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A Phase II Clinical Trial of Oral Valproic Acid in Patients with Castration-Resistant Prostate Cancers Using an Intensive Biomarker Sampling Strategy

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Abstract

Oral valproic acid (VPA), which is a histone deacetylase inhibitor, was used in a phase II trial to treat patients with castration-resistant prostate cancer (CRPC). Ten patients with CRPC were treated with oral VPA. Oral VPA was not well tolerated in this patient population at a dose targeted to a serum level less than 50 μg/L. The main toxicities were grades 1 and 2 neurologic events and grades 1 and 2 fatigue that caused interruption in the administration of oral VPA and dose delays. Two (20%) of 10 patients had prostate-specific antigen (PSA) responses, and one response was durable. Intensive biomarker collections (weekly) revealed that PSA levels were inversely correlated with total VPA levels. Histone acetylation could not be consistently observed in peripheral lymphocytes using oral VPA. Oral VPA can be administered to CRPC patients with resultant PSA responses. However, oral VPA cannot be administered reliably to achieve consistent levels or duration to be useful in the treatment of CRPC patients. It is unlikely that PSA responses from oral VPA are related to histone deacetylase inhibition. Development of oral VPA in prostate cancers is not recommended using an oral formulation. An intensive biomarker strategy is useful to develop clinical hypotheses in patients with CRPCs in small numbers of patients.

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Introduction

Castration-resistant prostate carcinoma (CRPC) is an aggressive disease with limited treatment options. Mitoxantrone plus prednisone is an established chemotherapy regimen that has been shown to improve pain in symptomatic patients but does not improve survival. Several randomized studies have compared docetaxel-based regimens to the mitoxantrone-based regimens and demonstrated a modest improvement in survival, making this treatment a standard of care for this disease [1–4]. Despite these advances, the median survival of CRPC remains only 18 to 20 months [2]. There is a clear need for further improvements in therapy for CRPC. The investigation of novel targeted agents is required because none of the above-mentioned therapies are curative and do not offer long periods of palliation.

Histone deacetylase (HDAC) inhibition provides a novel approach for cancer treatment. Histones are part of the core proteins in structures called nucleosomes. Acetylation and deacetylation of histones plays a role in the regulation of gene expression. Deacetylated histones are highly charged and bind tightly to DNA, inhibiting transcription. This is presumably a result of limited access of transcription factors and RNA polymerases to DNA. Acetylation, conversely, neutralizes the charge of histones and generates a more open DNA conformation [5]. This conformation allows transcription factors and associated transcription apparatus access to the DNA, promoting expression of the corresponding genes. A balance between the activity of two groups of enzymes, histone acetyltransferase and HDAC, controls cell-specific patterns of gene expression. Several lines of evidence suggest that aberrant recruitment of HDAC may result in modification of chromatin and may play a role in changes in gene expression seen in transformed cells [6–12]. Histone deacetylase inhibitors (HDAIs) have been shown to induce differentiation and cell cycle arrest or apoptosis in cultured tumor cells and to inhibit the growth of tumors in animal models [13–20]. In addition, HDAIs have been shown to induce expression of p21, a key mediator of G_1 arrest and differentiation [21,22].

Valproic acid (VPA) is a commonly used antiseizure medication. It has recently been shown to be an HDAI [23]. Similar to more widely studied HDAIs, VPA can cause growth arrest and induce differentiation

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of transformed cells in culture. In addition, these authors found that VPA and its analogs inhibit multiple HDACs from class I and class II (but not HDAC6 or HDAC10). Valproic acid and its analogs also induced hyperacetylation of core histones H3 and H4 in intact cells with an order of potency that parallels *in vitro* inhibition. Valproic acid and VPA analogs induced differentiation in hematopoietic cell lines in a p21-dependent manner, and the order of potency for induction of differentiation paralleled the potencies for inhibition and for acetylation of histones associated with the p21 promoter, supporting the argument that differentiation caused by VPA was mediated through inhibition of HDACs.

In another recently published study [24], VPA was used for therapy for prostate cancer cell lines and also administered *in vivo* in prostate cancer xenografts. The authors demonstrated that in contrast to acute treatment of VPA that increased net histone H3 acetylation and up-regulated p21, androgen receptor (AR), and cytosolic prostate-specific antigen (PSA) expression, chronic treatment led to marked decreases in the net proliferation rate, correlating with increased caspase-2 and caspase-3 activation and reversal of effects on AR and PSA expression. Chronic administration of VPA also reduced tumor burdens in xenograft models.

In the current study, we tested the feasibility and efficacy of treating men with CRPCs with oral VPA. In this particular trial, we attempted to study the relationship between VPA levels, serum testosterone, serum PSA, and histone acetylation using an intensive (weekly) sampling strategy for biomarkers.

Materials and Methods

Patients

Men at least 18 years, who signed informed consent and were diagnosed with prostatic adenocarcinoma, were considered for this study. The clinical study was started in 2005 and was approved by institutional review boards. All patients were enrolled after signing an informed consent. Eligibility requirements included no more than two prior chemotherapies, castrate levels of serum testosterone below 50 ng/ml, PSA progression (including baseline PSA levels at least 5.0 ng/ml), Karnofsky performance status of 70% or greater, and adequate organ function. Men were excluded if they had a history of liver disease, human immunodeficiency virus infection, seizure disorders, or brain metastases. Additionally, men were excluded if they had a myocardial infarction within the previous 6 months or evidence of uncontrolled congestive heart failure. Men who were chemically castrated were required to continue luteinizing hormone–releasing hormone agonist therapy while on study.

Treatment

Valproic acid syrup was dispensed to patients orally at a concentration of 50 mg/ml. Drug was taken by mouth on a daily basis according to the targeted dose level. The initial VPA dose was 10 mg/kg per day. Within-patient dose escalation occurred weekly by 5 to 10 mg/kg per day up to a maximum of 60 mg/kg per day provided that the total VPA blood level of did not exceed 50 μg/L. This is consistent for dosing for seizures (for which VPA is commonly used) because doses above this level are commonly associated with neurologic toxicity). Valproic acid dose reductions and delays were based on hematologic and nonhematologic toxicities as described in the label of VPA for its seizure indication. Treatment administration continued until disease progression, unacceptable toxicity, or until withdrawal of consent by the patient.

Assessments

Patients were seen weekly during the first 8 weeks of VPA therapy and at the investigator's discretion thereafter. Assessments made during study visits included performance status, physical examination, hematology, and clinical chemistry.

Biomarker Assessment Strategy

To study the relationship among PSA, serum testosterone, VPA blood levels, and histone acetylation, samples were collected before treatment and weekly (while on therapy). This strategy allowed large numbers of samples to test for variation of these parameters throughout the course of therapy and statistical analyses of treatment effects.

Histone Acetylation

Histone acetylation was studied using a modification of an ELISA assay already described [25]. Patient samples were collected and lymphocytes extracted using standard mononuclear cell extraction tubes and stored at −80°C. For histone extraction, cells were resuspended in triton extraction buffer (TEB: PBS containing 0.5% Triton X-100, 2 mM phenylmethanesulfonyl fluoride, 0.02% NaN_3) at a cell density of approximately 1×10^7 cells per milliliter. Cells were lysed on ice for 10 minutes with gentle mixing and then centrifuged at 4800*g* for 10 minutes at 4°C. The supernatant was discarded, and the cell pellet was washed with TEB. The pellet was resuspended in 0.2 N HCl at a cell density of approximately 4×10^7 cells per milliliter, and histones were acid-extracted overnight at 4°C. The supernatant containing histones was collected by centrifugation at 4800*g* for 10 minutes and stored at 4°C, and protein concentration was determined using the BCA assay (23235; Pierce, Rockford, IL). Histone extracts, 100 ng per well, were added to duplicate wells of Immulon Microtiter 2HB ELISA plates (3455; Thermo, Waltham, MA), resuspended in 0.2 N HCl to a final 50-μl volume. Plates were incubated overnight at 4°C on a rocking platform shaker at 300 rpm. After incubation, the plates were washed three times with 150 μl 1× PBS + 0.05% Tween, blocked with 100 μl PBST + 3% nonfat dry milk + 1% goat serum, and incubated for 1 hour at room temperature on a rocking platform shaker at 300 rpm. The antibodies were diluted in antibody dilution buffer, PBST + 1% goat serum, at a concentration of 0.05 μg/ml for acetylated H3 (06-599; Upstate, Billerica, MA) and 1:1000 for Histone H3 (9715; Cell Signaling Technology, Danvers, MA) and 50 μl of the antibody solution is added to each well. The plates were incubated for 2 hours at room temperature on a rocking platform shaker at 300 rpm. The plates were washed with PBST three times at 5 minutes each on a shaker at 300 rpm. Goat anti-rabbit HRP (SAB-300; Stressgen, Ann Arbor, MI) secondary antibody, diluted to 1:10,000 in the antibody dilution buffer, was added at 50 μl per well. The plates were incubated for 1 hour at room temperature on a shaker at 300 rpm subsequently washed with 150 μl PBST four times at 5 minutes each time, on a shaker at 300 rpm. The ELISA was visualized using the TMB Peroxidase EIA Substrate Kit (172-1066; BioRad, Hercules, CA) according to the manufacturer's instruction. The plate was incubated on a rocking platform shaker on low speed until development, and approximately 10 minutes later, the plate was read at 655 nm on the Biotek μQuant plate reader (Biotek Instruments, Winooski, VT). Positive controls were used using cell lines (NCI H2452) treated with various HDAIs including VPA. Additional positive controls included treatment of extracted mononuclear cells *ex vivo* with VPA. Appropriate negative controls were also used to optimize the assay.

Table 1. Patient and Baseline Disease Characteristics (*N* = 10).

Age (years)	
Median (range)	71 (48-80)
Race (no. patients)	
White	10
Performance status (no. patients)	
100	2
90	2
80	4
70	1
60	1
Diagnosis (no. patients)	
Prostatic adenocarcinoma	10*
Stage (no. patients)	
Metastatic	9
Gleason score (no. patients)	
5	1
6	1
7	2
8	2
9	1
n/a	3
Prior treatment (no. patients)	
Chemotherapy	9
Surgery	3
Radiation	4
PSA (ng/ml)	
Median (range)	33.8 (5.5-731.6)
Testosterone (ng/dl)	
Median (range)	26 (16-48)

*Pathology report for two patients unavailable.

Statistical Considerations

This was an open-label, single-institution, single-arm, two-stage phase II study in patients with CRPCs. If at least one responder among the first 19 patients was observed, an enrollment of an additional 20 patients was planned for stage 2. This study had approximately 85% power to exclude a response rate of 15% in favor of a 35% response rate with a nominal false-positive rate of 0.05.

Prostate-specific antigen response to therapy and disease progression was based on the criteria of Bubley et al. [26]. A PSA response required at least a 50% reduction with a confirmation of at least 4 weeks later. The response duration was calculated from the time of the first 50% reduction until the time of 50% increase in PSA over nadir, provided the increase was at least 5 ng/ml (or returned to baseline) and was confirmed. Nonresponding patients were progressed at the time of 25% increase over baseline or nadir, provided the increase was at least 5 ng/ml and was confirmed.

Kaplan-Meier techniques [27] were used to summarize time to PSA progression. Repeated measures analysis including patient as a random factor was conducted to compare PSA values between responders and nonresponders. Spearman correlation coefficients were calculated to assess factors that were individually associated with PSA levels. Factors included VPA exposure (both VPA levels and duration of treatment exposure) and serum testosterone. Multiple regression analysis using both stepwise and backward elimination techniques (α = 0.10 for variable entry and elimination) were used to identify factors that were independently correlated with PSA.

Results

Patient Characteristics

From April 22, 2005 to July 24, 2006, 19 men signed informed consent and 10 received VPA. Nine patients were ineligible because they withdrew consent or did not have PSA elevations consecutively as required by the study. A summary of patient characteristics for the 10 treated patients is shown in Table 1. All patients were hormone refractory at time of enrollment. Nine of 10 patients had metastatic disease at the time of enrollment.

Table 2. Average Daily Dose of VPA and Days on Treatment.

	Average Daily Dose (mg/kg per day)	Days of Treatment (no. days)
Including skipped doses, median (range)	13.4 (10-19)	51 (14-633)
Excluding skipped doses, median (range)	14.9 (10-19)	38.5 (14-626)

Study Drug Administration

A summary of dose administration is shown in Table 2. All patients received at least 7 consecutive days of 10 mg/kg per day of VPA before their first intrapatient dose escalation. Including days of omitted or head doses, the average daily dose ranged from 10 to 19 mg/kg per day. A summary of the number of days of treatment by dose level is shown in Table 3. Seven patients either omitted doses or had doses held. These patients had a median of 7 days of omitted doses, ranging from 1 to 21 days. At least seven of the ten patients were escalated to 20 mg/kg per day of VPA.

Adverse Events

The main toxicity of VPA was neurologic and constitutional. There were numerous [11] drug-related grade 1 and 2 neurologic events (Table 4). These ranged from confusion, dizziness, fatigue, somnolence to tremor, and frank drowsiness. These events seemed to be unrelated to the serum levels of VPA (total or free). Neurologic events were the most likely reasons for dose delays and withheld doses in the study. Constitutional symptoms such as fatigue was common. Fatigue seemed to be most related to length of administration of VPA. One patient died during the treatment administration period. This death was related to disease progression and was not study drug–related. An additional patient experienced a serious adverse event (hypoxia). Although possible relationship to VPA could not be ruled out, it is more likely that the hypoxia was related to smoking history.

Overall, VPA seemed to be tolerated relatively poorly in this older population and constant dose titration was required. Study accrual was terminated because of poor tolerance of VPA and inability to consistently titrate the dose of VPA to maintain the. within-patient total VPA blood level of <50 μg/L.

Efficacy

Individual patient data for PSA response and progression is shown in Table 5. Among the 10 evaluable patients, one patient (patient

Table 3. Days of Treatment by Dose Level (*N* = 10).

Dose Level (mg/kg per day)	Number of Patients	Number of Days of Treatment		
		Minimum	Median	Maximum
10	10	7	14	424
15	8	7	12.5	181
20	7	7	13	49
25	4	2	7	14

Table 4. Summary of Common Toxicity Criteria Adverse Events.

Event	Grades 1 and 2	Grade 3	Grade 4
Laboratories			
Hemoglobin	1	1	0
Lymphopenia	1	0	0
Glucose, elevated (serum)	1	0	0
Neurology			
Confusion	2	0	0
Dizziness	3	0	0
Memory impairment	1	0	0
Neurology other (drowsiness)	2	0	0
Somnolence	2	0	0
Tremor	1	0	0
Gastrointestinal			
Anorexia	5	0	0
Constipation	1	0	0
Dental (periodontal disease)	1	0	0
Dry mouth	1	0	0
Other – thirst	1	0	0
Heartburn	1	0	0
Nausea	2	0	0
Vomiting	2	0	0
Constitutional			
Fatigue	5	2	0
Fever	1	0	0
Rigors	0	1	0
Weight loss	1	0	0
Cardiac			
Cardiac arrhythmia (other tachycardia)	1	0	0
Pulmonary			
Dyspnea	3	0	0
Hypoxia	1	1	0
Lymphatics			
Edema (limb)	3	0	0
Musculoskeletal			
Extremity, lower (gait)	2	0	0
Pain			
Pain (musculoskeletal)	4	0	0
Dermatology			
Pruitis	1	0	0

Number of patients with possibly drug-related events, N = 10.

number 5) had a confirmed PSA response with no evidence of subsequent progression. However, after a 29-day documented remission period, the patient withdrew consent due to unacceptable neurologic toxicity and was lost to follow-up. A second patient (patient number 6) demonstrated a PSA response with a duration of 471 days. This patient was stable for 632 days and, after which, withdrew consent because of constitutional symptoms. The remaining eight patients had documented PSA disease progression, seven of whom did not have a PSA nadir. With 80% of events observed, the median time to progression was 17.5 days, and the interquartile range was 7 to 292 days.

Table 5. Summary of Response and Time to Progression, N = 10.

Patient Number	Time to PSA Progression (days)	PSA Progression Occurred	PSA Response	Response Duration (days)
1	14	Yes	No	–
2	21	Yes	No	–
3	7	Yes	No	–
4	7	Yes	No	–
5	77	No	Yes	29
6	632	No	No*	–
7	74	Yes	No	–
8	14	Yes	No	–
9	292	Yes	No	–
10	7	Yes	No	–

*Patient 6 had an unconfirmed (single value) PSA response with censored duration of 471 days.

Pharmacodynamics

Figure 1 shows levels of total histone 3 and acetylated histone 3, expressed as a percent of baseline total histone 3. Acetylated histone 3 did not increase over time suggesting that oral administration of VPA may not be able to consistently cause histone acetylation in lymphocytes.

Biomarker Analysis

A plot of PSA as a percent of baseline is shown in Figure 2. A repeated-measures analysis showed responding patients on treatment had significantly lower PSA (P = .011) values on average. In a recent publication from the prostate cancer clinical trials working group [28], it was recommended that the percentage of change in PSA from baseline to 12 weeks (or earlier for those who discontinue therapy) and the maximum decline in PSA that occurs at any point after treatment be reported for each patient using a waterfall plot. Accordingly, the waterfall plot is depicted in Figure 3 for this trial. Accordingly, the waterfall plot is depicted in Figure 3. As is evident from this plot, three patients had any decline in PSA in this trial with only two patients having a <50% decline.

Table 6 shows correlations relating PSA levels to VPA exposure (levels and duration) and serum testosterone levels. Increasing VPA levels were associated with decreasing PSA (Figure 4); however, the correlation was stronger with total VPA than with free VPA. The duration of VPA exposure was also negatively correlated with PSA values. Concurrent with decreasing (increasing) PSA values was a trend for increasing (decreasing) serum testosterone levels.

These individual correlations were examined further to identify factors that independently correlated with PSA. Multiple regression analyses, using both stepwise and backward elimination techniques, resulted in a final model that included total VPA (P < .001) and duration of VPA exposure (P = .002) as independent predictors of declining PSA. Serum testosterone was not independently predictive of PSA (P = .399).

Threshold analyses were conducted for total VPA level and duration of exposure to identify VPA and duration values resulting in the largest dichotomy between high and low PSA values (Figure 5). The geometric mean of the PSA values for samples corresponding to total VPA less than or equal to 46 mg/L was 59.6 ng/ml and was 11.7 ng/ml

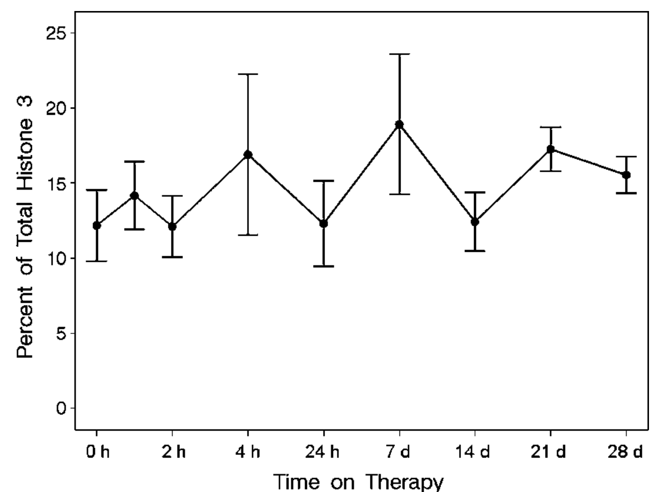

Figure 1. Acetylated histone 3 as a percentage of total histone 3 over time. Values are averages of n = 8 patients ± SE.

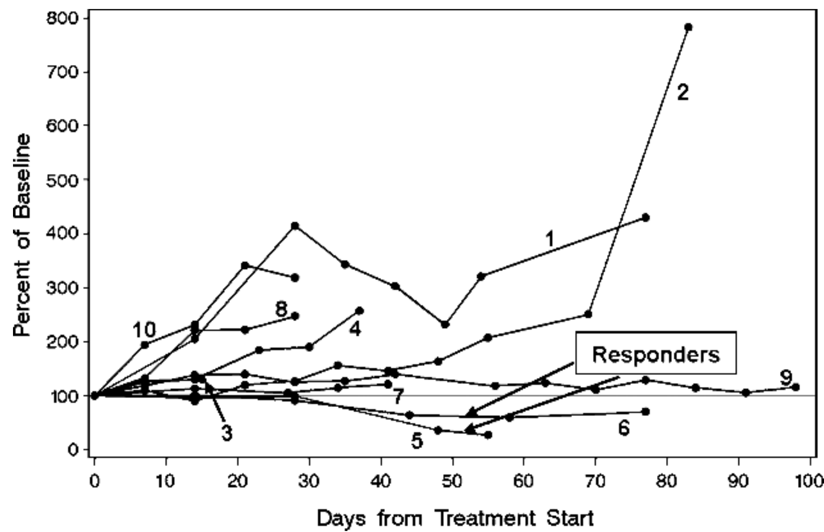

Figure 2. Prostate-specific antigen as percent of baseline *versus* time on study therapy (truncated at 100 days).

for samples corresponding to VPA greater than 46 mg/L ($P < .001$). Prostate-specific antigen was higher when VPA exposure did not exceed 55 days, geometric mean = 55.0 ng/ml, compared with PSA values from samples corresponding to greater than 55 days of VPA exposure, geometric mean = 9.0 ng/ml ($P < .001$).

Conclusions

In this study, we administered a putative HDAI (oral VPA) to patients with CRPC. There is preclinical data from several laboratories that suggests that VPA has anticancer activity *in vitro* and *in vivo* [23,24]. In the current study, we used an intensive biomarker strategy to explore the potential efficacy for oral VPA in prostate cancer. We were interested in exploring the therapeutic potential of this agent in prostate cancers.

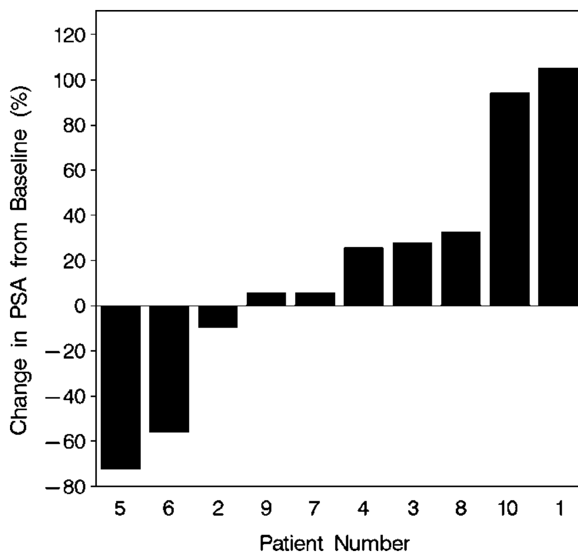

Figure 3. Waterfall plot showing maximal PSA decrease as a percent of baseline. For each patient, the minimum among all postbaseline PSA values was identified and expressed as a percent change from baseline. This is interpreted as the best PSA response. For patients with a postbaseline nadir, plotted values are maximum PSA decrease. For patients without a postbaseline nadir, plotted values are minimum PSA increase.

We noted that oral VPA was difficult to administer reliably to older patients with prostate cancer. The median age of our patients on trial was 71 years, and this patient population experienced high degree of short-term (neurologic) or long-term (fatigue) toxicity from oral VPA. In addition, it was difficult to use total or free serum VPA levels to guide us during this trial. Total and free serum VPA levels did allow us to prevent severe toxicity from oral VPA as we did not have grade 3 or 4 toxicity, but we were unable to use these levels to guide us in preventing prolonged grade 1 or 2 toxicities. Base on safety observations, the trial was discontinued early.

Although we treated only 10 patients with prostate cancer, several interesting trends emerged from the study because intensive biomarker analysis was carried out. Surprisingly, 2 (20%) of 10 patients had PSA responses to oral VPA, and this was durable in 1 patient. When we designed the trial, we were concerned that VPA would alter testosterone levels, causing PSA levels to rise or fall depending on the T levels. Thus, we repeatedly measured serum testosterone, although all patients had medical or surgical castration. Although there were interesting fluctuations in the testosterone levels that we initially reported were related to PSA level [29], final analysis of the data shows that such fluctuations did not statistically influence the PSA levels.

Nevertheless, PSA levels correlated inversely with total VPA levels. This relationship was confirmed by many statistical analyses and was also strongly positive in a multivariate analysis.

In addition, we measured histone acetylation in lymphocytes. We were unable to detect reliable histone acetylation in the peripheral blood in this current trial. Based on these observations, it is likely

Table 6. Spearman Correlation Coefficients for PSA, VPA, and Testosterone.

		Total VPA	Free VPA	Testosterone	Time
PSA	Correlation	−0.54	−0.32	−0.30	−0.49
	P	<.001	.002	.003	<.001
	N	98	94	94	109

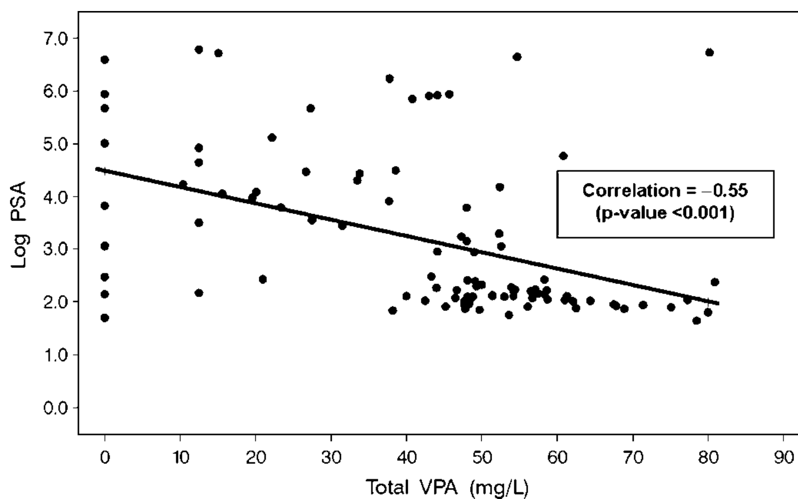

Figure 4. Scatter plot between log PSA and total VPA levels.

that the effect on PSA is unlikely to be related to HDAC inhibition). Valproic acid has been shown to induce differentiation of cancer cell lines and leukemia cells *in vivo*, and this may be a putative anticancer mechanism in this study. Conversely, as demonstrated by Xia et al., chronic low-dose administration (as opposed to acute administration) of VPA has significant antitumor activity. Our observations are consistent with this preclinical observation. There is a small possibility that tumor HDAC inhibition was observed regardless of lack of acetylation in peripheral lymphocytes. However, this is quite unlikely because we have seen comparable levels of acetylation *ex vivo* in lymphocytes treated with oral VPA (manuscript in preparation).

In a recent study of VPA in solid tumors [30], 26 patients with pretreated and progressive malignant disease were enrolled in escalating dose-level cohorts of 3 patients. The patients received VPA as a 1-hour infusion split in two doses daily for 5 consecutive days. After a 2-week therapy-free interval, the treatment was repeated. The starting dose in this study was 30 mg/kg per day and escalated to 60, 75, 90, and 120 mg/kg. The main toxicity was neurological (grade 3/4) occurring in 9 of 26 patients at dose levels of 75, 90, and 120 mg/kg. No hematological toxicity grade 3/4 toxicity was observed. The maximum tolerated dose of infusional VPA was 60 mg/kg. Biomonitoring of peripheral blood cells demonstrated the induction of hyperacetylation in most patients. Neurotoxicity was the dose-limiting factor of infusional VPA in the treatment of patients with advanced cancer. In our current study, we administered VPA orally because oral VPA is used commonly as an antiseizure medication, and patient convenience would be higher with an oral route of administration. Pharmacokinetic differences would seem to favor an intravenous route of administration but this strategy is potentially limited by inability to modulate HDACs on a continuous basis.

In summary, oral VPA can be administered to CRPC patients with resultant PSA responses. Total VPA levels and duration of VPA exposure were independent predictors for declining PSA. However, oral VPA is not well tolerated in this patient population because of grade 1 and 2 neurologic symptoms and fatigue. Total and free VPA levels are useful for preventing severe toxicities from oral VPA but are unable to protect against lower-grade toxicities. It is unlikely that PSA responses from oral VPA are related to HDAC inhibition but are likely to be due to alternative mechanisms. Further development of oral VPA in prostate cancers is not recommended using an oral formulation. Finally, an intensive biomarker strategy is useful to develop clinical hypotheses in patients with CRPCs.

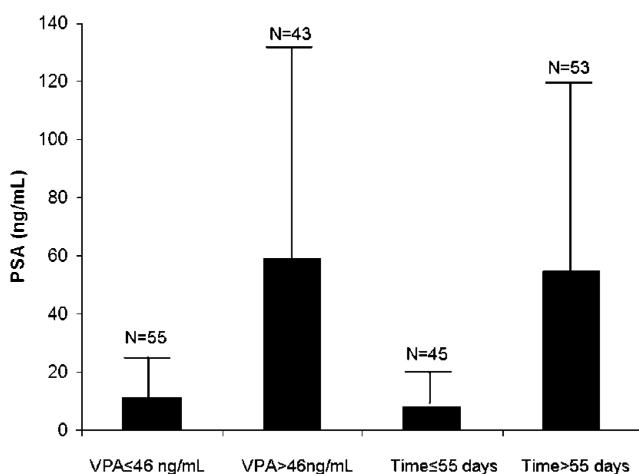

Figure 5. Prostate-specific antigen values by VPA levels and by duration of VPA exposure. Vertical bars represent geometric means of PSA values for high and low VPA levels, and for shorter and longer duration of VPA treatment (Time). Geometric means were calculated as $\exp(X^{-})$, where X^{-} is the average of the log PSA values. Error bars represent geometric mean plus one standard error, calculated as $\exp(X^{-} + SE)$, where SE is the standard error of the mean of log PSA values.

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