Published in final edited form as:
Pediatr Blood Cancer. 2009 September ; 53(3): 505–508. doi:10.1002/pbc.21988.

Initial Testing (Stage 1) of Vorinostat (SAHA) by the Pediatric Preclinical Testing Program

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Abstract

Vorinostat, a histone deacetylase inhibitor, was evaluated against the *in vitro* and *in vivo* childhood solid tumor and leukemia models in the Pediatric Preclinical Testing Program (PPTP). *In vitro* testing was performed by the DIMSCAN cytotoxicity assay. *In vivo,* vorinostat was administered intraperitoneally to mice bearing xenografts. Vorinostat demonstrated 2-log cell growth inhibitory activity *in vitro,* but generally at concentrations not sustainable in the clinic. No objective responses were observed for any of the solid tumor or acute lymphoblastic leukemia xenografts. Preclinical studies with appropriate drug combinations may provide direction for further clinical evaluations of vorinostat against selected pediatric cancers.

Keywords

Preclinical Testing; Developmental Therapeutics; Vorinostat

INTRODUCTION

Vorinostat (suberoylanilide hydroxamic acid or SAHA) is a potent histone deacetylase (HDAC) inhibitor that inhibits both class I and class II enzymes [1]. The former group includes HDACs 1, 2, 3 and 8 and are primarily localized to the nucleus, while the latter group includes HDACs 4, 5, 6, 7, 9, and 10 and are primarily found in the cytoplasm [1]. HDAC inhibitors induce hyperacetylation of a number of proteins, resulting in a plethora of downstream effects. Increased levels of acetylated histones generally result in a more open

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chromatin conformation that is associated with active gene expression [2,3]. HDAC inhibitors also impair mitotic progression, producing defects in chromosome condensation, segregation, and kinetochore assembly [1,2]. Non-histone proteins that show increased acetylation in response to HDAC inhibition include numerous transcription factors (e.g., E2F-1, NF-kappaB, GATA-1, Bcl-6, etc.), Hsp90, Ku70, and α-tubulin [1,2]. Not yet understood is which of these biological effects of HDAC inhibitors is primarily associated with anticancer activity [1,2].

Vorinostat induces differentiation, growth arrest, or apoptosis in a broad range of cancer cell lines [2–4]. It has also demonstrated anti-tumor activity in a number of *in vivo* models including leukemia, lymphoma, prostate, and breast cancer [2,5–7]. Like other HDAC inhibitors under clinical evaluation [4,5], vorinostat's greatest clinical activity has been against cutaneous T-cell lymphoma [6–8]. Vorinostat was approved by FDA in 2006 for the treatment of cutaneous manifestations of advanced cutaneous T-cell lymphoma [9]. Objective responses were also reported in vorinostat phase 1 studies for patients with mesothelioma, thyroid cancer, laryngeal cancer, Hodgkin disease, and diffuse large B-cell lymphoma [10,11]. Vorinostat has entered phase 1 evaluation in children with cancer.

MATERIALS AND METHODS

In vitro

Cell sensitivity was determined using DIMSCAN. Cells were incubated in the presence of vorinostat for 96 hours at concentrations from 0.01 μM to 100 μM and analyzed as previously described [12].

In vivo

CB17SC-M *scid*$^{-/-}$ female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [15–17]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid* $^{-/-}$ mice as described previously [18]. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Tumor volumes (solid tumor xenografts) or percentages of human CD45-positive (hCD45) cells (ALL xenografts) were determined as previously described [13]. Responses were determined using three activity measures as previously described [13]. A detailed description of the analysis methods is included in the Supplemental Response Definitions.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation

Vorinostat was provided to the Pediatric Preclinical Testing Program by Merck through the Cancer Therapy Evaluation Program (NCI). For *in vivo* testing vorinostat was dissolved in DMSO (final concentration 10%) and diluted in PEG400 (final concentration 45%) in water and administered intraperitoneally daily × 5 for 6 weeks at a dose of 125 mg/kg. Vorinostat was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

In vitro testing

Vorinostat was uniformly able to inhibit growth of the cell lines from the PPTP *in vitro* panel (Table I). The median IC_{50} for the entire panel was 1.44 μM with a range of 0.48 μM to 9.77 μM. The median IC_{90} for the panel was 4.86 μM with a range of 1.84 to 61.32 μM.

In vivo testing

Vorinostat was evaluated in 45 xenograft models. Eight of 424 (1.9%) mice died in the vehicle control arm and 53 of 431 (12.3%) in the vorinostat treatment arm. Seven lines (BT-29, KT-14, Rh28, BT-36, GBM2, OS-2, and OS-33) were excluded from analysis due to toxicity greater than 25 percent. A complete summary of results is provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values. No objective responses were observed in any of the models. The best responses observed were nine examples of PD2 (progressive disease with growth delay). These included TC-71 (Ewing), NB-Ebc1 (neuroblastoma) and Rh41 (rhabdomyosarcoma) xenografts that were also tested *in vitro* and whose IC_{90} values were the lowest in the Ewing, neuroblastoma, or rhabdomyosarcoma cell line panels. Vorinostat induced significant differences in EFS distribution compared to controls in 16 of 38 evaluable xenografts (Table II), although no xenografts met the criteria for intermediate or high activity (EFS T/C value > 2.0 and a significant difference in EFS distribution).

DISCUSSION

Phase I clinical trials of vorinostat demonstrated that the agent can be administered safely for a prolonged period of time at doses that inhibit HDAC activity [9]. Major adverse events for vorinostat administered orally or intravenously were fatigue, diarrhea, anorexia, dehydration, myelosuppression and thrombocytopenia [10].

The antitumor activity of vorinostat was studied in the PPTP *in vitro* and *in vivo* panels, to determine if this agent had significant activity against pediatric leukemias or solid tumors. Vorinostat achieved cell growth inhibition in all tested cell lines *in vitro;* IC_{50} values ranged from 0.48 to 9.8 μM. This range is in agreement with growth inhibitory effect of vorinostat demonstrated in other *in vitro* models [2–4]. However, drug concentrations that resulted in 1 log inhibition (IC_{90}) were generally beyond the clinically achievable levels (1–2 μM).

For *in vivo* testing, vorinostat was administered at the dose and schedule that has previously been shown to induce acetylation of histones H3 and H4, pharmacodynamic markers of HDAC inhibition [2,4]. Vorinostat induced differences in EFS distribution in solid tumor xenografts, but no objective responses were observed in either the ALL or the solid tumor panels. Thus, vorinostat as a single agent did not show significant anti-tumor activity in either the *in vitro* or *in vivo* panels.

In spite of the lack of activity by single agent vorinostat in the PPTP models, it does remain an anti-tumor agent of interest for use in drug combinations. For example, HDAC1 overexpression has recently been shown to be one mechanism of multi-drug resistance in neuroblastoma cell lines that can be reversed with HDAC inhibition[14]. Combinations of HDAC inhibitors with retinoids are also of interest in the pediatric setting based on previously published work for the activity of retinoids as single agents against certain diagnoses (e.g., for neuroblastoma and medulloblastoma) and based on the activity described for combinations of retinoids and HDAC inhibitors [15–20]. Thus, while

vorinostat did not show promising single agent activity in PPTP testing, preclinical studies with appropriate drug combinations may provide direction for further clinical evaluations of vorinostat against selected pediatric cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NO1-CM-42216, CA21765, and CA108786 from the National Cancer Institute and used vorinostat provided by Merck. In addition to the authors, the report represents work contributed by the following: Sherry Ansher, Catherine A. Billups, Joshua Courtright, Edward Favours, Henry S. Friedman, Debbie Payne-Turner, Charles Stopford, Mayamin Tajbakhsh, Chandra Tucker, Joe Zeidner, Ellen Zhang, and Jian Zhang. Children's Cancer Institute Australia for Medical Research is affiliated with the University of New South Wales and Sydney Children's Hospital.

Reference List

1. Glaser KB. HDAC inhibitors: Clinical update and mechanism-based potential. Biochem Pharmacol. 2007
2. Richon VM. Cancer biology: mechanism of antitumour action of vorinostat (suberoylanilide hydroxamic acid), a novel histone deacetylase inhibitor. British journal of cancer. 2006; 95:5.
3. Richon VM, Sandhoff TW, Rifkind RA, et al. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97(18):10014–10019. [PubMed: 10954755]
4. Piekarz RL, Robey R, Sandor V, et al. Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. Blood. 2001; 98(9): 2865–2868. [PubMed: 11675364]
5. Prince HM, George D, Patnaik A, et al. Phase I study of oral LBH589, a novel deacetylase (DAC) inhibitor in advanced solid tumors and non-Hodgkin's lymphoma. J Clin Oncol. 2007; 25(18S) Abstr #3500.
6. Duvic M, Talpur R, Ni X, et al. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood. 2007; 109(1):31–39. [PubMed: 16960145]
7. Duvic M, Vu J. Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma. Expert opinion on investigational drugs. 2007; 16(7):1111–1120. [PubMed: 17594194]
8. Olsen EA, Kim YH, Kuzel TM, et al. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. J Clin Oncol. 2007; 25(21):3109–3115. [PubMed: 17577020]
9. Mann BS, Johnson JR, He K, et al. Vorinostat for treatment of cutaneous manifestations of advanced primary cutaneous T-cell lymphoma. Clin Cancer Res. 2007; 13(8):2318–2322. [PubMed: 17438089]
10. 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(17): 3923–3931. [PubMed: 15897550]
11. Kelly WK, Richon VM, O'Connor O, et al. Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously. Clin Cancer Res. 2003; 9(10 Pt 1): 3578–3588. [PubMed: 14506144]
12. Houghton PJ, Morton CL, Kolb EA, et al. Initial testing (stage 1) of the proteasome inhibitor bortezomib by the pediatric preclinical testing program. Pediatr Blood Cancer. 2007
13. Houghton PJ, Morton CL, Tucker C, et al. The pediatric preclinical testing program: Description of models and early testing results. Pediatr Blood Cancer. 2006

14. Keshelava N, Davicioni E, Wan Z, et al. Histone deacetylase 1 gene expression and sensitization of multidrug-resistant neuroblastoma cell lines to cytotoxic agents by depsipeptide. Journal of the National Cancer Institute. 2007; 99(14):1107–1119. [PubMed: 17623797]

15. Thiele CJ, Reynolds CP, Israel MA. Decreased expression of N-myc precedes retinoic acid-induced morphological differentiation of human neuroblastoma. Nature. 1985; 313(6001):404–406. [PubMed: 3855502]

16. Reynolds CP, Matthay KK, Villablanca JG, et al. Retinoid therapy of high-risk neuroblastoma. Cancer letters. 2003; 197(1–2):185–192. [PubMed: 12880980]

17. Coffey DC, Kutko MC, Glick RD, et al. Histone deacetylase inhibitors and retinoic acids inhibit growth of human neuroblastoma in vitro. Medical and pediatric oncology. 2000; 35(6):577–581. [PubMed: 11107121]

18. Kutko MC, Glick RD, Butler LM, et al. Histone deacetylase inhibitors induce growth suppression and cell death in human rhabdomyosarcoma in vitro. Clin Cancer Res. 2003; 9(15):5749–5755. [PubMed: 14654560]

19. Hallahan AR, Pritchard JI, Chandraratna RA, et al. BMP-2 mediates retinoid-induced apoptosis in medulloblastoma cells through a paracrine effect. Nat Med. 2003; 9(8):1033–1038. [PubMed: 12872164]

20. Gumireddy K, Sutton LN, Phillips PC, et al. All-trans-retinoic acid-induced apoptosis in human medulloblastoma: activation of caspase-3/poly(ADP-ribose) polymerase 1 pathway. Clin Cancer Res. 2003; 9(11):4052–4059. [PubMed: 14519626]

Table I

Activity of Vorinostat against Cell Lines in the PPTP *in Vitro* Panel

Cell Line	Histology	IC_{50} (μM)	Median IC_{50} Ratio	IC_{90} (μM)
RD	Rhabdomyosarcoma	6.06	0.24	51.75
Rh41	Rhabdomyosarcoma	0.88	1.63	2.81
Rh18	Rhabdomyosarcoma	9.77	0.15	21.60
Rh30	Rhabdomyosarcoma	1.72	0.83	4.36
BT-12	Rhabdoid	7.70	0.19	20.33
CHLA-266	Rhabdoid	2.49	0.58	15.45
TC-71	Ewing sarcoma	1.28	1.13	2.64
CHLA-9	Ewing sarcoma	1.30	1.11	4.50
CHLA-10	Ewing sarcoma	2.48	0.58	8.75
CHLA-258	Ewing sarcoma	0.61	2.37	12.22
GBM2	Glioblastoma	1.59	0.90	9.86
NB-1643	Neuroblastoma	1.44	1.00	61.32
NB-EBc1	Neuroblastoma	2.50	0.57	4.69
CHLA-90	Neuroblastoma	2.81	0.51	20.90
CHLA-136	Neuroblastoma	2.40	0.60	25.79
NALM-6	ALL	1.78	0.81	4.97
COG-LL-317	ALL	0.55	2.62	1.84
RS4;11	ALL	0.62	2.33	5.16
MOLT-4	ALL	0.68	2.11	2.42
CCRF-CEM	ALL	1.30	1.11	4.75
Kasumi-1	AML	0.80	1.79	3.67
Karpas-299	ALCL	0.48	2.97	2.12
Ramos-RA1	NHL	1.16	1.24	2.71
Median		**1.44**	**1.00**	**2.46**
Minimum		**0.48**	**0.15**	**1.84**
Maximum		**9.77**	**2.97**	**61.32**

Table II

Activity for Vorinostat against the PPTP *in Vivo* Panel

Xenograft Line	Histology	KM Estimate of Median Time to Event	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	Overall Group Response	T/C Volume Activity	EFS Activity	Response Activity
KT-10	Wilms	12.0	0.032	1.3	>4	0.67	0.15	PD1	Low	Low	Low
KT-11	Wilms	17.4	0.004	1.9	>4	0.34	<0.001	PD2	Int	Low	Int
KT-13	Wilms	12.8	0.197	1.3	>4	0.71	0.05	PD1	Low	Low	Low
SK-NEP-1	Ewing	11.6	0.034	1.7	>4	0.61	0.03	PD2	Low	Low	Int
EW5	Ewing	13.8	0.044	1.1	>4	1.02	0.50	PD1	Low	Low	Low
EW8	Ewing	15.3	<0.001	1.6	>4	0.57	0.00	PD2	Low	Low	Int
TC-71	Ewing	10.4	0.143	1.8	>4	0.48	0.04	PD2	Low	Low	Int
CHLA258	Ewing	10.4	0.349	1.2	>4	0.90	0.37	PD1	Low	Low	Low
Rh30	ALV RMS	20.3	0.611	1.0	>4	0.84	0.97	PD1	Low	Low	Low
Rh30R	ALV RMS	20.9	0.012	1.3	>4	0.63	0.04	PD1	Low	Low	Low
Rh41	ALV RMS	19.1	<0.001	1.6	>4	0.56	<0.001	PD2	Low	Low	Int
Rh18	EMB RMS	19.8	0.063	1.5	>4	0.57	0.01	PD1	Low	Low	Low
Rh36	EMB RMS	24.4	0.005	1.4	>4	0.89	0.36	PD1	Low	Low	Low
BT-28	Medulloblastoma	5.7	0.052	1.2	>4	0.82	0.08	PD1	Low	Low	Low
BT-45	Medulloblastoma	16.6	0.026	1.3	>4	0.84	0.10	PD1	Low	Low	Low
BT-46	Medulloblastoma	7.3	0.625	1.2	>4	0.91	0.58	PD1	Low	Low	Low
BT-44	Ependymoma	26.1	0.357	0.7	>4	1.61	0.02	PD1	Low	Low	Low
BT-39	Glioblastoma	10.0	0.918	1.0	>4	1.04	0.74	PD1	Low	Low	Low
D645	Glioblastoma	8.6	0.891	1.0	>4	1.03	0.53	PD1	Low	Low	Low
D456	Glioblastoma	6.0	0.872	1.1	>4	0.90	0.66	PD1	Low	Low	Low
NB-SD	Neuroblastoma	> EP	0.225	> 1.5	3.0	1.18	1.00	PD2	Low	NE	Int
NB-1771	Neuroblastoma	35.2	<0.001	1.4	>4	0.31	<0.001	PD1	Int	Low	Low
NB-1691	Neuroblastoma	10.7	0.076	1.1	>4	0.85	0.06	PD1	Low	Low	Low
NB-EBc1	Neuroblastoma	10.4	0.012	1.6	>4	0.69	0.02	PD2	Low	Low	Int
CHLA-79	Neuroblastoma	27.5	0.041	1.1	>4	0.78	0.48	PD1	Low	Low	Low
NB-1643	Neuroblastoma	12.9	0.021	1.3	>4	0.48	0.00	PD1	Low	Low	Low

Xenograft Line	Histology	KM Estimate of Median Time to Event	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	Overall Group Response	T/C Volume Activity	EFS Activity	Response Activity
OS-1	Osteosarcoma	34.5	0.062	1.1	>4	0.77	0.10	PD1	Low	Low	Low
OS-17	Osteosarcoma	24.2	0.004	1.8	>4	0.49	<0.001	PD2	Low	Low	Int
OS-9	Osteosarcoma	> EP	0.002	> 1.6	3.2	0.67	0.03	PD2	Low	NE	Int
OS-31	Osteosarcoma	21.1	0.003	1.3	>4	0.58	0.01	PD1	Low	Low	Low
ALL-2	ALL B-precursor	9.8	0.269	0.8	>25	.		PD1		Low	Low
ALL-3	ALL B-precursor	8.2	0.396	0.8	>25	.		PD1		Low	Low
ALL-4	ALL B-precursor	2.3	0.731	1.3	>25	.		PD1		Low	Low
ALL-7	ALL B-precursor	2.0	1.000	1.0	>25	.		PD1		Low	Low
ALL-8	ALL T-cell	6.7	0.519	1.1	>25	.		PD1		Low	Low
ALL-16	ALL T-cell	15.8	0.072	1.4	>25	.		PD1		Low	Low
ALL-17	ALL B-precursor	2.0	1.000	1.0	>25	.		PD1		Low	Low
ALL-19	All B-precursor	1.0	0.674	1.0	>25	.		PD1		Low	Low