Published in final edited form as:

Pediatr Blood Cancer. 2018 July ; 65(7): e27023. doi:10.1002/pbc.27023.

Phase I study of vorinostat in combination with isotretinoin in patients with refractory/recurrent neuroblastoma: A new approaches to Neuroblastoma Therapy (NANT) trial

Navin Pinto¹, Steven G. DuBois², Araz Marachelian³, Scott J. Diede⁴, Agne Taraseviciute¹, Julia L. Glade Bender⁵, Denice Tsao-Wei⁶, Susan G. Groshen⁶, Joel M. Reid⁷, Daphne A. Haas-Kogan⁸, C. Patrick Reynolds⁹, Min H. Kang⁹, Meredith S. Irwin¹⁰, Margaret E. Macy¹¹, Judith G. Villablanca³, Katherine K. Matthay^{12,13}, and Julie R. Park¹

¹Seattle Children's Hospital, University of Washington, Seattle, Washington

²Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, Massachusetts

³Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, California

⁴Global Clinical Development—Oncology, Merck Research Laboratories, North Wales, Pennsylvania

⁵Division of Pediatric Hematology, Oncology, and Stem Cell Transplantation, Columbia University Medical Center, New York City, New York

⁶Department of Preventative Medicine, University of Southern California, Los Angeles, California

⁷Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, Minnesota

⁸Department of Radiation Oncology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

⁹Cancer Center and Cell Biology, Texas Tech University Health Sciences Center School of Medicine, Lubbock, Texas

¹⁰Division of Haematology/Oncology, The Hospital for Sick Children, University of Toronto, Toronto, Canada

Correspondence: Julie R. Park, Seattle Children's Hospital, 4800 Sand Point Way NE, M/S MB.8.501, Seattle, WA 98115. julie.park@seattlechildrens.org.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ORCID

Navin Pinto <http://orcid.org/0000-0002-8775-6710>

Steven G. DuBois <http://orcid.org/0000-0003-0882-738X>

Meredith S. Irwin <http://orcid.org/0000-0002-2452-5181>

Margaret E. Macy <http://orcid.org/0000-0001-6446-9180>

Judith G. Villablanca <http://orcid.org/0000-0002-7174-669X>

¹¹Pediatric Hematology/Oncology/Bone Marrow Transplant, University of Colorado Anschutz Medical Campus, Aurora, Colorado

¹²UCSF Benioff Children's Hospital, University of California, San Francisco, California

¹³UCSF School of Medicine, University of California, San Francisco, California

Abstract

Background—Vorinostat combined with retinoids produces additive antitumor effects in preclinical studies of neuroblastoma. Higher systemic exposures of vorinostat than achieved in pediatric phase I trials with continuous daily dosing are necessary for *in vivo* increased histone acetylation and cytotoxic activity. We conducted a phase I trial in children with relapsed/refractory neuroblastoma to determine the maximum tolerated dose (MTD) of vorinostat on an interrupted schedule, escalating beyond the previously identified pediatric MTD.

Methods—Isotretinoin (cis-13-retinoic acid) 80 mg/m²/dose was administered by mouth twice daily on days 1–14 in combination with escalating doses of daily vorinostat up to 430 mg/m²/dose (days 1–4; 8–11) in each 28-day cycle using the standard 3 + 3 design. Vorinostat pharmacokinetic testing and histone acetylation assays were performed.

Results—Twenty-nine patients with refractory or relapsed neuroblastoma were enrolled and 28 were evaluable for dose escalation decisions. Median number of cycles completed was two (range 1–15); 11 patients received four or more cycles. Three patients experienced cycle 1 dose-limiting toxicities. A total of 18 patients experienced grade 3/4 toxicities related to study therapy. The maximum intended dose of vorinostat (430 mg/m²/day, days 1–4; 8–11) was tolerable and led to increased histone acetylation in surrogate tissues when compared to lower doses of vorinostat ($P=0.009$). No objective responses were seen.

Conclusions—Increased dose vorinostat (430 mg/m²/day) on an interrupted schedule is tolerable in combination with isotretinoin. This dose led to increased vorinostat exposures and demonstrated increased histone acetylation. Prolonged stable disease in patients with minimal residual disease warrants further investigation.

Keywords

histone acetylation; neuroblastoma; pharmacokinetics; phase I clinical trial

1 INTRODUCTION

Neuroblastoma, a neoplasm of the sympathetic nervous system, is the most common solid tumor of early childhood. Clinical and tumor biologic factors ascertained at diagnosis of neuroblastoma identify those children who are at risk for poor long-term outcome despite intensive multimodal therapy (defined as high-risk neuroblastoma), and outcome for high-risk patients who fail to respond to or relapse after upfront therapy remains poor.¹ In a phase III randomized trial, administration of isotretinoin (cis-13-retinoic acid) at 80 mg/m²/dose administered twice daily for 2 weeks monthly over a period of 6 months following consolidation therapy improved 3-year event-free survival from 29% in the control cohort to 45% in patients receiving isotretinoin.^{2,3} Isotretinoin is therefore a standard agent for high-

risk neuroblastoma. Novel therapies that enhance the efficacy of isotretinoin may lead to further improvement in survival for patients with this disease.

Deregulation of histone acetylation (HA) has been implicated in the development of a range of malignancies. Thus, there is considerable interest in the use of inhibitors of histone deacetylases (HDAC) as a potential therapeutic modality in the treatment of hematological and solid tumor malignancies.⁴ Inhibitors of HDACs may relieve transcriptional repression caused by the products of certain oncogenes, leading to gene reactivation of tumor suppressors or death pathway genes, which have been abnormally silenced in the generation of the malignant phenotype. This reactivation may lead to cell cycle arrest, induction of apoptosis, differentiation, or autophagy.⁵ Neuroblastoma is thought to arise from defects in neural crest differentiation caused by aberrant gene regulation. Thus, compounds that affect chromatin structure, such as HDAC inhibitors, are potentially useful agents for the treatment of neuroblastoma.

Retinoic acid receptors (RARs) function as modulators of transcription through the recruitment of coregulator complexes. In the absence of a ligand, RARs recruit corepressor (CoR) complexes with HDAC activity, resulting in histone deacetylation, chromatin compaction, and subsequent repression of gene transcription. In contrast, RAR agonist binding causes a conformational change that destabilizes the binding of CoR and creates a new interaction surface that recruits co-activator complexes with histone acetyltransferase activity, leading to increased HA, local chromatin decondensation, and transcriptional activation.⁶ The antitumor activity of retinoic acids (RAs) are thought to be derived from their ability to induce differentiation and/or apoptosis of cancer cells. These findings have led to considerable interest in combining an HDAC inhibitor with a retinoid (such as isotretinoin) for synergistic activity in the treatment of hematological and solid malignancies. Both *in vitro* and *in vivo* xenograft models have confirmed the cooperative actions of retinoids and HDAC inhibitors. The combination of the HDAC inhibitor, *m*-carboxycinnamic acid bis-hydroxamide (CBHA), with all-trans RA (ATRA) led to synergistic cytotoxicity against neuroblastoma cell lines *in vitro* and xenograft *in vivo* models.^{7,8} Subsequent studies using the combination of ATRA with the HDAC inhibitors TSA, sodium butyrate, or vorinostat also demonstrated additive effects on inhibition of growth of neuroblastoma cell lines *in vitro*.⁹

Vorinostat (suberoylanilide hydroxamic acid [SAHA]) is a potent inhibitor of HDAC activity, binding directly to the catalytic pocket of HDAC enzymes and ultimately blocking enzymatic deacetylation at low nanomolar concentrations. The NIH-sponsored Pediatric Preclinical Testing Program has also evaluated vorinostat in mouse xenografts established from six different neuroblastoma cell lines. Xenografts derived from four of these lines showed statistically significant prolongation in event-free survival compared to control-treated mice.¹⁰ A phase I clinical trial of vorinostat plus isotretinoin in patients with relapsed solid tumors was performed by the Children's Oncology Group (COG) (NCT00217412). The maximum tolerated dose (MTD) of vorinostat alone was 230 mg/m²/day dosed continuously and 180 mg/m²/day four times per week when combined with 80 mg/m²/dose of isotretinoin twice daily on days 1–14 of each 28 day cycle (standard neuroblastoma dosing). No HA, a potential surrogate of vorinostat *in vitro* activity, was observed in

peripheral blood mononuclear cells (PBMC) at the 180 mg/m²/dose level, raising concern that this vorinostat dose may be insufficient to result in HDAC inhibition.¹¹

Based on the lack of PBMC HA observed on the COG trial, the New Approaches to Neuroblastoma Therapy (NANT) Consortium sought to explore an alternative vorinostat schedule. Adult clinical trials support the feasibility of delivering higher daily doses of vorinostat given for shorter durations compared to standard dosing of 400 mg once daily continuously.^{12–15} Based on these observations, a phase I trial was designed to assess whether an interrupted schedule of escalating doses of vorinostat (dosed on days 1–4 and 8–11 of every 28-day cycle) would allow a higher MTD in combination with standard-dose isotretinoin and would lead to improved HA.

2 METHODS

2.1 Patients

Patients were required to meet standard NANT eligibility criteria (outlined in Supplementary Material S1). Once the MTD of vorinostat was determined, an expansion cohort was opened at this dose level in order to further characterize the pharmacokinetic (PK) and pharmacodynamic profiles of the intermittent vorinostat dosing regimen. This cohort sought to enroll an additional six patients. Eligibility criteria for the expansion cohort were modified to also allow patients who had entered a second complete remission after relapse to enroll with no evidence of disease. Patients with measurable/evaluable disease were also allowed to enroll on the expansion cohort, with the same eligibility requirements outlined in Supplementary Material S1.

All patients were required to meet standard organ function criteria before enrolling (outlined in Supplementary Material S1).

Patients were excluded if they were pregnant, breastfeeding, or had active infection. Patients who required scheduled specific strong inducers of hepatic cytochrome enzymes (enzyme inducing anticonvulsants, pentamidine, or azole antifungals) were excluded. Patients previously treated with vorinostat or isotretinoin were eligible, provided the two drugs had not been previously used in combination.

Patients and/or legal guardians provided written informed consent, with assent obtained as appropriate. The institutional review board of each NANT site approved the study.

2.2 Protocol therapy

Patients received fixed doses of isotretinoin at 80 mg/m²/dose (2.67 mg/kg/dose for patients < 12 kg) given twice daily for the first 14 days of each 28-day cycle. Vorinostat was administered once daily on days 1–4 and days 8–11 of each 28-day cycle. The starting vorinostat dose was 180 mg/m²/dose; planned subsequent doses 230, 300, 360, and 430 mg/m²/dose (maximum absolute dose capped at 800 mg).

In the absence of clinical/radiographic or bone marrow evidence of progression, patients could continue to receive up to 24 cycles of therapy (effective with Amendment 9,

previously 12 cycles) as long as the patient did not have tumor progression and met laboratory and clinical parameters prior to the start of each cycle of protocol therapy. Patients who met the criteria for permanent discontinuation of vorinostat due to toxicity were removed from protocol therapy.

2.3 Toxicity assessment

Toxicity was assessed and reported for all patients who began vorinostat therapy using the Common Terminology Criteria for Adverse Events criteria, version 4.0. Patients who, during the first cycle, (1) received less than 75% of the planned doses of vorinostat for reasons other than toxicity and (2) did not experience a dose-limiting toxicity (DLT) were replaced for the purposes of evaluating the dose level for dose escalation/de-escalation decisions (that is, not evaluable for DLT). DLT was defined as events that were possibly, probably, or definitely attributable to the combination of vorinostat and isotretinoin. Hematologic DLT was defined as grade 4 thrombocytopenia (platelet count $< 25,000/\text{mm}^3$) or neutropenia (absolute neutrophil count $< 500/\text{mm}^3$) resulting in delays of greater than 7 days in protocol directed therapy, grade 4 thrombocytopenia requiring one or more platelet transfusions, or any adverse event requiring omission of three or more doses of vorinostat. Patients with known bone marrow metastatic disease were eligible for study but were not evaluable for hematologic toxicity. Additional DLT criteria are outlined in Supplementary Material S1.

2.4 Response evaluation

Patients underwent disease evaluation at baseline, after cycles 2 and 4, and then every three cycles. Overall response was graded according to the NANT Response Criteria (v1.0), as previously described.¹⁶ Overall responses of complete response (CR) or partial response (PR) were considered objective responses. Patients were considered evaluable for response if they received at least 75 percent of vorinostat in one course or experienced a DLT at any time after at least one dose of vorinostat. Patients who had progressive disease were considered to be evaluable for response regardless of drug administration.

Local radiographic (anatomical imaging and methyliodobenzyl-guanidine [MIBG]) and bone marrow reports (aspirates and biopsies) were centrally reviewed at NANT Operations Center for accuracy so they were consistent with assigned overall response after any queries were resolved. MIBG scans from baseline were centrally reviewed for all patients to accurately and consistently describe MIBG avid disease at study entry.

2.5 PK and pharmacodynamic studies

All patients were required to submit serial samples during cycle 1 for vorinostat PK studies. Three ml of blood were drawn into a serum separator tube before the first dose of vorinostat on day 1 and then at hours 0.5, 1, 1.5, 2, 4, 6, 8, and 24 (before the vorinostat dose on day 2). Samples were processed and batch analyzed as previously described.¹⁷

For optional isotretinoin PK studies, 5 ml of blood were collected into sodium heparin tubes 4 hr after the last morning dose of isotretinoin during cycle 1 (day 14). Samples were processed and batch analyzed as previously described.¹⁸

The study included an optional vorinostat pharmacodynamics aim to correlate PBMC HA with vorinostat PKs. 5 mL of blood were collected in a sodium heparin tube at four time points: cycle 1, day 1 pre-dose, and at 1, 6, and 24 hr post the first dose. ELISA was used to quantify acetylated histone H3 as previously described.¹⁹

2.6 Study design and statistical methods

Evaluation of vorinostat dose levels followed the 3 + 3 dose escalation design.²⁰ Dose escalation considerations were based on cycle 1 data only. The MTD was the highest dose level tested at which 0/6 or 1/6 patients experienced DLT with at least 2/3 or 2/6 patients encountering DLT at the next higher dose. Once the MTD was determined, an expansion cohort was opened at that dose level in order to further characterize the PK and pharmacodynamic profiles of the intermittent vorinostat dosing regimen.

Progression-free survival (PFS) was calculated as time from start of treatment to first episode of disease progression or death from any cause, whichever was observed first; patients who were alive and without progression were censored at last follow-up. For one patient who withdrew from treatment and died 2 months later, with date of progression unknown, the date of progression was taken as 1 month after withdrawal from treatment. Kaplan–Meier plots were used to summarize PFS and overall survival (OS).

For vorinostat PK measurements, geometric means and associated 95% confidence intervals were calculated; for isotretinoin PK measurements, because of the limited numbers of values, medians and ranges are presented. For each patient, at each time point, the HA color intensity values were measured in triplicate; observed color intensity values were log-transformed and the averages of the three readings were calculated. In addition, for each patient, the baseline average HA value was subtracted from the average HA value at 1, 6, and 12 hr. For presentation, the HA differences (and means and associated 95% confidence intervals) were transformed back into percent change. In a final analysis to explore associations, the maximum percent change in HA was determined for each patient. To obtain a quantitative assessment of the associations between vorinostat and isotretinoin PK's, and dose level and percent change in HA levels, the Pearson correlation was calculated; for all other associations, the Spearman correlation was calculated. Scatterplots were drawn and inspected whenever possible. To evaluate the association between HA levels (the averages and the differences from baseline) over time and over the dose levels, a generalized linear model was used with patient as a random effect and dose level and time as fixed effects. All *P*-values are two-sided.

3 RESULTS

3.1 Patient characteristics

Twenty-nine patients enrolled from January 2011 through October 2013. No patients were determined to be ineligible after enrollment. Characteristics of the 29 eligible patients are provided in Table 1. One patient on dose level 1 missed three doses of vorinostat (due to concurrent infection) and was deemed inevaluable for dose escalation decisions and was replaced. Ten patients were inevaluable for response. Reasons for inevaluability included

taking less than 75% of prescribed protocol therapy and withdrawal from protocol therapy (n = 2), incomplete restaging evaluation (n = 1), off treatment due to unacceptable toxicity and disease evaluation was not completed (n = 1), and entering protocol therapy in a CR with no evaluable disease in the expansion cohort (n = 6). Twenty-three patients were previously exposed to isotretinoin and two patients were previously exposed to vorinostat.

3.2 Dose escalation and toxicity

A summary of the dose escalation is provided in Table 2. At dose level 1, one patient developed a DLT (grade 3 pain in back and extremities, grade 3 pain in skin, and grade 3 chelitis). On dose level 5, one patient developed a DLT (grade 3 creatinine increase). Dose level 5 (vorinostat dose 430 mg/m²) was the maximum intended dose and did not exceed toxicity threshold with 1/6 DLT's; therefore, this was defined as the MTD.

An additional seven patients (six patients in CR and one patient with evaluable disease at study entry) were enrolled in the expansion cohort at the MTD. The patient with evaluable disease at study entry experienced a DLT (grade 3 maculopapular rash).

The 29 eligible patients received a total of 132 cycles of therapy. Nine patients had bone marrow involvement at study entry. No patients experienced hematologic DLT. Hematologic toxicity in all patients was the most common grade 3 or higher toxicity but was not dose limiting; nine patients developed maximum grade 3 and two developed grade 4 neutropenia; six patients developed maximum grade 3 and one grade 4 thrombocytopenia. Three patients had grade 3 decreased white blood cells. Nonhematologic DLTs were the following: two patients had grade 3 pain and two patients experienced grade 3 elevations in alanine aminotransferase level. There were no toxic deaths. One patient in the MTD expansion cohort required a dose reduction in cycle 4 due to a grade 1 prolonged QTc. Information on toxicity grade 3 is summarized in Table 3.

3.3 Vorinostat/isotretinoin PKs

Vorinostat and isotretinoin PK were collected during cycle 1 of therapy. A total of 28 patients provided samples for vorinostat PK; 23 provided all required specimens. Table 4 summarizes the vorinostat PK results.

The mean apparent oral vorinostat clearance was 151 l/hr (range 58–435 l/hr) on the interrupted dosing schedule. Vorinostat clearance and half-life (2.8 hr, range, 1.2–10.1 hr) appeared stable across dose levels, consistent with linear PK ($P=0.60$ and $P=0.39$, respectively). There was substantial interpatient variability in SAHA maximum concentration (mean 428 ng/ml, range 158–980 ng/ml) and area under the curve (AUC) (median 2174 ng/ml/hr, range 930–5776 ng/ml/hr) values across dose levels, and both increased with dose ($P<0.001$ and $P=0.003$, respectively).

An optional single isotretinoin PK sample was drawn 4 hr after the last dose of isotretinoin in cycle 1 in 12 out of 29 patients. The isotretinoin median serum concentration was 0.83 $\mu\text{g/ml}$ (range 0.19–1.40 $\mu\text{g/ml}$) and the 4-oxo-13-cisRA median serum concentration was 3.17 $\mu\text{g/ml}$ (range 0.47–5.84 $\mu\text{g/ml}$). There was no apparent association between vorinostat

dose and either the RA or the 4-oxo-13-cisRA serum levels ($P=0.68$ and $P=0.39$, respectively), nor any association with the vorinostat PK levels.

3.4 Vorinostat pharmacodynamics

Twenty-three of 29 patients consented to the optional vorinostat pharmacodynamic assessments. Fifty-three adequate PBMC samples were obtained from 16 patients (nine treated at dose levels 1–4 and seven treated at dose level 5/expansion cohort). Overall, the percent change from baseline in HA levels at 1 hr post treatment was significantly greater in dose level 5 compared to dose levels 1–4 ($P=0.009$) and this difference persisted at hours 6 and 24 (Table 5). After controlling for the dose level, there was an association between the vorinostat AUC and clearance values and the increase in HA levels ($P=0.021$ for both AUC and clearance).

3.5 Antitumor activity

No objective responses were seen on this study. A median of two cycles were administered (range 1–15). Eleven (38%) of the 29 patients completed 4 (median 11, range 4–15) cycles without tumor progression. Nineteen patients were evaluable for full response assessment; six patients were in CR at study entry and four were classified as inevaluable for response (one patient received only 50% of prescribed therapy and three were not re-evaluated after start of treatment). Of those evaluable for response, 5/19 (17%) patients achieved prolonged stable disease (SD) (median 12 cycles, range 4–15). A median of 7.5 cycles were received (range 2–12 cycles) by the six patients who enrolled in second or greater CR and five of these six patients (83%) completed a median of 11 cycles without tumor progression (range 4–12). There was not a statistically significant difference in PFS between patients treated at (a) dose levels 1–4 ($n=16$), (b) dose level 5 who presented with measurable/evaluable disease ($n=7$), and (c) dose level 5 who presented without measurable/evaluable disease ($n=6$), $P=0.35$. There was also no statistically significant difference in PFS for patients previously exposed to isotretinoin or vorinostat when compared to the entire cohort.

A total of 20 patients have progressed; one patient died without disease progression of infectious complications related to a secondary malignancy and 16 died following progression. The median PFS for the entire cohort of 29 patients is 2.0 months (95% confidence interval, 1.6–11.0 months). The 6-month PFS rate was 0.36 ± 0.09 . The median OS for the entire cohort of patients is 30.8 months (95% confidence interval of 13.9–47.0 months; Figure 1).

4 DISCUSSION

The administration of isotretinoin in the setting of minimal residual disease has improved outcomes for children with high-risk neuroblastoma and is considered standard-of-care as a component of post-consolidation maintenance therapy. Preclinical data suggest that the HDAC inhibitor vorinostat has modest single-agent activity in neuroblastoma xenograft experiments,¹⁰ and may have additive antitumor effects when combined with retinoids.^{7–9} We demonstrate that an interrupted schedule of higher dose vorinostat can be safely combined with standard doses of isotretinoin in children with high-risk neuroblastoma,

achieving *in vivo* pharmacodynamic activity as determined by PBMC HA without excess toxicity.

Preclinical studies suggest that a higher systemic exposure to vorinostat may be necessary to achieve maximal antitumor activity.¹⁰ In a previous pediatric phase I trial, single agent MTD of vorinostat (180 mg/m²/dose for 14 days) was not tolerable with the standard dose of isotretinoin (80 mg/m²/dose twice daily for 14 days).¹¹ However, we demonstrate the tolerability of intermittent vorinostat dosing combined with standard dosing of isotretinoin at all vorinostat dose levels tested, escalating to the maximum intended dose of 430 mg/m². In addition to safely delivering a higher dose of vorinostat, higher AUC values (mean 2174 ng/ml/hr, range 930–5776 ng/ml/hr) were achieved, consistent with demonstrated linear PKs.¹¹ Additional PKs were similar to those previously reported.¹¹ Pharmacodynamic effects of vorinostat were observed with the maximal effect on modulation of PBMC HA observed at the MTD. This is in contrast to the COG study of isotretinoin and vorinostat, where consistent PBMC HA was only seen at the highest continuous dose level of vorinostat (300 mg/m²/dose), a dose determined to be too toxic in combination with isotretinoin. At the continuous vorinostat MTD (230 mg/m²/dose), only transient PBMC HA was observed. RA and 4-oxo-13-cisRA serum concentrations were similar to previously reported levels in patients with neuroblastoma^{21,22} without evidence for drug interactions.

Although no objective responses were seen, 11/29 (38%) patients were able to continue the combination for four or more cycles without clinical evidence of progression and 7/29 (24%) received 11–15 cycles of therapy. In addition, 83% (5/6) of patients who entered with no evidence of disease were able to complete four or more cycles without disease progression. Given the favorable prolonged SD rate, this combination warrants further investigation. A randomized phase III clinical trial (COG ANBL0032, NCT00026312) demonstrated a survival benefit of postconsolidation GD-2-directed chimeric antibody immunotherapy (dinutuximab) with cytokines (alternating cycles of IL-2 and GM-CSF) and isotretinoin for patients with neuroblastoma who had achieved at least a PR to induction and consolidation therapies.²³ Recent investigations have demonstrated that HDACis like vorinostat may enhance cancer immunotherapy through an immunostimulatory effect. Tumor bearing syngenic mice with solid tumors or lymphoma have significantly improved tumor responses to vorinostat and panobinostat with an intact immune system, when compared to immunodeficient mice.²⁴ Further trials incorporating intermittent vorinostat dosing in combination with isotretinoin and dinutuximab as a postconsolidation maintenance strategy should be considered given the preclinical signal^{7–9} as well as manageable toxicity profile and potential activity in children with neuroblastoma who are in a minimal residual disease state.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded by NIH (grant no. P01CA081403), St. Baldrick's Foundation, Children's Neuroblastoma Cancer Foundation, and a National Cancer Institute Cancer Clinical Investigator Team Leadership Award to A.M. (3P30CA014089-41S2).

Funding information

National Cancer Institute, Grant/Award Numbers: 3P30CA014089-41S2, P01CA081403; St. Baldrick's Foundation; Children's Neuroblastoma Cancer Foundation

Grant sponsor: NIH, Grant number: P01CA081403, Grant sponsor: National Cancer Institute, Grant number: 3P30CA014089-41S2, Grant sponsors: St. Baldrick's Foundation, Children's Neuroblastoma Cancer Foundation

Abbreviations

ATRA	all-trans retinoic acid
AUC	area under the curve
COG	Children's Oncology Group
CoR	corepressor
CR	complete response
DLT	dose-limiting toxicity
HA	histone acetylation
HDAC	histone deacetylase
MIBG	methyliodobenzylguanidine
MTD	maximum tolerated dose
NANT	New Approaches to Neuroblastoma Therapy
OS	overall survival
PBMC	peripheral blood mononuclear cell
PFS	progression-free survival
PK	pharmacokinetic
PR	partial response
RA	retinoic acid
RARs	retinoic acid receptors
SAHA	suberoylanilide hydroxamic acid
SD	stable disease

References

1. Pinto NR, Applebaum MA, Volchenboum SL, et al. Advances in risk classification and treatment strategies for neuroblastoma. *J Clin Oncol*. 2015; 33:3008–3017. [PubMed: 26304901]
2. Matthay KK, Reynolds CP, Seeger RC, et al. Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a Children's Oncology Group study. *J Clin Oncol*. 2009; 27:1007–1013. [PubMed: 19171716]
3. Matthay KK, Villablanca JG, Seeger RC, et al. Children's Cancer Group. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. *N Engl J Med*. 1999; 341:1165–1173. [PubMed: 10519894]
4. Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene*. 2007; 26:5541–5552. [PubMed: 17694093]
5. Shahbazian MD, Grunstein M. Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem*. 2007; 76:75–100. [PubMed: 17362198]
6. Altucci L, Gronemeyer H. The promise of retinoids to fight against cancer. *Nat Rev Cancer*. 2001; 1:181–193. [PubMed: 11902573]
7. Coffey DC, Kutko MC, Glick RD, et al. The histone deacetylase inhibitor, CBHA, inhibits growth of human neuroblastoma xenografts *in vivo*, alone and synergistically with all-trans retinoic acid. *Cancer Res*. 2001; 61:3591–3594. [PubMed: 11325825]
8. Coffey DC, Kutko MC, Glick RD, et al. Histone deacetylase inhibitors and retinoic acids inhibit growth of human neuroblastoma *in vitro*. *Med Pediatr Oncol*. 2000; 35:577–581. [PubMed: 11107121]
9. De los Santos M, Zambrano A, Sanchez-Pacheco A, Aranda A. Histone deacetylase inhibitors regulate retinoic acid receptor beta expression in neuroblastoma cells by both transcriptional and posttranscriptional mechanisms. *Mol Endocrinol*. 2007; 21:2416–2426. [PubMed: 17622583]
10. Keshelava N, Houghton PJ, Morton CL, et al. Initial testing (stage 1) of vorinostat (SAHA) by the pediatric preclinical testing program. *Pediatr Blood Cancer*. 2009; 53:505–508. [PubMed: 19418547]
11. Fouladi M, Park JR, Stewart CF, et al. Pediatric phase I trial and pharmacokinetic study of vorinostat: a Children's Oncology Group phase I consortium report. *J Clin Oncol*. 2010; 28:3623–3629. [PubMed: 20606092]
12. Kelly WK, O'Connor OA, Krug LM, et al. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J Clin Oncol*. 2005; 23:3923–3931. [PubMed: 15897550]
13. Garcia-Manero G, Tambaro FP, Bekele NB, et al. Phase II trial of vorinostat with idarubicin and cytarabine for patients with newly diagnosed acute myelogenous leukemia or myelodysplastic syndrome. *J Clin Oncol*. 2012; 30:2204–2210. [PubMed: 22585696]
14. Ramalingam SS, Parise RA, Ramanathan RK, et al. Phase I and pharmacokinetic study of vorinostat, a histone deacetylase inhibitor, in combination with carboplatin and paclitaxel for advanced solid malignancies. *Clin Cancer Res*. 2007; 13:3605–3610. [PubMed: 17510206]
15. Munster PN, Marchion D, Thomas S, et al. Phase I trial of vorinostat and doxorubicin in solid tumours: histone deacetylase 2 expression as a predictive marker. *Br J Cancer*. 2009; 101:1044–1050. [PubMed: 19738609]
16. DuBois SG, Marachelian A, Fox E, et al. Phase I study of the aurora A kinase inhibitor alisertib in combination with irinotecan and temozolomide for patients with relapsed or refractory neuroblastoma: a NANT (New Approaches to Neuroblastoma Therapy) trial. *J Clin Oncol*. 2016; 34:1368–1375. [PubMed: 26884555]
17. Parise RA, Holleran JL, Beumer JH, Ramalingam S, Egorin MJ. A liquid chromatography-electrospray ionization tandem mass spectrometric assay for quantitation of the histone deacetylase inhibitor, vorinostat (suberoylanilide hydroxamic acid, SAHA), and its metabolites in human serum. *J Chromatogr B Anal Technol Biomed Life Sci*. 2006; 840:108–115.
18. Vane FM, Stoltenberg JK, Bugge CJ. Determination of 13-cis-retinoic acid and its major metabolite, 4-oxo-13-cis-retinoic acid, in human blood by reversed-phase high-performance liquid chromatography. *J Chromatogr*. 1982; 227:471–484. [PubMed: 6460777]

19. DuBois SG, Groshen S, Park JR, et al. Phase I study of vorinostat as a radiation sensitizer with ¹³¹I-metaiodobenzylguanidine (¹³¹I-MIBG) for patients with relapsed or refractory neuroblastoma. *Clin Cancer Res*. 2015; 21:2715–2721. [PubMed: 25695691]
20. Storer BE. Design and analysis of phase I clinical trials. *Biometrics*. 1989; 45:925–937. [PubMed: 2790129]
21. Khan AA, Villablanca JG, Reynolds CP, Avramis VI. Pharmacokinetic studies of 13-cis-retinoic acid in pediatric patients with neuroblastoma following bone marrow transplantation. *Cancer Chemother Pharmacol*. 1996; 39:34–41. [PubMed: 8995497]
22. Veal GJ, Cole M, Errington J, et al. Pharmacokinetics and metabolism of 13-cis-retinoic acid (isotretinoin) in children with high-risk neuroblastoma—a study of the United Kingdom Children’s Cancer Study Group. *Br J Cancer*. 2007; 96:424–431. [PubMed: 17224928]
23. Yu AL, Gilman AL, Ozkaynak MF, et al. Children’s Oncology Group. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med*. 2010; 363:1324–1334. [PubMed: 20879881]
24. West AC, Mattarollo SR, Shortt J, et al. An intact immune system is required for the anticancer activities of histone deacetylase inhibitors. *Cancer Res*. 2013; 73:7265–7276. [PubMed: 24158093]

FIGURE 1. Kaplan–Meier plots for progression-free survival and overall survival for all patients (n = 29)

TABLE 1

Patient demographics

Patient and disease characteristics (n = 29)	Number of patients (%)
Male:female	16:13
Median age (years) at study enrollment (range)	10.3 (3.3–19.4)
Median age (years) at diagnosis of high risk neuroblastoma (range)	5.0 (2.0–14.5)
Median months from diagnosis of high risk neuroblastoma to enrollment (range)	47.9 (1.6–141.1)
MYCN status at diagnosis	
Amplified	4 (14%)
Nonamplified	22 (76%)
Unknown	3 (10%)
Response to frontline therapy	
Recurrent/progressive disease	24 (83%)
Refractory disease	5 (17%)
Persistent disease	0 (0%)
Therapy received prior to enrollment	
Prior isotretinoin	23 (79%)
Prior vorinostat	2 (7%)
Prior myeloablative stem cell transplant	24 (83%)
Sites of tumor at enrollment	
No measurable/evaluable tumor sites	6 (21%)
Measurable/evaluable disease	23 (79%)
Disease sites in patients with measurable/evaluable disease (n = 23)	
Bone marrow ^a	
Positive	9 (39%)
% Bone marrow tumor for patients positive at baseline	
>0% to <5%	3
5%	2
50%	1
Positive, % not specified	3
Negative	14 (61%)
CT/MRI ^a	
Measurable soft tissue disease	14 (61%)
Median sum of longest diameters at baseline in cm (range)	8.2 (1.7–20.1)
No measurable soft tissue disease	9 (39%)
MIBG status at baseline ^a	
Positive	18 (78%)
Median Curie score at baseline among those with positive MIBG (range)	12 (1–20)
Negative	3 (13%)

Patient and disease characteristics (n = 29)	Number of patients (%)
Known MIBG nonavid	2 (9%)
Method of isotretinoin intake	
Swallowed/chewed whole capsule	27 (93%)
All other methods	2 (7%)

^aPatients with measurable/evaluable disease.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

TABLE 2

Planned and evaluated dose levels of vorinostat and isotretinoin

Dose level	Vorinostat (mg/m ² /dose)	Isotretinoin (mg/m ² /dose)	Number of patients entered	Number of patients evaluable for DLT ^a	Number of patients evaluable with DLT
1	180	80	7	6	1 (grade 3 extremity/back/skin pain, grade 3 cheilitis)
2	230	80	3	3	0
3	300	80	3	3	0
4	360	80	3	3	0
5	430	80	6	6	1 (grade 3 creatinine)
Expansion cohort 1 (age < 21 years)	430 (MTD)	80	7	7	1 (grade 3 maculopapular rash)

^aTo be evaluable for DLT, during first cycle, patient had to: (1) receive 75% or more of the planned doses of vorinostat or (2) experience DLT.

TABLE 3

Grade 3+ toxicities observed in the first cycle of therapy according to dose level

Vorinostat dose level (mg/m ² /dose)	Toxicity	Number of patients with maximum toxicity grade observed	
		Grade 3+ toxicities observed on first cycle	Grade 3+ toxicities observed over all cycles
1 (180) (n = 7)	Back pain	1	1
	Cheilitis	1	1
	Decreased neutrophil count	0	1
	Pain in extremity	1	1
	Pain of skin	1	1
2 (230) (n = 3)	Decreased lymphocyte count	1	1
	Decreased neutrophil count	0	1
	Hypertriglyceridemia	0	1
	Nausea	1	1
	Vomiting	1	1
3 (300) (n = 3)	Decreased neutrophil count	1	2
	Decreased white blood cell count	0	1
	Pain in extremity	0	1
4 (360) (n = 3)	Decreased neutrophil count	0	1
	Decreased platelet count	1	1
	Decreased white blood cell count	0	1
5 (430) (n = 6)	Alanine aminotransferase	1	1
	Aspartate aminotransferase	1	1
	Anemia	1	1
	Decreased neutrophil count	0	2a
	Decreased platelet count	2	3
	Decreased white blood cell count	0	1
	Dehydration	0	1
	Fever	0	1
	Increased creatinine	0	1
Expansion Cohort 1 (430) (n = 7)	Upper respiratory infection	0	1
	Alanine aminotransferase	1	1
	Decreased neutrophil count	0	4a
	Decreased platelet count	2	3a
	Headache	0	1
	Maculopapular rash	1	1

^aIncluded one patient with grade 4 on each.

Toxicities attributed as unlikely or unrelated to protocol therapy are not shown. Grade 4 or 5 toxicities were not observed in the first cycle of therapy at any dose level on this trial.

TABLE 4

Vorinostat PK summaries by dose level

Geometric mean (95% confidence interval)	AUC _{inf} (ng/ml/hr) (n = 23)	C _{max} (ng/ml) (n = 28)	t _{1/2} (hr) (n = 23)	Clearance (Cl F obs) (l/hr) (n = 23)
Dose level 1 (n = 6/7) ^a	1351 (904, 2020)	287 (208, 397)	2.6 (1.7, 4.0)	152 (93, 250)
Dose level 2 (n = 2/3)	1671 (832, 3353)	226 (138, 369)	2.4 (1.2, 4.9)	181 (76, 427)
Dose level 3 (n = 3/3)	2638 (1494, 4660)	552 (337, 903)	2.5 (1.4, 4.5)	129 (64, 260)
Dose level 4 (n = 3/3)	1653 (936, 2919)	448 (274, 734)	2.3 (1.3, 4.2)	322 (159, 649)
Dose level 5—MTD (n = 9/12)	3253 (2342, 4517)	587 (459, 751)	3.2 (2.3, 4.5)	118 (79, 177)
All dose levels (n = 23/28)	2174 (1696, 2787)	428 (348, 526)	2.8 (2.3, 3.4)	151 (115, 197)
Range of values—all dose levels (n = 23/28)	930–5776	158–980	1.2–10.1	58–435
P-value (Pearson correlation between PK parameter & dose level)	r = 0.59, P = 0.003	r = 0.62, P < 0.001	r = 0.19, P = 0.39	r = -0.11, P = 0.60

^aNumber of patients with complete PK determinations/number of patients enrolled. C_{max}, maximum concentration.

TABLE 5

Percent change in histone acetylation (HA) levels from baseline

Cohort	Hour	Number of patients	Mean percent change	95% confidence interval
Dose levels 1–4	1	7	–3.9%	(–14.3%, 7.8%)
	6	5	–2.9%	(–15.9%, 12.2%)
Dose level 5	24	7	–5.7%	(–16.0%, 5.8%)
	1	6	18.5%	(2.6%, 37.0%)
	6	6	21.9%	(5.4%, 40.9%)
	24	5	14.4%	(–2.5%, 34.2%)

The percent changes in HA levels were greater in the dose level 5 cohort ($P = 0.009$ for the main effect of cohort).

For both cohorts, the changes were not different at the three different time points ($P = 0.76$ for the main effect of hour).