Abstract C226

Initial Testing of the Histone Deacetylase Inhibitor Vorinostat by the Pediatric Preclinical Testing Program

Peter J. Houghton[1], John M. Maris[2], Joshua Courtright[2], Henry S. Friedman[3], Stephen T. Keir[3], Richard B. Lock[4], Hernan Carol[4],Richard Gorlick[5], E. Anders Kolb[6], Nino Keshelava[7], C. Patrick Reynolds[7], Christopher Morton[1], Malcolm A. Smith[8].
[1]St. Jude Children's Research Hospital, [2]Children's Hospital of Philadelphia, [3]Duke University, [4]Children's Cancer Inst., Australia, [5]Albert Einstein College of Medicine, [6]A.I. duPont Hospital for Children, [7]Children's Hospital of Los Angeles, [8]CTEP/NCI.

Abstract

Background: Vorinostat is a small molecule inhibitor of histone deacetylase (HDAC), which like other HDAC inhibitors induces hyperacetylation of a number of proteins, resulting in a multitude of downstream effects. Increased levels of acetylated histones are associated with activation of expression of selected genes as well as with impaired mitotic progression. HDAC inhibitors also induce increased acetylation of non-histone proteins, including numerous transcription factors, Hsp90, Ku70, and α-tubulin. Vorinostat has been approved by the U.S. Food and Drug Administration for the treatment of cutaneous manifestations in patients with CTCL who have progressive, persistent or recurrent disease on or following two systemic therapies.
Methods: The PPTP includes a molecularly characterized *in vitro* panel of cell lines (n=27) and *in vivo* panel of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood ALL. Vorinostat *in vitro* testing used media containing 20% FCS and evaluated concentrations from 10 nM to 100 μM. Vorinostat was tested against the xenografts of the PPTP *in vivo* panels using intraperitoneal administration for six weeks (5-days on, 2-days off) at a dose of 125 mg/kg. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).
Results: Vorinostat was uniformly active against the PPTP *in vitro* panel with a median IC50 of 1.44 μM (range, 0.45 μM to 8.5 μM). Toxicity rates for treated and control animals were 12.7% and 1.7%, respectively, with 7 of 43 xenografts excluded from analysis for excessive toxicity. Vorinostat induced significant differences in EFS distribution compared to controls in 16 of 30 evaluable solid tumor xenografts, but in none of 6 evaluable acute lymphoblastic leukemia (ALL) xenografts. Although there were significant differences in EFS distribution in many of the PPTP solid tumor xenografts, no xenograft met the criteria for intermediate activity (EFS T/C > 2) for the time to event activity measure. No objective responses were observed in any of the solid tumor *in vivo* panels or in the ALL panel. The best response observed was PD2 (progressive disease with growth delay), which was noted in 9 of 30 evaluable solid tumor xenografts.
Conclusions: Vorinostat has little activity as a single agent against the xenografts of the PPTP's *in vivo* panel. These results do not exclude potential activity for vorinostat against a biological subtype of a pediatric cancer that is not represented within the PPTP panel. Given the limited *in vivo* activity observed for vorinostat, further preclinical work will focus on identifying vorinostat combinations with increased potential for having a favorable therapeutic index when translated into the pediatric clinical setting. Combinations of vorinostat with agents that modify transcriptional activity (e.g., retinoids and demethylating agents) are of particular interest for evaluation in pediatric preclinical models. (Supported by NCI NO1CM42216)

PPTP *In Vitro* Testing Methods

Methods: ***In vitro* testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates (Keshelava, et al. Methods Mol.Med., *110*: 139-153, 2005). Testing was for 96 hours at concentrations from 10 nM to 100 μM with replicates of 6 per data point. Data were analyzed using Kaleidagraph (Synergy), fitting a non-linear regression, sigmoidal dose-response model to the response, relative fluorescence values *vs.* the concentration. The PPTP in vitro panel contains cell lines for neuroblastoma (4), Ewing sarcoma (4), rhabdomyosarcoma (4), ALL (5), NHL (2), and others.**

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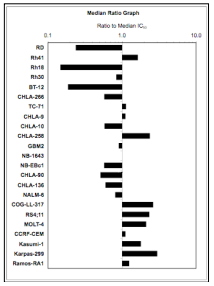
Vorinostat *In Vitro* Activity

- **The median IC_{50} for the panel was 1.44 μM with the maximal inhibition of growth approaching 100% for all lines tested.**
- **The Median IC_{50} Ratio Graph (below) illustrates the relative sensitivity of the PPTP cell lines by plotting the ratio of the panel's median IC_{50} to the IC_{50} for individual cell lines. Lines to the right indicate greater sensitivity.**
- **There was a tendency for lower IC_{50} values for the ALL panel (median = 0.7 μM).**
- **Median IC_{50} values for the rhabdomyosarcoma (3.9 μM) and neuroblastoma (2.5 μM) panels were higher than the panel's median IC_{50} value.**

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



Methods for PPTP *In Vivo* Testing

Stage 1 testing involves testing an agent across the entire PPTP panel of childhood cancer xenograft lines at its MTD or at a dose selected based on PK/PD studies using adult preclinical models.

Solid tumor testing: For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm^3. Two perpendicular tumor diameters were measured at once weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula (π/6)×d3, where d represents the mean diameter.

Acute lymphoblastic leukemia testing: For each xenograft line, 8 mice were inoculated with 3-5 x 10^6 mononuclear cells purified from the spleens of secondary recipient mice. Engraftment was monitored weekly by flow cytometry, and treatment was initiated when the proportion of human CD45+ cells in the peripheral blood reached 1%. The proportion of human CD45+ cells in the peripheral blood was monitored weekly throughout the course of treatment.

Drug: Vorinostat was dissolved in DMSO (final concentration 10%) and diluted in PEG400 (final concentration 45%) in water and administered intraperitoneally daily x 5 for 6 weeks at a dose of 125 mg/kg. Vorinostat was provided to each testing site in coded vials for blinded testing according the PPTP's standard operating procedures. Vorinostat was provided by Merck through the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Solid Tumor Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	>25% ↑ in tumor volume, TGD value ≤1.5	0
PD2 (Progressive Disease 2)	>25% ↑ in tumor volume, TGD value >1.5	2
SD (Stable Disease)	<25% ↑ in tumor volume, <50% regression	4
PR (Partial Response)	≥50% regression, but no CR	6
CR (Complete Response)	<0.1 cm3 tumor volume	8
MCR (Maintained CR)	<0.1 cm3 tumor volume at the end of study	10

Leukemia Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	No PR & TGD value of ≤1.5 & events at EOS	0
PD2 (Progressive Disease 2)	No PR & TGD value >1.5 & events at EOS	2
SD (Stable Disease)	No PR and no events at EOS	4
PR (Partial Response)	CD45% <1% for only 1 week	6
CR (Complete Response)	CD45% <1% for 2 consecutive weeks	8
MCR (Maintained CR)	CD45% <1% for last 3 weeks of study	10

Median Group Response: Each individual mouse in the treatment group was assigned a response score (see Tables above) and a median score for the treatment group was calculated and then each treatment group was assigned an overall response according to the table below.

If Median Score (MS) from (1):	Overall Group Response
0 ≤ MS ≤1	PD1
1 < MS ≤3	PD2
3 < MS ≤5	SD
5 < MS ≤7	PR
7 < MS ≤9	CR
9 < MS	MCR

Statistical Methods: Event-free survival (EFS) distributions of each treatment group were compared to the EFS distribution of the respective control group using the exact log rank test. P-values were 2-sided & were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant.

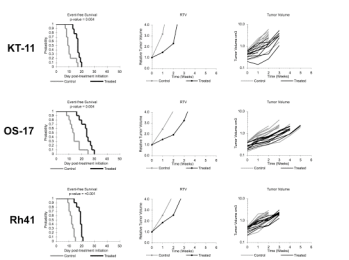
Vorinostat *In Vivo* Activity

- **Vorinostat induced significant differences in EFS distribution compared to controls in 16 of 30 evaluable solid tumor xenografts, but in none of 6 evaluable ALL xenografts.**
- **Although there were significant differences in EFS distribution in many of the PPTP solid tumor xenografts, the EFS T/C values were below the criteria for intermediate activity for the time to event measure of activity in all evaluable lines (EFS T/C > 2).**
- **No objective responses were observed in any of the solid tumor panels or in the ALL panel. Progressive disease with growth delay (PD2) was observed in 9 of 30 evaluable solid tumor xenografts and in 0 of 6 ALL xenografts. The PD2 responses were concentrated in the Ewing sarcoma (3 of 5 xenografts), neuroblastoma (2 of 6 xenografts), and osteosarcomaa panels (2 of 4 xenografts).**

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	Overall Group Response
KT-10	Wilms	0.032	1.3	>4	0.67	0.146	PD1
KT-11	Wilms	0.004	1.9	>4	0.34	<0.001	PD2
KT-13	Wilms	0.197	1.3	>4	0.71	0.054	PD1
SK-NEP-1	Ewing	0.036	1.7	>4	0.61	0.029	PD2
EW5	Ewing	0.044	1.1	>4	1.02	0.497	PD1
EW8	Ewing	<0.001	1.6	>4	0.57	0.004	PD2
TC-71	Ewing	0.143	1.8	>4	0.48	0.002	PD2
CHLA258	Ewing	0.349	1.2	>4	0.90	0.371	PD1
Rh30	ALV Rhabdomyosarcoma	0.611	1.0	>4	0.84	0.968	PD1
Rh30R	ALV Rhabdomyosarcoma	0.012	1.3	>4	0.63	0.043	PD1
Rh41	ALV Rhabdomyosarcoma	<0.001	1.6	>4	0.56	<0.001	PD2
Rh18	EMB Rhabdomyosarcoma	0.063	1.5	>4	0.57	0.010	PD1
Rh36	EMB Rhabdomyosarcoma	0.005	1.4	>4	0.89	0.356	PD1
BT-28	Medulloblastoma	0.052	1.2	>4	0.82	0.083	PD1
BT-45	Medulloblastoma	0.026	1.3	>4	0.84	0.101	PD1
BT-46	Medulloblastoma	0.625	1.2	>4	0.91	0.579	PD1
BT-44	Ependymoma	0.357	0.7	>4	1.61	0.015	PD1
BT-39	Glioblastoma	0.918	1.0	>4	1.04	0.739	PD1
D645	Glioblastoma	0.891	1.0	>4	1.03	0.529	PD1
D456	Glioblastoma	0.872	1.1	>4	0.90	0.661	PD1
NB-SD	Neuroblastoma	0.225	> 1.5	3.0	1.18	1.000	PD2
NB-1771	Neuroblastoma	0.006	1.4	>4	0.31	<0.001	PD1
NB-1691	Neuroblastoma	0.076	1.1	>4	0.85	0.063	PD1
NB-EBc1	Neuroblastoma	0.012	1.6	>4	0.69	0.019	PD2
CHLA-79	Neuroblastoma	0.041	1.1	>4	0.78	0.481	PD1
NB-1643	Neuroblastoma	0.021	1.3	>4	0.48	0.002	PD1
OS-1	Osteosarcoma	0.062	1.1	>4	0.77	0.095	PD1
OS-17	Osteosarcoma	0.004	1.8	>4	0.49	<0.001	PD2
OS-9	Osteosarcoma	0.002	> 1.6	3.2	0.67	0.026	PD2
OS-33	Osteosarcoma	0.003	1.3	>4	0.58	0.006	PD2
ALL-3	ALL B-precursor	0.396	0.8	>25	.	.	PD1
ALL-4	ALL B-precursor	0.731	1.3	>25	.	.	PD1
ALL-7	ALL B-precursor	1.000	1.0	>25	.	.	PD1
ALL-8	ALL T-cell	0.519	1.1	>25	.	.	PD1
ALL-16	ALL T-cell	0.072	1.4	>25	.	.	PD1
ALL-17	ALL B-precursor	1.000	1.0	>25	.	.	PD1

- Tumor Volume T/C: Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days).
- Red shading in the p-value columns indicates a statistically significant difference between treated and control groups.
- Shading in the EFS columns indicates xenografts that have either high (dark blue), intermediate (light blue), or indeterminate (gray) activity.

Vorinostat *In Vivo* Activity



CONCLUSIONS

- **Vorinostat was active against each of the cell lines of the PPTP *in vitro* panel. The concentrations required for half-maximal inhibition (median IC_{50} 1.44 mM) approximate the peak concentrations achieved with vorinostat at its approved 400 mg dose in adults, though these concentrations are maintained for a relatively short period.**
- **Vorinostat was tested at its MTD against the PPTP's solid tumor xenografts. Previous preclinical studies have documented increases in acetylated histones in tumor tissue following vorinostat treatment at doses lower than those used for PPTP in vivo testing.**
- **The lack of vorinostat activity against the ALL panel was disappointing given the *in vitro* sensitivity of the ALL cell lines and given preclinical and clinical activity for vorinostat against adult lymphoid malignancies. Preclinically, vorinostat is active against Eu-myc lymphomas and induces apoptosis in cutaneous T-cell lymphoma cell lines. Clinically, vorinostat induces objective responses in approximately 30% of patients with advanced cutaneous T-cell lymphoma.**
- **Given the limited single agent *in vivo* activity observed for vorinostat, further preclinical work is needed to identify vorinostat combinations with increased potential for having a favorable therapeutic index when translated into the pediatric clinical setting.**
- **The combination of vorinostat with demethylating agents such as azacitidine and decitabine is of interest because of the potential for this combination to maximally reverse the epigenetic changes associated with gene silencing. The combination of HDAC inhibition and retinoid therapy is of interest in the pediatric setting, particularly for neuroblastoma and medulloblastoma. Combination testing is planned as part of the PPTP Stage 2 testing plan for vorinostat.**

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