Clinical and Biological Effects of Valproic Acid as a Histone Deacetylase Inhibitor on Tumor and Surrogate Tissues: Phase I/II Trial of Valproic acid and Epirubicin/FEC

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Abstract

Purpose: The aim was to study the biological and molecular effects of the histone deacetylase (HDAC) inhibitor, valproic acid, in patients with solid tumor malignancies.

Experimental Design: A phase I dose escalation of valproic acid given on days 1 to 3 followed by epirubicin (day 3) was followed by a dose expansion of valproic acid combined with 5-fluorouracil, epirubicin, and cyclophosphamide (FEC100). Pharmacodynamic and pharmacokinetic studies entailed valproic acid and epirubicin plasma levels and their interaction, the effects of valproic acid on histone acetylation in peripheral blood mononuclear cells (PBMC) and tumor cells at baseline and day 3, and baseline expression of HDAC2 and HDAC6 as therapeutic targets.

Results: Forty-four patients were enrolled in the phase I part, with a disease-specific cohort expansion of 15 breast cancer patients (median age, 55 years; range, 28-66 years) receiving 120 mg/kg/day valproic acid followed by FEC100. Partial responses were seen in 9 of 41 (22%) patients during the phase I part. Objective responses were seen in 9 of 14 (64%) evaluable patients at the dose expansion with a median number of 6 administered cycles. Predominant toxicities were valproic acid–associated somnolence and epirubicin-induced myelosuppression. Valproic acid plasma levels were associated with short-term, reversible depletion of WBC and neutrophils within 48 hours. Histone acetylation in tumor samples and in PBMCs correlated with valproic acid levels and was further linked to baseline HDAC2 but not to HDAC6 expression.

Conclusion: Valproic acid is a clinically relevant HDAC inhibitor, and PBMCs may serve as a surrogate for tumor histone acetylation in solid tumor malignancies. HDAC2 should be further considered as a relevant therapeutic target.

Histone acetylases and deacetylases (HDAC) play an important role in the epigenetic control of tumor cells and are involved in the regulation of cell growth, differentiation, and oncogenesis (1). Several HDAC inhibitors are currently undergoing clinical testing. Although there is activity in hematologic malignancies with several HDAC inhibitors and the first agent in its class has recently been approved for the treatment of cutaneous T-cell lymphoma (2–13), there is considerably less efficacy seen with the current HDAC inhibitors in solid tumor malignancies (14–17). However, the global effects induced by these drugs may be more effectively exploited when used in combination with either hormonal or chemotherapies (18–28). To date there are only limited data available on pharmacologic markers, and no biomarkers have been established. The evaluation of histone hyperacetylation in peripheral blood mononuclear cells (PBMC) has been shown to correlate with HDAC inhibitor drug concentrations in some but not all clinical trials. Few data are available, however, on whether HDAC inhibitor plasma concentrations further correlate with histone acetylation in tumors, particularly in solid tumors (10, 29, 30).

The anticonvulsant, valproic acid, has HDAC inhibitory activity (26, 31–33). In cell culture models, exposure to valproic acid results in dose-dependent, reversible cell cycle arrest and chromatin decondensation and cellular differentiation (22, 34–36). Several reports have suggested that HDAC inhibitors can synergize with cytotoxic or biological anticancer agents (19–23, 25). A sequence-specific administration of a HDAC inhibitor followed by a topoisomerase II inhibitor resulted in synergistic cytotoxic effects (21). Mechanistic studies suggested that HDAC inhibitor–induced chromatin decondensation facilitated binding of topoisomerase II inhibitors to the DNA substrate and consequently increased DNA strand breaks by recruitment of topoisomerase IIβ (37). In xenograft models,

Translational Relevance

Histone deacetylase (HDAC) inhibitors are a novel class of anticancer agents. Preclinical data suggest that HDAC inhibitors sensitize cancer cells to DNA targeting agents. The current study summarizes the pharmacokinetic and pharmacodynamic effects of the HDAC inhibitor, valproic acid, given in combination with epirubicin, and their effects on tumor and surrogate tissues.

This proof-of-principle study suggests that a combination of an HDAC inhibitor and an anthracycline is safe, tolerable, and feasible. The noteworthy antitumor efficacy seen in anthracycline-sensitive as well as in anthracycline-resistant tumors suggests that this combination may have broad application. Further studies with valproic acid or more potent HDAC inhibitors may entail a promising strategy to potentiate DNA-damaging agents. Detailed analysis of tumor and surrogate tissues further indicate that HDAC2 may be the relevant therapeutic target in this interaction; hence, HDAC2 may deserve further consideration as a biomarker or selective target.

valproic acid potentiated epirubicin-induced cell death without exacerbating toxicity (38). An earlier report summarized the safety, toxicity, and feasibility of a phase I trial involving valproic acid followed by the anthracycline, epirubicin. Given the efficacy in breast cancer and preclinical data suggesting a potential role of HDAC2 as an important determinant of histone deacetylation, the cohort at the maximally tolerated dose for the combination was expanded for 15 patients with locally advanced or metastatic breast cancer with more extended correlative studies in PBMCs and tumor cells obtained before and after valproic acid administration.

Here, we describe the biological effects of valproic acid on histone acetylation and the relevance of HDAC2 in PBMCs and tumors cells in a clinical trial evaluating HDAC inhibitors to potentiate the effects of epirubicin.

Patients and Methods

Eligibility. Patients with advanced solid tumor malignancies with Eastern Cooperative Oncology Group performance status of 0 to 2 and adequate organ function (hemoglobin >9.0 g/dL, absolute neutrophil count >1,500 cells/mm^3, platelets >100,000 cells/mm^3, normal creatinine and bilirubin levels, and liver enzymes within 1.5× the upper level of the institutional upper normal range) were eligible. Patients with an ejection fraction of <50%, or long QT-syndrome, ventricular tachycardia, or fibrillation were excluded. Prior anthracycline use was allowed if <300 mg/m^2 doxorubicin or equivalent. For the dose expansion portion of the trial, 15 patients were required to have measurable locally advanced (IIIC) or metastatic (IV) breast cancer. Patients were allowed any number of prior therapies including anthracyclines (<300 mg/m^2 doxorubicin or doxorubicin equivalent, e.g. 1.8× for epirubicin). The patient had to be willing to undergo a pre- and post–valproic acid tumor biopsy. The study opened on April 5, 2004, and the last patient was enrolled on May 7, 2007, with the last patient remaining on study until September 14, 2007. Informed consent was obtained from patients in accordance with good clinical practice and federal and institutional guidelines governing registered clinical trials (ClinicalTrials.gov number NCT00246103).

Study treatment. A loading dose of valproic acid (15, 30, 45, 60, 75 90, 100, 120, 140, 160 mg/kg/d) was followed by 5 oral doses in 2 divided doses (7.5, 15, 22.5, 30, 37.5, 45, 50, 60, 70, 80 mg/kg; Depakote, Abbott Laboratories) given every 12 h starting 4 h after the loading dose. Epirubicin (Ellence; Pfizer) was given i.v. on day 3, 4 h after the last valproic acid dose. The dose expansion phase was limited to patients with breast cancer, and the drugs 5-fluorouracil and cyclophosphamide were added to accommodate a more typical breast cancer regimen (FEC100). A -1 dose step down from the maximum tolerated dose was built into the protocol to avoid excessive toxicity upon addition of 5-fluorouracil and cyclophosphamide. Patients in the dose expansion group were treated with a 120 mg/kg/d valproic acid loading dose followed by 60 mg/kg given every 12 h for 5 doses followed by 100 mg/m^2 epirubicin, 500 mg/m^2 cyclophosphamide, and 500 mg/m^2 5-fluorouracil (FEC100) repeated every 3 wk with a predetermined maximum of seven cycles for anthracycline-naïve patients and four cycles for those with prior adjuvant doxorubicin (if <300 mg/m^2) treatment.

Treatment assessment. Baseline safety and toxicity evaluations included a history, physical examination, and complete blood counts with differential, metabolic, hepatic, and renal function assessment at baseline and on days 3, 10, 17, and 21, and then every 3 wk. Other laboratory tests, including pancreatic enzymes or platelet functions, were evaluated as clinically indicated. Disease restaging was done every two cycles (6 wk). Valproic acid effects on blood cells were assessed as a fraction of baseline.

Pharmacokinetics. A 4-h postloading dose and day 3 pre-epirubicin valproic acid samples were obtained in cycles 1 and 4. Blood samples (5 mL) were collected in heparinized tubes, processed within 30 min after collection, and stored at -20°C. Total and free valproic acid levels were measured by commercially available tests (Nichols Institute). Plasma samples were assayed similarly to those previously described to determine epirubicin peak concentrations (39). Plasma samples (0.2 mL) were extracted using 96-well solid phase extraction (C18), reconstituted in mobile phase (0.1% acetic acid-methanol), and separated on a Zorbax SB-C18 column. Epirubicin was then detected by mass spectrometry via selected ion monitoring at m/z 544. Blood samples were drawn at 0, 24, and 48 h after epirubicin infusion and at day 10 of cycle 1.

Histone acetylation and HDAC expression. PBMCs were isolated by Ficoll centrifugation (Ficoll-Paque; GE HealthCare) and then adhered to glass slides using CytoSpin (Shandon) funnels. PBMCs and tumor cells obtained by fine-needle aspiration were fixed with acetic acid-ethyl alcohol (5-95%) for 1 min. Slides were dual stained with antiacetylated histone H3 or H4 (Upstate Biotechnology; polyclonal, 1:200, #06-599 or #06-946) and anti-lamin (BD Biosciences; monoclonal, 1:200), as well as HDAC2 (Upstate Biotechnology) and HDAC6 (Abcam) for 1 h, and developed with antirabbit Alexa-Fluor 546 and antimouse Alexa-Fluor 488 (Molecular Probes) and bisbenzimide (0.5 mg/mL) for 1 h. Images were acquired by confocal microscopy and analyzed as described previously (37, 38). Histone acetylation and HDAC2 and HDAC6 expression were evaluated by Western blot analysis as described previously (40). Each lane contained 25 μg lysate obtained from baseline PBMC and/or tumor cells with an internal control of 25 μg lysate from MCF-7 cells derived from the same source for all Western blots. HDAC2 and HDAC6 expression in PBMC and MCF-7 samples were normalized to lamin (Millipore) expression and depicted relative to HDAC2 and HDAC6 expression in the control MCF-7 cells (set at a relative level of 1000). Histone acetylation was plotted against HDAC2 and HDAC6 expression.

Statistical analyses. Descriptive statistics were used to summarize patient results. Toxicity was graded by CTCAE version 3.0, and objective tumor response was defined by Response Evaluation Criteria in Solid Tumors (RECIST) guidelines as described by Therasse et al. (41). Responses were reviewed by an independent study radiologist. All patients at the dose expansion had to have measurable disease by RECIST criteria.

Objective response rate and its 95% confidence interval were estimated based on exact binomial distributions. The pharmacodynamic

effects of valproic acid as a function of valproic acid plasma levels were analyzed using descriptive statistics, including graphical illustrations. Spearman correlation coefficient methods were used to estimate correlation between two variables and to carry out the test of significance of the estimated correlation. No formal comparisons and multiple comparison adjustments were attempted due to the nature of phase I study.

Results

Patient characteristics, dose delivery, and toxicities

The phase I part of the study enrolled 44 patients with solid tumors who received at least one cycle of therapy (41 evaluable for response); the phase II dose expansion enrolled a planned 15 patients with locally advanced (IIIC) or metastatic (IV) breast cancer (14 evaluable for response). One patient received unplanned radiation therapy of the spine for pain control prior to restaging and was withdrawn from assessment; two patients withdrew consent from study after one cycle due to personal reasons in the absence of clinical progression. The inevaluable patient at the dose expansion had a nonstudy related infection on day 3 of cycle 1 and did not receive the chemotherapy regimen; she subsequently withdrew consent. Table 1A and B summarize patient demographics and tumor characteristics. The phase I safety, adverse events, and dose-limiting toxicity

Table 1.

A. Demographics and Treatment and Responses

|	Dose escalation	Dose expansion
No. of patients	44	15
Evaluable for response	41	14
Median age in y (range)	54 (21-78)	58 (28-66)
Gender: Female	26 (59%)	15 (100%)
Eastern Cooperative Oncology Group performance status		
0	24 (55%)	1 (7%)
1	20 (45%)	14 (93%)
Histology		
Melanoma	11 (25%)	
Head and neck	4 (9%)	
Small cell lung cancer	4 (9%)	
Sarcoma/Colon/Gastric	6 (14%)	
Prostate	2 (5%)	
Other	7 (16%)	
Breast	10 (23%)	15 (100%)
Number of prior systemic therapies	4 (0-10)	0 (0-5)
Prior exposure to anthracycline	11 (25%)	2 (17%)
Responses (%)	9/41 (22%)	9/14 (64%)
Confidence Intervals	11%-38%	35%-87%
CR/PR:	0/9	1/8
Stable disease ≥12 wk	13 (45%)	3 (15%)
Median number of cycles delivered	4 (1-11)	6 (1-7)*

B. Detailed tumor characteristics treatment and responses for dose expansion

Patient number	Stage	ER status	HER2 status	Prior doxorubicin	No. of cycles delivered	Best Response
1	IV	+	A	No	7	PR
2	IV	+	N A	No	7	SD (NED)
3	IIIC	-	A	No	6	PR
4	IV	+	N A	No	1	NE
5	IV	+	A	No	7	PR
6	IV	+	N A	No	7	PR
7	IV	-	N A	No	7	CR
8	IV	-	N A	Yes	2	POD
9	IIIC	-	A	No	1	PR
10	IV	-	A	Yes	4	PR
11	IV	+	N A	No	6	PR
12	IV	+	N A	No	2	SD
13	IV	-	N A	No	4	SD
14	IV	+	N A	No	7	PR
15	IV	+	N A	Yes	2	POD
All	IV: 13 IIIC: 2	ER+ 9 (60%) ER- 6 (40%)	A: 5 (30%) NA: 14 (70%)	Yes: 3 (20%) No: 12 (80%)		CR: 1 (7%) PR: 8 (57%) SD: 3 (21%) POD: 2 (14%)

Abbreviations: A, amplified; NA, not amplified; ER, estrogen receptor; HER2, human epidermal growth factor receptor; NED, no evidence of disease; SD, stable disease; PR, partial response; POD, progression; CR, complete response rate.
*For a maximum of seven cycles.

Fig. 1. Waterfall plot of best response separated by tumor histology. Responses measured by RECIST criteria depicted in a Waterfall plot and separated by tumor histologies. Bars reaching the shaded areas depict objective responses.
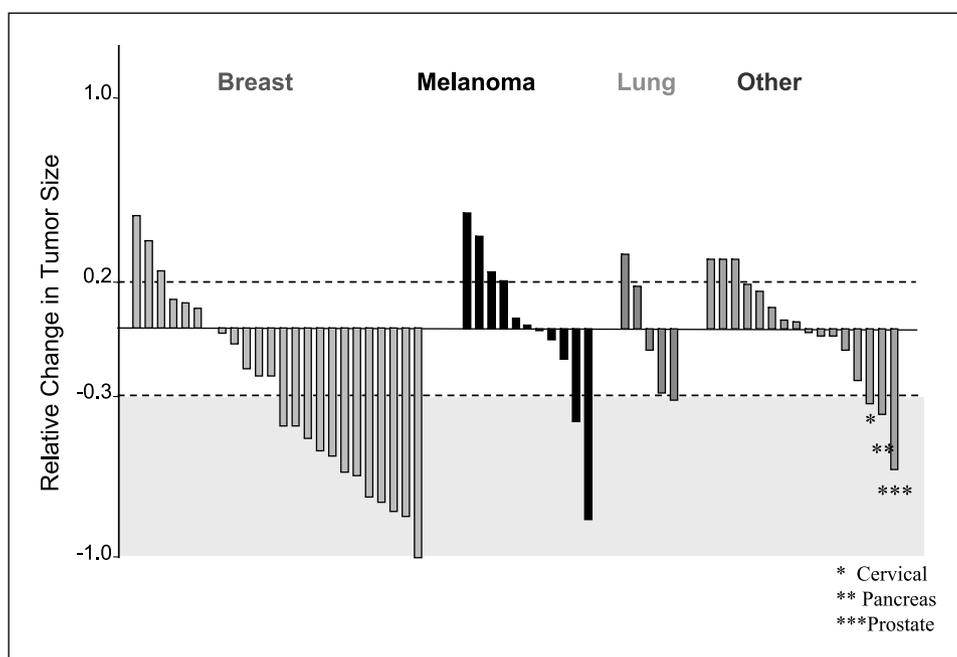
(DLT) data were reported in detail previously (42). The maximally administered valproic acid dose was reached at 160 mg/kg/day in 2 divided doses ×5 followed by epirubicin. DLTs included grade III neurovestibular symptoms (somnolence, confusion, hallucinations, hearing loss, dizziness), as well as diarrhea with electrolyte imbalance and myelosuppression.

The maximally tolerated dose was 140 mg/kg/day for valproic acid and epirubicin. For the dose escalation, valproic acid was lowered to 120 mg/kg/day for a planned six patients prior to escalation to 140 mg/kg/day. Although there were no DLTs in the 120 mg/kg valproic acid dose at the dose expansion, this dose was associated with a 20% grade III somnolence in the post-DLT period. Based on responses seen with lower doses of valproic acid and the pharmacokinetic analysis (see below), patients in the dose expansion group were treated with a 120 mg/kg/day valproic acid loading dose followed by 60 mg/kg given every 12 hours for 5 doses followed by FEC100. Whereas a median of 4 cycles was delivered during the dose escalation part, a median of 6 (range, 1-7) of 7 maximal cycles was delivered at the dose expansion (Table 1A and B). Grade III and IV non–dose-limiting treatment-related toxicities included nausea/vomiting (grade III, 3 of 15 patients; 20%), neurovestibular symptoms (grade III, 2 of 15 patients; 13%), grade III/IV neutropenia during any cycle (12 of 15 patients; 80%), grade III thrombocytopenia (1 of 15 patients; 7%), and febrile neutropenia (3 of 15 patients; 20%) in any cycle (median 6). Unlike results reported with long-term administration of valproic acid, no electrolyte imbalances or liver enzyme function changes (>grade I) were seen. Dose adjustments for valproic acid at the dose expansion were required for the loading dose (120 mg/kg) in the post-DLT period for 3 of 15 patients (20%). Two patients had somnolence and one patient was unable to tolerate the excessive number of tablets. The loading dose of 120 mg/kg/day valproic acid included doses ranging from 6,000 to 14,500 mg (e.g., up to 28 500-mg tablets). The regular valproic acid doses were reduced from 60 to 50 mg/kg (80%) in 2 of 15 patients (13%) with a mean total dose delivery of 95% of expected dosing (Table 1B). The patients requiring dose adjustments all had valproic acid levels >200 μg/mL. A 25% dose reduction for epirubicin was required in 1 of 15 patients (7%) for prolonged neutropenia and fatigue.

Antitumor efficacy of the combined treatment

In the dose escalation part of the trial, objective responses were seen in 9 of 41 patients (22%). At dose expansion, 9 of 14 breast cancer patients (64%) showed an objective response. Also, one patient with an inoperable local recurrence was rendered operable after seven cycles, although that patient's tumor did not meet the criteria for response. One patient with a local recurrence and prior anthracycline therapy had a partial response by computed tomography measurements after four cycles, but that patient had no discernible tumor at surgical resection. Figure 1 shows percent changes in tumor size by RECIST criteria by Waterfall plot.

Pharmacokinetic results for valproic acid and epirubicin

Because the pharmacokinetic profile of valproic acid is well described and valproic acid in this study was used to potentiate epirubicin, pharmacokinetic sampling was limited to a post-loading dose sample and a trough sample on day 3 to reflect the doses over the 48 hours of pretreatment (43). Table 2A and Fig. 2 display pharmacokinetic details for total and free valproic acid on day 3 ($n = 59$). The valproic acid doses and day 3 plasma levels were highly correlated ($P < 0.0001$) for both total valproic acid (Fig. 2A) and free valproic acid (Fig. 2B). The C_{max} samples were obtained at 4 hours. Given the excessive numbers of pills (up to 29 pills of 500 mg) prescribed at the higher doses, the interpretability of the C_{max} was limited (43). The free-to-total valproic acid ratio (Fig. 2B) increased over the dose escalation range with a sharp increase in the 160 versus the 140 mg/kg/day dose to 0.70, which may at least in part contribute to the vastly increased toxicities seen at 160 mg/mg/day, in which both patients had multiple DLTs, including bone

Table 2. Day 3 total and free valproic acid pharmacokinetics

A. Day 3 Total vaproic acid (VPA) levels

VPA dose level (mg/kg/d)	n	Median (μg/mL)	Mean (μg/mL)	Standard deviation	Minimum (μg/mL)	Maximum (μg/mL)
15	3	78.00	71.67	15.50	54	83
30	3	98.00	91.00	27.18	61	114
45	3	131.00	164.67	70.78	117	246
60	6	141.80	141.42	53.06	53	216
75	9	155.40	145.86	28.36	91	180
75 oral	3	121.00	141.17	54.62	100	203
90 oral	3	155.00	178.00	49.67	144	235
100 oral	3	175.20	181.07	32.20	152	216
120 oral	3+15	220.50	219.43	73.57	109	393
140 oral	6	148.00	168.52	53.25	111	241
160 oral	2	257.70	257.70	59.82	215	300

B. Day 3 free VPA levels

VPA dose level (mg/kg/d)	n	Median (μg/mL)	Mean (μg/mL)	Standard deviation	Minimum (μg/mL)	Maximum (μg/mL)
15	3	13.50	14.57	3.72	12	19
30	3	19.10	18.80	7.16	12	26
45	3	29.80	32.40	19.93	14	54
60	6	43.15	44.98	16.12	21	70
75	9	61.20	57.29	19.94	16	82
75 oral	3	28.80	47.73	34.19	27	87
90 oral	3	46.60	67.20	45.17	36	119
100 oral	3	77.00	77.27	2.61	75	80
120 oral	3+15	88.85	108.23	77.03	26	265
140 oral	6	51.65	67.37	39.83	35	142
160 oral	2	192.10	192.10	132.80	98	286

marrow suppression. The ratios were only weakly associated with age. Epirubicin levels were obtained at a limited sampling rate at the end of infusion and at 24, 48, and 168 hours (5 half-lives). Neither valproic acid dose nor plasma level influenced epirubicin peak concentration or its 24-, 48-, and 168-hour plasma levels (Fig. 2C).

Pharmacodynamic endpoints

Effect of valproic acid–induced toxicity on WBC, neutrophils, and platelets. We have previously reported that valproic acid had no effect on epirubicin-induced myelosuppression (WBC, hemoglobin, or platelets) or cardiac effects (change in left ventricular ejection fraction; ref. 42). However, valproic acid plasma levels were associated with a dose-dependent depletion of WBC (ratio of day 3 to day 1 cell count; $P = 0.0017$ for total valproic acid level, $P = 0.0092$ for free valproic acid level) and absolute neutrophil count ($P = 0.0048$ for total valproic acid level, $P = 0.0307$ for free valproic acid level; Fig. 3 A and B). The effects occurred within 48 hours of valproic acid administration and were quickly reversible. In contrast, valproic acid levels had no effects on the decrease in WBC on day 17 ($P = 0.94$ for total, $P = 0.44$ for free valproic acid; 14 days after epirubicin infusion), which is the expected WBC nadir induced by epirubicin (Fig. 3C). As described by others, valproic acid was also associated with thrombocytopenia (day 10:day 1 ratio; $P = 0.001$ for total valproic acid level, $P = 0.0002$ for free valproic acid level; Fig. 3D). Valproic acid plasma levels had no significant effects on hemoglobin on day 3 ($P = 0.10$), 10 ($P = 0.14$), or 17 ($P = 0.62$).

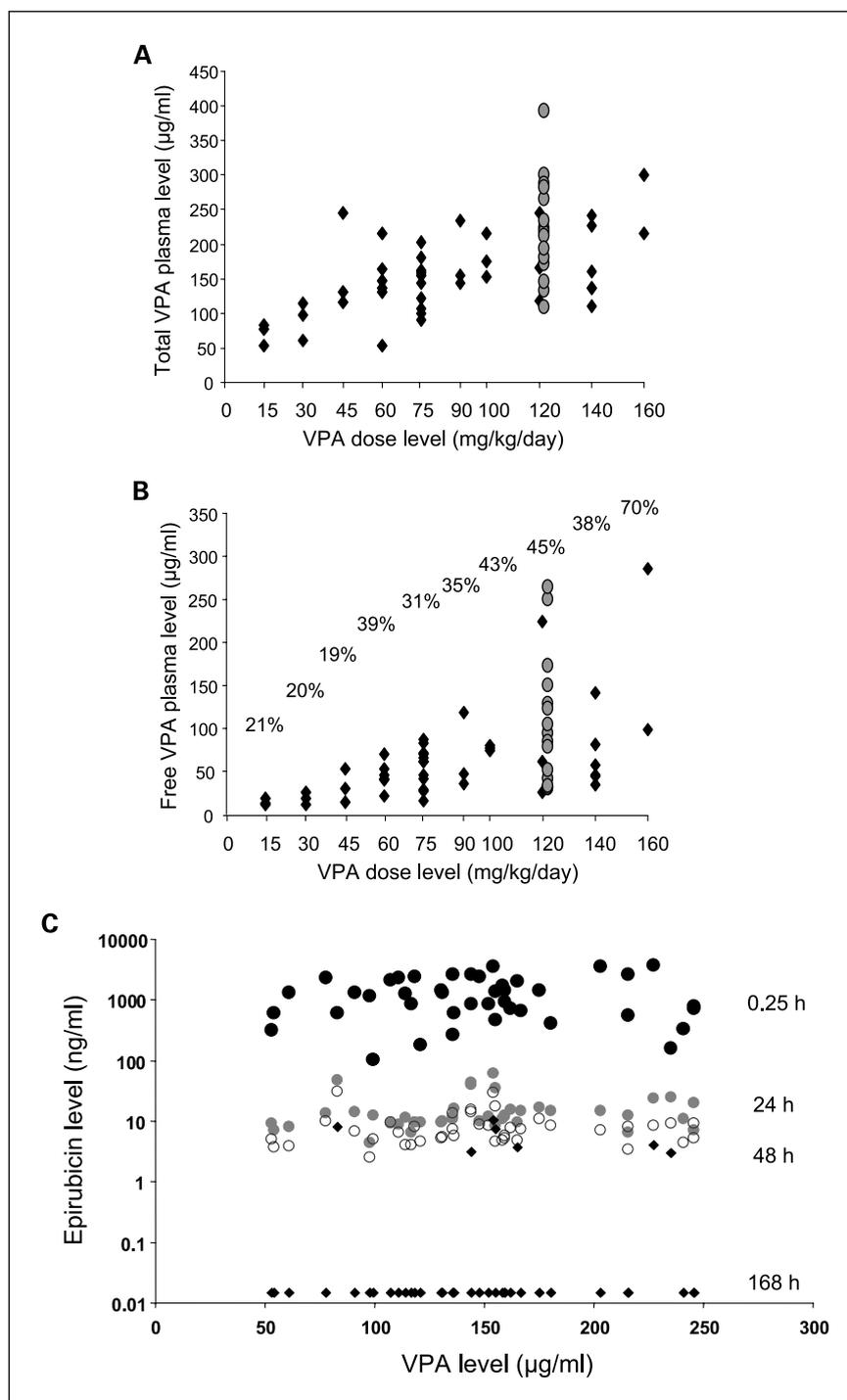*Valproic acid–induced histone acetylation.* Histone acetylation was evaluated by immunofluorescence in PBMC and compared with tumor samples on days 1 and 3 of cycle 1. Histone acetylation was expressed as change compared with baseline and normalized to the housekeeping gene *lamin*. This study suggested a significant correlation between histone H4 and H3 acetylation and valproic acid dose (data not shown) as well as valproic acid concentrations (Fig. 4A and data not shown), despite the notable interpatient variability ($P = 0.0001$). Tumor histone acetylation was obtained in 15 patients at the dose expansion phase; however, although there was a trend, there was no statistically significant correlation between H4 and H3 histone acetylation and valproic acid dose or concentrations (Fig. 4B and data not shown).

A comparison of the median increase in H3 and H4 histone acetylation in tumor cells versus the PBMCs derived from the patients treated at the dose expansion cohort suggested a comparable induction of histone acetylation seen in MCF-7 breast cancer cells treated with 2 mmol/L valproic acid (acetyl-H3: tumor: 1.66 ± 0.23-fold versus PBMC: 2.58 ± 0.22-fold versus MCF-7 cells: 2.20 ± 0.35-fold and acetyl-H4: tumor 2.17 ± 0.44-fold versus PBMC 2.66 ± 0.27-fold versus MCF-7 cells: 2.04 ± 0.14-fold). The median for day 3 valproic acid concentrations was calculated at 217 ± 37.5 μg/mL (1.51 mmol/L; Fig. 4D).

Relevance of HDAC enzymes as therapeutic targets

The HDAC inhibitors are divided into different classes by their structure, but more importantly they may distinguish themselves by their differential activity against individual HDAC enzyme targets. In extensive preclinical studies, we have shown that HDAC inhibitor–induced chromatin decondensation, which forms the basis of the proposed synergy (19, 21, 22, 38), is

Fig. 2. Scatter diagrams of day 3 free and total vaproic acid (VPA) pharmacokinetics and epirubicin plasma concentrations plotted against VPA plasma concentrations. Scatter diagram of (*A*) total and (*B*) free VPA plasma concentrations for the dose escalation (◆) and dose expansion cohorts (◉) plotted against VPA doses (mg/kg/d given in 2 divided doses) and free-to-total VPA ratios (%). Scatter diagram of epirubicin concentrations (*C*) were measured at 0.25 (●) at end of infusion, and at 24 (◉), 48 (○), and 168 hours (◆) after epirubicin infusion and plotted against VPA plasma concentrations. No interactions between epirubicin and VPA dose or plasma concentrations were observed.

mainly mediated through the HDAC2 enzyme.[3] HDAC2 is one of the most sensitive targets of valproic acid (33, 44). Although we observed a correlation between valproic acid concentrations and histone acetylation, there was a further strong correlation between HDAC2 expression (relative expression to MCF-7 cells) and histone acetylation ($P = 0.0063$ for H4 and $P = 0.0427$ for H3) assessed by Western blot (Fig. 4C and data not shown). We

found no correlation between HDAC6 expression and histone acetylation ($P = 0.9006$ for HDAC6/H4, $P = 0.8018$ for HDAC6/H3).

Discussion

Several HDAC inhibitors are now being tested in cancer patients in trials ranging from early phase I to randomized phase III either as single agents or in combination with cytotoxic biologic agents (2, 5, 6, 8–14, 16, 30, 42, 45–48).

[3] Marchion et al. Mol Cancer Ther 2009. In press.

However, unlike the activity seen in hematologic malignancies, the single agent activity seems more limited in solid tumors. This trial describes the results of a rationally designed, sequence-specific administration of a HDAC inhibitor followed by an anthracycline based on extensive *in vitro* and *in vivo* studies. The chromatin decondensation induced by the HDAC inhibitor facilitates DNA access of the anthracycline and recruits topoisomerase IIβ, thereby potentiating DNA strand breaks and cell death (19–22, 37, 49, 50). Preclinical studies further indicated that these occur predominantly in tumor cells (38).

The data presented suggest that combined valproic acid and epirubicin, as well as FEC100, an approved regimen for breast cancer, has an acceptable toxicity profile and antitumor efficacy. At dose expansion, 9 of 14 evaluable patients had an objective response. One patient had a complete clinical response in all target lesions as well as pathologic complete response in the breast and lymph nodes. One patient had a partial response by computed tomography criteria but a pathologic complete response by surgical assessment, and one patient presenting with inoperable chest wall disease was deemed stable by radiologic assessment but was amenable to surgical complete resection after treatment (Table 1B). Despite the promising degree of efficacy, the interpretation of this trial is limited by its small numbers. The efficacy of the combination as primary therapy is currently being further explored in a phase II trial (NCT00437801). It is interesting to note that 8 of the 12 patients with metastatic breast cancer had tumors expressing estrogen receptors. A phase II trial is planned to further delineate the efficacy of the combination involving a new formulation of valproic acid (pulse enhanced acetylation; TopoTarget) in melanoma.

Despite its low potency compared with other HDAC inhibitors, valproic acid was initially chosen for its extensive safety and toxicity profile. However, much higher than expected doses were tolerated when administered for 48 hours. Between 140 and 160 mg/kg/day valproic acid, there was a drastic decrease in tolerability, with both patients experiencing multiple DLTs. We also noted a much steeper increase in the free valproic acid level (mean 192 versus 67 µg/mL; 286% increase) compared with the total valproic acid level (mean 258 versus 169 µg/mL; 153% increase) between the 160 and 140 mg/kg/day dose (Table 2A and B). Furthermore, the "free-to-total" ratio of valproic acid increased to 0.7 over the last cohort (Fig. 2B). This may further explain the drastic increase in the toxicities seen at the 160-mg/kg/day dose as the free valproic acid level has been more commonly associated with toxicity (Food and Drug Administration package insert). Epirubicin plasma levels (Fig. 2C) or its toxicity (typically seen as day 14-21 myelosuppression; Fig. 3C and data not shown) was not linked to valproic acid dose or level, and there was no increase in the epirubicin-induced bone marrow suppression related to valproic acid dose (42). Although valproic acid–induced thrombocytopenia is well established, the dose- and plasma level–dependent short-term effects on WBC (Fig. 3A, B, and D) and particularly neutrophils are a new finding and could be a pharmacologic guide in this setting.

The pharmacokinetic profile suggests that sufficient plasma levels could be achieved for histone acetylation. Acetylation of H4 and H3 histones in PBMCs, a surrogate tissue, and in tumor
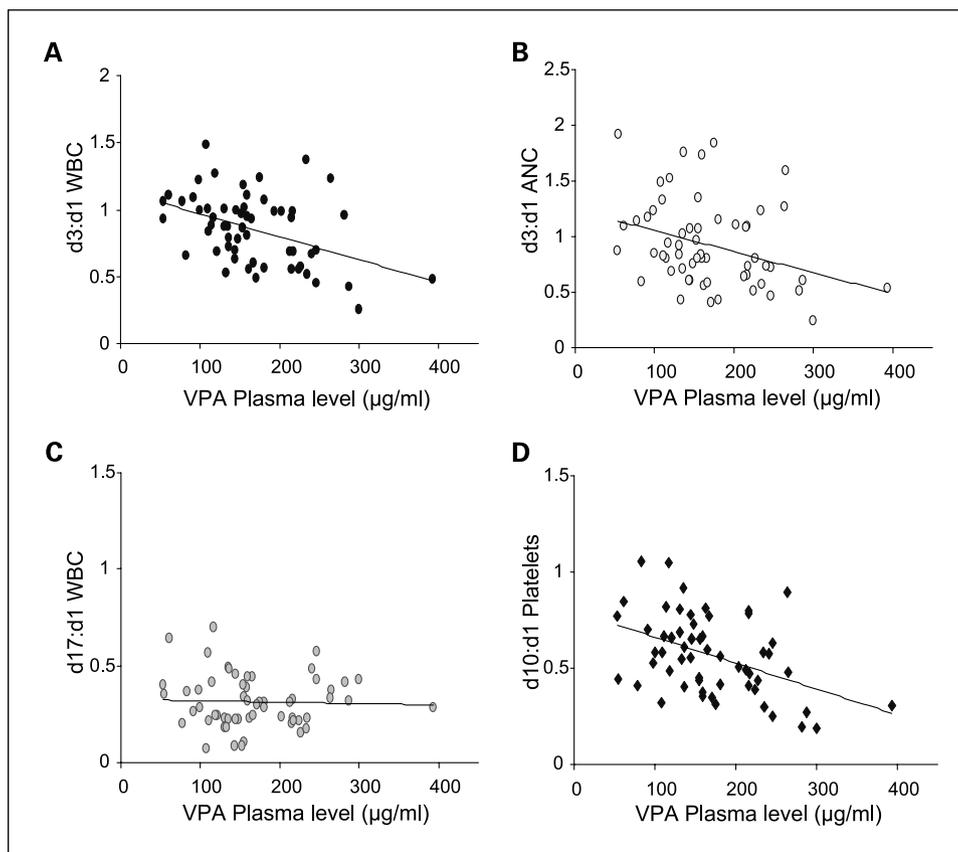
Fig. 3. VPA effects on WBC, neutrophils (ANC), and platelets. VPA effects on day 3 WBC (*A*), day 3 ANC (*B*), day 17 WBC (*C*), and day 10 platelets (*D*) depicted as ratio to baseline. Epirubicin was administered on day 3. VPA plasma levels were associated with a significant decrease in day 3 WBC (*P* = 0.0017) and ANC (*P* = 0.0048) and day 10 platelet count (*P* = 0.0010), but not with the WBC on day 17 (*P* = 0.94).

Fig. 4. Histone acetylation in PBMC and tumor cells and correlation between HDAC enzyme expression and histone acetylation in PBMC. Scatter plots depicting relative changes on day 3 compared with baseline in H4 histone acetylation by immunofluorescence (*A*) in PBMC (*n* = 59; *P* = 0.0001; correlation coefficient = 0.484) and (*B*) in tumor cells (*n* = 15; *P* = 0.292; correlation coefficient = 0.291). Scatter plots depicting baseline expression of (*C*) HDAC2 plotted against *x*-fold change in histone H4 acetylation in PBMCs by Western blot analysis (*n* = 32). Spearman's correlation suggested a significant interaction between HDAC2 and H4 acetylation (*P* = 0.0063; correlation coefficient = 0.496). *D*, comparison of relative increase (medians) in histone H3 and H4 acetylation (day 3 versus day 0) in the PBMCs and tumor cells from the dose expansion (*n* = 15), and MCF-7 cells treated with VPA (2 mmol/L).
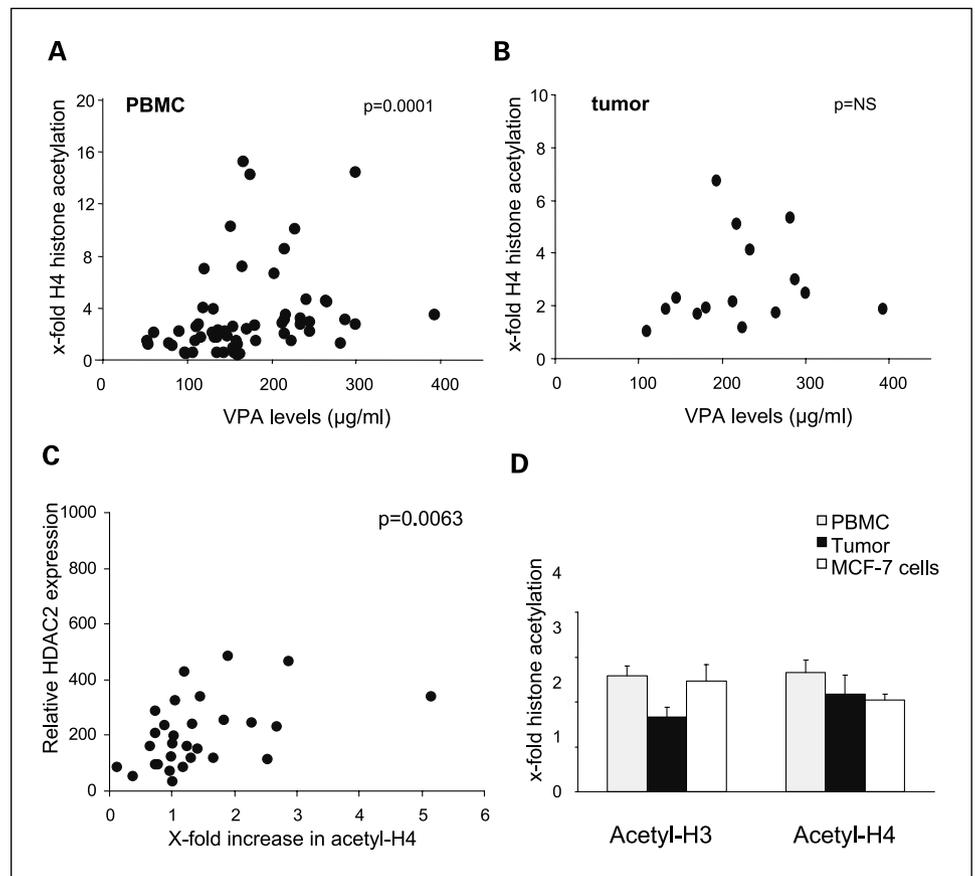
samples increased with the valproic acid dose and was statistically correlated with valproic acid plasma concentrations (Fig. 4A and B and data not shown) in PBMCs. Although a correlation seems to exist between valproic acid level and tumor histone acetylation, this did not reach statistical significance, which at least in part may be explained by the low number of patients. The accompanying correlative studies indicated that, despite the low potency of valproic acid, histone acetylation in surrogate and tumor tissues is attainable and comparable with the changes in histone acetylation seen with valproic acid in breast cancer cell lines (Fig. 4D). Preclinical data suggested that the effects of valproic acid on chromatin remodeling are due to the inhibitory effects on HDAC2 rather than other HDAC enzymes. There was a statistically significant interaction between the HDAC2 expression in PBMCs (Fig. 4C and data not shown) and the degree of histone acetylation, suggesting that the inhibition of HDAC2 may be the most relevant and the testing of its expression should be considered as a biomarker when using HDAC inhibitors in this setting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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