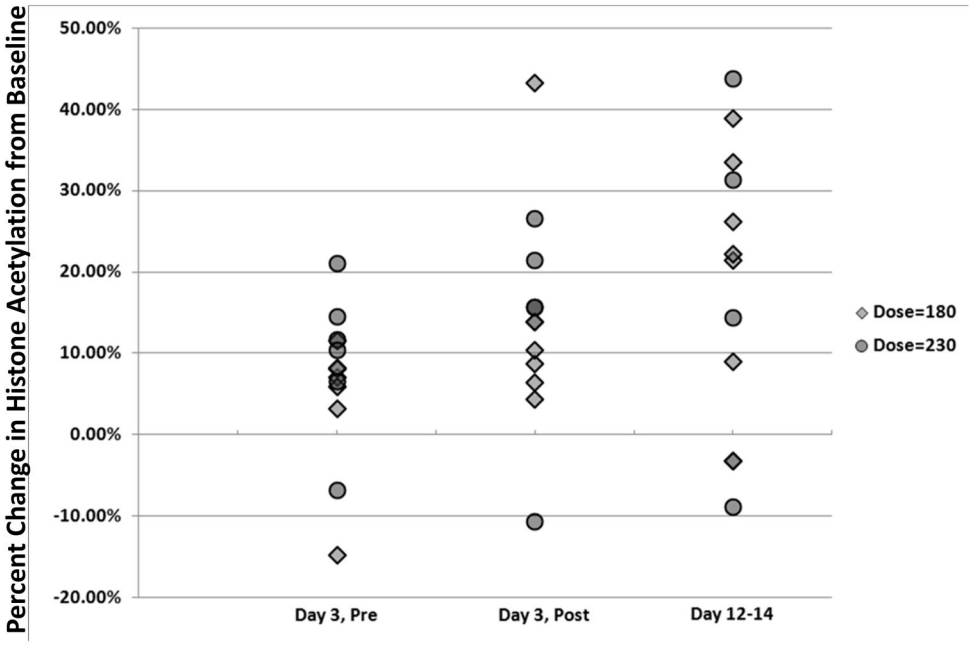

Figure 1.

Relative percent change in histone acetylation from baseline in peripheral blood mononuclear cells from 15 patients treated with vorinostat (180 or 230 mg/m^2) and MIBG. Values from Day 3 pre-vorinostat, Day 3 one hour post-vorinostat, and Day 12–14 pre-vorinostat were normalized to baseline values obtained on Day 1.

Table 1

Characteristics of 27 enrolled patients.

Median age at study entry (range)	6.6 years (3.0 – 18.4)
Median time from diagnosis to entry (range)	25 months (5 – 66)
Male : Female	20 : 7
Relapsed disease[#]	20
Primary refractory disease	7
Prior myeloablative therapy	20/26[*]
***MYCN* amplified tumor**	8/25[**]
Bone marrow involved at study entry	17
Soft tissue disease at study entry	19

[#] Patients who had a history of progression/relapse at any time prior to study enrollment.

[*] Data missing for 1.

[**] Data missing for 2.

Table 2

Planned and evaluated dose levels of vorinostat and ^{131}I-MIBG. Dose level 6 was the planned highest dose level, but exceeded the maximum tolerated dose level and was therefore never evaluated.

Dose Level	^{131}I-MIBG (mCi/kg)	Vorinostat (mg/m^2/dose)	Number Entered	Number Evaluable for DLT	Number Evaluable with DLT
1	8	180	5	3	0
2	12	180	4	3	0
3	12	230	3	3	0
4	15	230	7	6	1*
5	18	230	2	2	2**
5a	18	180	6	6	0
6	18	270	NA (not opened)	NA	NA

* Grade 4 hypokalemia.

** Grade 3 oral bleeding with delayed platelet engraftment (n = 1) and grade 5 CNS hemorrhage and grade 3 QT prolongation (n = 1).

Table 3

Grade ≥ 3 hematologic and non-hematologic toxicities observed in the first course of therapy according to dose level. Toxicities attributed as unrelated to protocol therapy are not shown.

Dose Level	Toxicity	Number of Patients with Maximum Toxicity Grade Observed		
		3	4	5
1 (n=5)	Lymphopenia	1	3	0
	Leukopenia	3	0	0
	Neutropenia	2	1	0
	Thrombocytopenia	0	2	0
	Infection with normal ANC or Grade 1 or 2 neutrophils	2	0	0
2 (n=4)	Lymphopenia	2	2	0
	Thrombocytopenia	1	2	0
	Leukopenia	2	1	0
	Neutropenia	2	1	0
	Anemia	1	0	0
3 (n=3)	Lymphopenia	0	3	0
	Leukopenia	2	1	0
	Neutropenia	2	1	0
	Thrombocytopenia	2	1	0
	Anemia	0	1	0
	Infection with normal ANC or Grade 1 or 2 neutrophils	1	0	0
	Pain (Abdomen NOS)	1	0	0
4 (n=7)	Neutropenia	1	5	0
	Thrombocytopenia	1	5	0
	Lymphopenia	2	3	0
	Leukopenia	3	2	0
	Anemia	4	0	0
	Hypokalemia	1	1*	0
	ALT elevation	1	0	0
	AST elevation	1	0	0
	Infection with normal ANC or Grade 1 or 2 neutrophils	1	0	0
	Amylase elevation	1	0	0
5 (n=2)	Leukopenia	0	2	0
	Lymphopenia	0	2	0
	Neutropenia	0	2	0
	Thrombocytopenia	0	2*	0
	Anemia	1	0	0
	Prolonged QTc interval	1*	0	0
	Dehydration	1	0	0

Dose Level	Toxicity	Number of Patients with Maximum Toxicity Grade Observed		
		3	4	5
	Nausea	1	0	0
	Vomiting	1	0	0
	Hemorrhage, CNS	0	0	1*
	Hemorrhage, GI (Oral cavity)	1*	0	0
	AST elevation	1	0	0
	Infection with Grade 3 or 4 neutrophils	2	0	0
	Amylase elevation	1	0	0
	Hyperglycemia	1	0	0
	Hypokalemia	1	0	0
	Pain (Head/headache)	1	0	0
5a (n=6)	Neutropenia	3	3	0
	Lymphopenia	0	5	0
	Thrombocytopenia	1	4	0
	Leukopenia	3	2	0
	Anemia	2	0	0
	Diarrhea	1	0	0

*Dose-limiting toxicity

Table 4

Best overall objective responses (CR and PR) by central review after completion of protocol therapy according to dose level and site(s) of disease evaluable for response.

Dose Level	Overall Response	MIBG Response	CT/MRI Response	Bone Marrow Response
1*	0/4	0/4	1/3	0/3@
2	0/4	0/4	0/2	0/2
3	0/3	0/3	0/3	0/3
4**	2/6	3/6	2/5	1/1
5	0/2	0/2	0/2	0/1@@
5a	1/6	4/6	1/3	1/6@
All Dose Levels	3/25 (12%)	7/25 (28%)	4/18 (22%)	2/16 (13%)

* Five patients enrolled to dose level 1, but one patient did not have disease evaluation after first course and is therefore inevaluable for response.

** Seven patients enrolled to dose level 4, but one patient did not receive MIBG and therefore is inevaluable for response.

@ Denominator includes one patient with no BM involvement at baseline, but who subsequently had tumor detected in the BM (PD).

@@ Post treatment BM not assessed in 1 of the 2 patients (and therefore not evaluable for BM response – although PD noted on CT).