Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Evidence from clinical trials for the use of valproic acid in solid tumors: focus on prostate cancer

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The progression of prostate cancer to an androgen-unresponsive state is challenged with unmet therapeutic needs. Valproic acid is a HDAC inhibitor that has recently been found to possess antitumor activity in diverse tumor types. Its role in prostate cancer has been explored in multiple clinical trials on solid tumors. Results from these trials have shown that it offers an encouraging avenue of treatment. Valproic acid was well tolerated in most of these trials without causing any significant life-threatening toxicities. The combination of valproic acid with other chemotherapy agents has been shown to be safe, tolerable and efficacious in prostate cancer. Multiple trials are underway combining various antineoplastic agents with valproic acid in an attempt to achieve a broad therapeutic index. Although the underlying mechanisms behind the efficacy of valproic acid remain to be elucidated, it entails a promising strategy for development of an effective anticancer therapy.

Keywords: cancer • HDAC inhibitor • prostate • valproic acid

Jatinder Goyal[1] & Ronald Rodriguez*[2]

[1]Department of Medicine, the University of Alabama at Birmingham, Birmingham, AL, USA
[2]Department of Urology, University of Texas Health Science Center San Antonio, 7703 Floyd Curl Drive, MC 7845, San Antonio, TX 78229-3900, USA
*Author for correspondence:
Tel.: +1 210 567 5643
Fax: +1 210 567 6868
E-mail: rrodrig@jhmi.edu

Prostate cancer is the second leading cause of cancer-related death in men in the USA. The progression of prostate cancer from an androgen-sensitive state to an androgen-resistant state is marked by unveiling of a new set of challenges in managing this complex disease. At that time, it is termed as castration-resistant prostate cancer (CRPC). Although docetaxel constitutes first-line treatment for these patients [1], the US FDA has recently approved four new agents for use in CRPC. These include the chemotherapeutic agent cabazitaxel [2], the immunotherapy product sipuleucel-T [3], the androgen biosynthesis inhibitor abiraterone [4], and the androgen receptor (AR) antagonist enzalutamide [5]. The radiopharmaceutical agent Radium-223 has shown promising results but is still pending FDA approval [6]. Despite these developments, there is still no curative treatment for CRPC and prognosis of these patients is poor.

A novel treatment strategy under active investigation for treatment of CRPC is HDAC inhibition. Histone proteins constitute an integral part of the core proteins in nucleosomes. Acetylation and deacetylation of histones plays a crucial role in regulation of vital biologic functions through regulation of cellular gene expression. This includes cell growth, differentiation and oncogenesis [3]. Altered activity of HAT and HDAC has been implicated in several types of cancers, a phenomenon that can be potentially modulated with HDAC inhibitors [7,8]. To this end, several HDAC inhibitors have been investigated under clinical trials for efficacy in various malignancies. Though the first successful results were demonstrated in hematological cancers [9–13], we have witnessed a recent spurt in trials exploring their role in solid tumors [14–24]. Two of these agents, vorinostat and romidepsin, were recently approved as monotherapy for use in cutaneous T-cell lymphoma [25,26]. Many studies have also demonstrated that HDAC inhibitors potentiate the antitumor effects of other chemotherapeutic agents [27–30], some of which have been formally tested in clinical trials [31,32].

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Valproic acid (VPA) is a commonly prescribed antiseizure medication with extensive pharmacological characterization [33]. It was found to possess HDAC inhibitory properties, making it a potential anticancer agent [6,34,35]. It has been shown to induce proteasomal degradation of HDAC2 leading to cellular differentiation, cell growth arrest, inhibition of angiogenesis and cell death *in vitro* and *in vivo* [36–38]. Several studies have tested the utility of VPA (often in combination with other chemotherapy agents) in various hematological [39–44] and solid tumors [45–50], prostate cancer being one of them [1,51–56]. Encouraging results have been reported from these trials, which have paved the way towards the development of viable treatment strategies. In this review, we have attempted to describe the emerging data both from preclinical studies, which have enhanced our understanding of the basic mechanisms through which VPA achieves antitumor effect, and from clinical trials in solid tumors including prostate cancer patients. The studies that have been summarized in this review are Phase I and II trials that have examined the clinical utility of VPA through determination of pharmacodynamic and pharmacokinetic features of the drug.

Evidence from preclinical studies

Oncogenesis is associated with dysregulation of enzyme-induced acetylation of histone proteins in cancer cells [57]. Since this epigenetic alteration is susceptible to reversal, newer agents targeting this cellular process have become an attractive area of cancer research. HDAC inhibitors alter the acetylation status of chromatin, producing changes in gene expression resulting in cell death, apoptosis, cell-cycle arrest and inhibition of angiogenesis and metastasis [58]. The various classes of these drugs include short-chain fatty acids, hydroxamic acids, cyclic peptides and benzamides. VPA belongs to the short-chain fatty acids class of HDAC inhibitors. The role of VPA as a HDAC inhibitor was first discovered in neuroblastoma and teratocarcinoma cells [6,34,35], and it was only later that this effect was linked to its potential as an antineoplastic agent [53]. It was postulated that the antitumor effect of VPA was different from its antiepileptic effect. VPA affects tumor progression by chromatin remodeling through isolated inhibition of HDAC2. This isoenzyme-specific targeting by VPA could facilitate development of novel antineoplastic agents that can augment tumor lysis in conjunction with VPA. It has been proposed that chromatin decondensation induced by VPA facilitates access for other DNA-targeting chemotherapeutic agents, thus causing cell death [59,60]. Recent data also suggest that VPA might restore HAT activity by repressing HAT-targeting oncoproteins such as ERG (ETS-related gene) [61–66].

Preclinical studies in prostate cancer cell lines demonstrates that it might be especially vulnerable to the effects of VPA [67–69]. Studies have corroborated the hypothesis that VPA may be inhibiting growth of prostate cancer cells *in vitro* and *in vivo* by modulating multiple pathways [70–72]. VPA inhibits tumor growth by multiple mechanisms including cell-cycle arrest, induction of differentiation, and inhibition of growth of tumor vasculature. We studied the effect of VPA on xenograft growth inhibition by studying expression of various markers [72]. We demonstrated that treatment with VPA causes cell-cycle arrest in prostate cancer cells *in vivo*, as determined by increase in p21 and p27 and decrease in cyclin D1 expression. Increased expression of cytokeratin-18 was also seen in xenografts. A reduction in AR expression was also observed. While decreased proliferation was found *in vitro*, increase in apoptosis was found to be the reason for decreased tumor growth *in vivo*. In addition, an antiangiogenic effect was observed after VPA treatment.

VPA successfully induced apoptosis in LNCaP cells (prostate cancer cell line) in an *in vitro* cell culture system [73]. Concomitantly, it produced significant inhibition of HDAC activity, expression of prostate-derived ETS transcription factor, and down-regulation of PSA to basal levels. Furthermore, it led to the upregulation of pro-apoptotic factor caspase-3, TIMP-3, and IGFBP3 in these prostate cancer cells. Sodium butyrate, another short-chain fatty acid, was found to induce apoptosis in androgen-resistant LNCaP cells [74]. Since progression of prostate cancer from an androgen-sensitive to an androgen-unresponsive state is seen in a considerable number of patients, this has potentially huge implications.

The authors have explored the effect of acute and chronic treatment with VPA through its effect on histone acetylation, p21 gene expression, AR expression, PSA expression and cell survival in prostate cancer cell lines [75]. The effect of VPA was also studied *in vivo* on tumor xenograft growth. It was found that, although acute treatment with VPA might have nominal effects on cell survival and proliferation in prostate cancer, chronic treatment results in profound decrease in cellular proliferation *in vitro*, independent of androgen regulation [75]. Chronic treatment of VPA was also associated with marked caspase-2 and -3 activation, along with a reversal in the upregulation of AR and PSA expression, which was seen with acute treatment. These results were seen in both AR positive cell lines (LNCaP and C4-2) and AR negative cell lines (DU145 and PC3). These were then verified with significant reduction in xenograft tumor models *in vivo* as well. VPA use has also been demonstrated to be associated with reduced microvessel proliferation in mouse xenograft tumors using prostate

cancer cell lines, providing further evidence of its role as an antiangiogenic agent [70].

Multiple combination drug therapy regimens, including VPA, have been tested in an attempt to produce improved antitumor efficacy. Data regarding the synergistic effect of VPA with other chemotherapeutic agents have been reported from various *in vitro* and *in vivo* studies. Studies have shown that PPARγ is overexpressed in prostate cancer cells [67]. The expression of PPARγ is important in repressing proliferation of malignant prostate cancer cells [68]. Inhibition of HDAC produced by VPA when combined with the activation of PPARγ might be a very potent treatment strategy. Encouraging results were reported when pioglitazone was tested with VPA in prostate cancer mouse xenograft models [76]. The combination treatment was able to produce higher tumor kill than either therapy alone. This study also showed that the combination was able to suppress bone invasive potential of prostate cancer cells. The expression of E-cadherin, a protein involved in control of cell migration and invasion is also highly upregulated in the combination arm, an effect that was not witnessed in either treatment arm alone. These studies have enhanced our current understanding on the underlying mechanisms through which VPA induces its antineoplastic effect. Further evidence suggests that VPA might act as a radiosensitizer for multiple tumor types including prostate cancer [77]. Low doses of VPA produced low cytotoxic effects but significantly enhanced radiation-induced apoptosis. Although not completely understood, VPA seems to stabilize an acetyl modification of the p53 tumor suppressor gene, resulting in a proapoptotic function at the mitochondrial membrane. Extensive experiments ruled out the role of p53 as a transcription factor in the process. The radiosensitizing effect of VPA might also be related to the decondensation effect induced by the drug, which might make it more vulnerable to ionizing radiation [78–86]. Recently, published data have suggested that VPA might produce an antitumor effect through upregulation of E-cadherin and resulting inhibition of cell migration [87]. This inhibitory effect on cellular migration and invasion was amplified when VPA was used in combination with the mTOR inhibitor rapamycin [88]. VPA has also been shown to enhance the cytotoxic effects of gossypol in prostate cancer, as increased DNA damage was seen on quantitative proteomic analysis [89]. Other agents including low-dose IFNα have also been found to enhance the antitumor properties of VPA. In one such study, IFN-α was found to distinctly elevate histone H3 acetylation caused by VPA [90]. This combinatorial effect was visible in terms of Akt phosphorylation, p21 and p27, and integrin α1, α3 and β1 expression.

Evidence from clinical trials

VPA was initially investigated for antitumor activity based on its low toxicity profile and availability, despite the fact that other HDAC inhibitors have demonstrated more promising antitumor effect. It has shown excellent tolerability within the serum range of 50–100 µg/ml based on experience from its use as an antiepileptic agent [33]. The worrisome significant toxicities associated with VPA, such as lethargy, coma, tachycardia, metabolic acidosis, and hypotension do not occur with serum concentrations <450 µg/ml [91].

Table 1 summarizes the data derived from clinical trials on the efficacy of VPA in patients with solid tumors, with a focus on prostate cancer. The first clinical trial to investigate the role of VPA in solid tumors was a dose-escalating Phase I trial in a heterogeneous cohort of 26 patients with refractory advanced cancer (one patient with prostate cancer) [51]. VPA was given as a 1-h infusion daily for 5 days in a 21-day cycle beginning with a dose of 30–120 mg/kg/day. The primary end point of the study was the maximum tolerated dose (MTD) of VPA and its toxicity profile. Neurocognitive impairment dominated the toxicity profile in the form of grade 3 or 4 side effects occurring in nine out of 26 patients. Seven of these patients had confusion or disorientation. Dose-limiting somnolence was seen in two patients. The MTD was determined to be 60 mg/kg/day. Pharmacokinetic studies showed that in patients who were administered doses of 90 or 120 mg/kg/day, the serum VPA levels were above 200 mg/l and individual levels were as high as 500 mg/l, which explained the dose-limiting toxicity (DLT). However, all side effects were reversible, an observation that has been confirmed in other trials. No objective clinical oncologic response was observed in the study. The trial also demonstrated that most patients had induction of hyperacetylation in peripheral blood mononuclear cells (PBMC) and down-modulation of HDAC2. Since the trial aimed to determine the MTD, it was successfully terminated after obtaining the above-mentioned results.

Another advantage with the use of VPA lies in its availability in an oral formulation. In a pivotal single institution Phase II clinical trial, the investigators used oral VPA for CRPC in ten patients [54]. The patients were treated with escalating doses of VPA beginning with 10 mg/kg up to a maximum of 60 mg/kg, aiming to target a serum level of <50 µg/l. Out of these ten patients, one had a confirmed PSA response with no evidence of disease subsequent progression. Another patient had a PSA response with duration of 471 days. This patient was found to be stable at 632 days but withdrew from the trial due to constitutional symptoms. The remaining eight patients progressed with a median time to disease progression of 17.5 days. The study found that

Table 1. Clinical trials investigating the efficacy of valproic acid in solid tumors with focus on prostate cancer.

Trial	Phase	Total patients (No. with PCA)	Treatment	Primary end point	Results	Other outcomes	Adverse effects	Ref.
Atmaca *et al.*	I	26 (1)	Escalating dose of VPA infusion daily for 5 days (30–120 mg/kg/day)	MTD	MTD was 60 mg/kg/day	VPA dose correlated with serum levels and DLT	Grade III or IV neurological toxicity in eight out of 26 patients (30.7%)	[52]
Munster *et al.*	I/II	44 (2)	Escalating dose of VPA (15–160 mg/kg/day) + Epirubicin on day 3. Dose expansion with FEC in breast cancer patients	VPA PK and tumor histone acetylation	Partial response in nine of 41 patients (22%)	Positive correlation between histone acetylation and HDAC2 expression	Somnolence with VPA in 20% patients in post-DLT phase. Dose-dependent decline in white blood cell and ANC with VPA	[54]
Sharma *et al.*	II	10 (10)	Oral VPA targeted to serum level <50 µg/l	PSA response	Two of ten patients had PSA responses with one stable response	PSA levels inversely correlated with VPA levels	Grade I and II neurocognitive events and fatigue	[55]
Braiteh *et al.*	I	55 (2)	Escalating doses of 5-AZA (20–94 mg/m^2) + VPA titrated to serum levels of 75–100 µg/ml	MTD	MTD of 5-AZA was 75 mg/m2. SD in 14 patients (25%) lasting 4–12 months	Histone acetylation seen with higher frequency in patients achieving SD ($p = 0.0003$)	DLT were neutropenic fever and thrombocytopenia at dose of 94 mg/m^2	[56]
Munster *et al.*	I	41 (2)	VPA on day 1–3 (15–160 mg/kg/day) + Epirubicin on day 3	MTD	MTD was 140 mg/kg/day; nine patients (22%) had partial response; 16 patients (39%) had SD/minor response	VPA concentrations correlated with dose and PBMC histone acetylation	Neurovestibular DLT as somnolence, confusion and hearing loss (n = 3); febrile neutropenia (n = 1)	[53]
David *et al.*[†]	I	9 (2)	Escalating doses of VPA + ATRA-IV	DLT	DLT could not be calculated	Disease stabilization in one patient	Grade II skin toxicity and thrombocytopenia seen. No grade III/IV toxicity	[57]
Bustinza-Linares *et al.*	I	32	Escalating doses of VPA, 5-AZA and carboplatin	MTD	MTD: 5-AZA 75 mg/m^2, VPA 20 mg/kg, and carboplatin AUC 3.0; SD/minor response in 18.8% of patients	One patient with PCA had stable disease for 11 months	DLT in six patients in the form of altered mental status, anemia, neutropenia and fever	[93]

[†]Study was prematurely terminated due to unavailability of ATRA-IV.

5-AZA: 5-azacytidine; ANC: Absolute neutrophil count; ATRA-IV: All trans retinoic acid; DLT: Dose-limiting toxicity; FEC: 5 Fluorouracil epirubicin cyclophosphamide; MTD: Maximum-tolerated dose; PBMC: Peripheral blood mononuclear cell; PCA: Prostate cancer; PK: Pharmacokinetics; SD: Stable disease; VPA: Valproic acid.

Table 1. Clinical trials investigating the efficacy of valproic acid in solid tumors with focus on prostate cancer (cont.).

Trial	Phase	Total patients (No. with PCA)	Treatment	Primary end point	Results	Other outcomes	Adverse effects	Ref.
Wheler *et al.*	I	52	Escalating doses of bevacizumab (2.5–11 mg/kg) + oral VPA (5.3–10 mg/kg)	MTD	MTD for bevacizumab and VPA was 11 and 5.3 mg/kg, respectively	SD in eight of 52 patients (15%); four patients had SD > 6 months; one patient with prostate cancer had SD of 7.6 months. *VEGF* genotypes correlated with SD > 6 months	DLT in two patients as grade III altered mental status	[94]

[†]Study was prematurely terminated due to unavailability of ATRA-IV.

5-AZA: 5-azacytidine; ANC: Absolute neutrophil count; ATRA-IV: All trans retinoic acid; DLT: Dose-limiting toxicity; FEC: 5 Fluorouracil epirubicin cyclophosphamide; MTD: Maximum-tolerated dose; PBMC: Peripheral blood mononuclear cell; PCA: Prostate cancer; PK: Pharmacokinetics; SD: Stable disease; VPA: Valproic acid.

increasing VPA levels were associated with decreasing PSA levels. In addition, the duration of treatment correlated negatively with PSA levels. These findings were confirmed on multiple regression analysis that showed that total VPA levels ($p < 0.001$) and duration of treatment ($p = 0.002$) independently predicted decline in PSA levels. Since this study did not find any reliable histone acetylation in peripheral lymphocytes – a surrogate measure for tumor tissue – the observed effect of VPA could not be attributed to HDAC inhibition in this study. There were 11 incidents of grade I/II neurologic toxicities, which ranged from confusion, dizziness, fatigue and somnolence, to tremor and frank drowsiness. These effects, however, did not seem to be related to the dose or length of VPA treatment. In addition, constitutional symptoms, such as fatigue, were commonly seen. The toxicities not only led to unwarranted delays in treatment but also contributed to the high dropout rate from the trial. This study was therefore terminated since the tolerance of the study population for the drug was found to be poor and constant titration of the dose to maintain therapeutic blood levels was cumbersome. Although the study succeeded in showing that there was some PSA response to VPA, it was not predictable and durable enough to justify treatment of more CRPC patients on the trial.

Combination with other agents

In another Phase I trial, 44 patients with different solid tumors, including two patients with prostate cancer, received increasing doses of oral VPA (15–160 mg/kg/day) on days 1–3 followed by epirubicin (day 3) in 3-week cycles [53]. DLTs were predominantly neurovestibular in the form of hearing loss, confusion and dizziness seen in three patients. Febrile neutropenia was seen in one patient. Since most DLTs were documented in patients receiving VPA doses of 160 mg/kg/day, this study recommended a maximum VPA dose of 140 mg/kg/day, since higher dosing consistently resulted in grade II neurovestibular toxicity. In addition, DLTs in the form of diarrhea and myelosuppression were also noted. Reassuringly, VPA did not induce exacerbation of epirubicin-related myelosuppression and/or cardiac complications. Furthermore, a partial oncologic response was seen in nine patients (22%) while stable disease/minor responses were seen in 16 patients (39%). One of the two patients with prostate cancer had a partial response to treatment (VPA dose of 100 mg/m^2) with no witnessed DLT. Although the study initially employed intravenous infusions of VPA, it was switched to oral formulations to avoid unwarranted toxicities. It is noteworthy, however, that this resulted in more variable VPA peak levels. Biomonitoring of PBMC through flow cytometry and western blot analysis confirmed induction of histone

hyperacetylation with downregulation of HDAC in the majority of the patients. However, this study found that total and free VPA plasma concentrations correlated with VPA dose and histone acetylation in PBMC. Although not determined in this study, it has been previously demonstrated in some studies that acetylated histones in PBMC correlated well with acetylated histones in tumor cells [11,92]. This study showed that not only was the heavy dose of VPA well tolerated, it was successful in achieving some clinical response in patients who had failed prior chemotherapy and some tumors that were traditionally believed to be resistant to epirubicin. Inspired by the positive results obtained from this study, the investigators carried out a Phase I/II trial to further investigate the therapeutic effects of this agent. The results of this study have been discussed below.

The effect of oral VPA in combination with a DNA-methyl transferase inhibiting agent 5-azacytidine (5-AZA) was explored in a Phase I trial that enrolled 55 patients with advanced cancers, including two prostate cancer patients [55]. These patients were treated with escalating doses of 5-AZA (from 20 to 94 mg/m^2) along with VPA titrated to a plasma level of 75–100 µg/ml daily. The MTD of 5-AZA was determined to be 75 mg/m^2. DLTs were seen in the form of neutropenic fever (two out of six patients) and thrombocytopenia (one out of six patients) at a 5-AZA dose of 94 mg/m^2. Although no partial or complete responses were seen in any patients, the disease process stabilized in 14 patients for a median duration of 6 months. One of these patients had prostate cancer and had stable disease for 6 months after being treated with a dose of 75 mg/m^2. The study did not find any difference in DNA hypomethylation between stable patients and progressing patients, thus suggesting the possibility of histone acetylation contributing to the antitumor effect. Histone acetylation was seen with a higher frequency in patients achieving stable disease than those not achieving it (seven out of ten vs 13 out of 23; p = 0.0003). Interestingly, however, no evidence has been found on the relationship between response and histone acetylation in previous studies on hematologic malignancies [39,40]. The study was terminated after achieving its objective of determining the MTD and exploring the therapeutic effect of the combination regimen. Building from the successes of their previous trial, Munster *et al.* conducted a Phase I/II trial investigating the biological and molecular effects of VPA with epirubicin in 44 patients with advanced solid malignancies [52]. This trial also involved a dose-expansion phase with 5-fluorouracil and cyclophosphamide in 15 patients with breast cancer. DLT was seen in the form of somnolence in three out of 15 (20%) patients receiving 120 mg/kg/day VPA in the post-DLT period. Although the thrombocytopenic effect of VPA is well documented, it was found that the drug also caused a dose-dependent depletion of white blood cells (p = 0.0092 for free VPA) and absolute neutrophil count (p = 0.03 for free VPA). Since these effects were seen within 48 h after VPA administration, they were considered to be independent of epirubicin-induced myelosuppression, which is expected to occur 2 weeks later. There was a much steeper increase in serum free VPA levels from 140 to 160 mg/kg/day, most likely accounting for the drastic rise in DLT seen with that dose. An objective response was seen in nine out of 41 patients (22%) during the dose-escalation period. Although the dose-expansion phase did not incorporate prostate cancer patients, a tumor response was seen in 64% of patients. Histone acetylation in PBMCs correlated with VPA dose and serum levels. The investigators also reported a correlation between histone acetylation and HDAC2 expression (p = 0.0063 for H4 and 0.0427 for H3), but not with HDAC6 expression. Consequently, this study suggested that HDAC2 expression could potentially be used as a biomarker for advanced cancers treated with HDAC inhibitors.

A Phase I study aiming to study the role of VPA in conjunction with a liposomal all-trans retinoic acid (ATRA) analog, ATRA-IV enrolled nine patients with metastatic solid malignancies, including two patients with prostate cancer [56]. VPA levels of up to 80–100 µg/ml were achieved safely and ATRA-IV was sequentially given to these patients. However, the trial could not determine the MTD of VPA owing to the lack of commercial availability of ATRA-IV (which was discontinued), prompting the premature closure of the study. Most side effects seen with the studied concentrations of VPA were less than grade II and mostly related to skin toxicity or thrombocytopenia. The best response was seen in a patient with prostate cancer who had a serum VPA level of 80–100 µg/ml and sequentially received an ATRA-IV dose of 60 mg/m^2. The patient had stabilization of disease for 16 weeks, but progressed thereafter due to serum PSA elevation, and managed to complete four treatment cycles [39,40,55].

In a recent Phase I trial, 32 patients with advanced solid tumors refractory to standard therapy were treated sequentially with VPA and azacytidine in combination with carboplatin with stair-step dose escalation [93]. The MTDs were identified as azacytidine 75 mg/m^2, VPA 20 mg/kg, and carboplatin AUC 3.0. Minor responses or stable disease lasting ≥4 months was achieved by six patients (18.8%), including one patient with prostate cancer (11 months). Grade III/IV toxicities developed in 78% of patients. DLT were seen in six patients, in the form of grade III altered mental status in four patients, grade IV neutropenia, grade III fever and grade III anemia in one patient and grade IV neutropenia in one patient. Overall, the most common toxicities were

fatigue in 78%, neutropenia in 63%, anemia in 47%, nausea in 44% and thrombocytopenia in 41% of the patients.

In a novel approach to combine VPA with another chemotherapy agent, a Phase I trial with anti-VEGF monoclonal antibody bevacizumab enrolled 52 patients with advanced malignancies including prostate cancer [94]. Patients were treated with escalating doses of bevacizumab from 2.5 to 11 mg/kg on days 1 and 15, and oral VPA at doses 5.3–10 mg/kg on days 1–28 every 28 days [94]. During the dose escalation, two patients experienced a DLT in the form of grade III altered mental status. The MTD was found to be 11 mg/kg for bevacizumab, and 5.3 mg/kg for VPA. Oncologic response as stable disease was documented in eight (15%) of 52 patients with four (8%) patients having stable disease for more than 6 months. Of these, one patient had prostate cancer with a stable disease for 7.6 months. The investigators also reported increased H3 acetylation on day-15 of cycle 1 compared with baseline in nine (41%) of 22 patients tested, independent of the dose of VPA administered. Furthermore, H3 acetylation was seen in all tested patients with stable disease >6 months as compared with only 35% of those who did not have stable disease >6 months. In 26 patients tested for VEGF genotype versus others, VEGF-2578 CA and VEGF-2578/-1154 CA/GA genotypes, compared with the others, were associated with increased stable disease >6 months ($p = 0.03$ and $p = 0.02$, respectively). These results were presented at the annual American Society of Clinical Oncology Meeting in 2011 and further details are as yet not available.

Based on our success in preclinical studies, we had designed a Phase II randomized controlled trial with VPA in non-metastatic prostate cancer patients with biochemical progression [101]. Eligible patients were those with asymptomatic non-metastatic disease after radical prostatectomy. The most recent PSA had to be >1.0 ng/ml with a PSA doubling time (PSADT) of <10 months. The primary end point of our study was to assess whether treatment with VPA can alter the kinetics of PSA progression in these patients. Concomitantly, we aimed to determine the duration of PSA response, assess the percentage of patients who achieve complete and partial responses, and assess the quality of life (QoL) of these patients. While the observation arm received standard of care monitoring, arm II received VPA twice daily for up to 1 year in the absence of disease progression or unacceptable toxicity. Patients completed QoL questionnaires at baseline, 6 months and 1 year. The target enrollment of the trial was 50 patients. Patient recruitment on the trial was halted after completion of 15 patients, as a result of the principal investigator changing institutions. Eight patients were recruited to the standard arm (no treatment) and seven patients to the treatment arm (VPA). The PSA and VPA levels were measured every month for up to 1 year and the the differences in PSADTs between the two groups calculated. On interim analysis, participants in the VPA arm experienced a slower PSA progression than the participants in the standard arm [unpublished results]. The mean on-study PSADT for VPA patients was higher than for standard patients, with values of 35.63 months and 6.02 months, respectively ($p = 0.16$). Furthermore, the mean difference between pre- and on-study PSADTs for VPA patients was 30.62 months in contrast to 0.59 months for standard patients ($p = 0.11$). In fact, four of seven VPA patients had an on-study PSADT that was greater than 10 months, which is indicative of a good prognosis; on the other hand, only one of seven standard patients had a good prognosis. QoL was assessed using FACT-P questionnaire [95], which was filled out by the participants at the beginning, middle and the end of the trial answering questions about their physical, emotional, social, functional, and prostate-specific well being. Most patients on VPA (four out of six) did not experience any change in QoL; however, two patients had a drop in their QoL. On plotting the total QoL scores of participants against their VPA levels, it was found that there was no correlation between the two variables. In addition, there was no correlation between blood VPA levels and fold change in PSA. In conclusion, our preliminary results showed that treatment of men with non-metastatic biochemical recurrence increases their PSA progression time. However, the results did not reach statistical significance because of inadequate power of the cohort, as the trial had to be prematurely halted due to relocation of the principal investigator of the trial.

The clinical trials discussed so far have utilized VPA either alone or in combination with certain agents that employ key intracellular pathways including DNA hypomethylation, DNA-damaging chemotherapy, and antiangiogenesis. Other agents with diverse mechanisms of action can be exploited in synergy with VPA, including hormonal therapy, Src inhibitors, PI3K inhibitors and tyrosine kinase inhibitors. A number of ongoing studies continue to explore the efficacy of combining VPA with various other antineoplastic drugs for the treatment of solid tumors (Table 2). The results of these studies have not been reported yet. Many of these employ combination with multiple drugs using different treatment schedules in an attempt to optimize the treatment regimen to derive maximal clinical benefit. Overall, these studies have not only shown that VPA in combination with other agents is well tolerated, but that it can produce clinical response in selected cases. However, these studies suffer from obvious drawbacks. Clinical evaluation so far is limited to data gathered from

Table 2. List of clinical trials exploring the role of valproic acid in solid tumors with a focus on prostate cancer.

NCT identifier	Title	Phase	Status	Ref.
NCT01552434	A Phase I trial of bevacizumab, temsirolimus alone and in combination with valproic acid or cetuximab in patients with advanced malignancy	I	Recruiting	[102]
NCT00496444	Phase I study of low-dose hypomethylating agent azacitidine combined with the HDAC inhibitor valproic acid in patients with advanced cancers	I	Completed	[103]
NCT00495872	A multi-arm complete Phase I trial of valproic acid-based 2-agent oral regimens for patients with advanced solid tumor	I	Ongoing but not recruiting	[104]
NCT00530907	Phase I study of valproic acid given in combination with bevacizumab in patients with advanced cancer to determine safety and tolerability	I	Recruiting	[105]
NCT00670046	Randomized, controlled Phase II study of valproic acid in patients with non-metastatic biochemical progression of prostate cancer	II	Terminated due to investigator relocation	[101]
NCT00404508	A Phase II study of epigenetic therapy with hydralazine and magnesium valproate to overcome chemotherapy resistance in refractory solid tumors	II	Completed	[106]

early-phase trials with only limited numbers of patients. No data are yet available from Phase III randomized placebo-controlled trials. Furthermore, patient cohorts comprising heterogeneous cancers limit the strength of any statistical inferences that can be possibly derived from these studies. Most of these trials have been on solid tumors with very few prostate cancer patients, limiting the applicability of the clinical data that have been gathered from them. In fact, the total number of prostate cancer patients on all trials combined is very few to derive robust clinical judgments for use in prostate cancer especially. More trials with higher number of patients composed of homogeneous patient groups are required in the future to allow any conclusions to be drawn with certainty about the efficacy of VPA in prostate cancer. Furthermore, the correlation between histone acetylation, VPA levels, and clinical response is inconsistent and varies between studies. This might be due to variability in the sensitivity of the assays used for experimentation. This might also suggest that the true effects of VPA might be downstream from histone acetylation and warrants further molecular analysis. Moreover, most studies have looked at histone acetylation in PBMCs and not in tumor cells. The correlation between acetylation seen in PBMCs and solid tumor cells has not been adequately evaluated and needs further characterization.

Vorinostat is another promising agent that has been used with some success in prostate cancer. It has been shown to target prostate cancer cell lines *in vitro* by reducing AR mRNA, an effect that is further amplified by androgen deprivation [83]. Further studies have shown that vorinostat retains this property in CRPC models as well, an effect that has considerable clinical merit [84]. This HDAC inhibitor was tested in a Phase I trial with docetaxel for patients with advanced and relapsed prostate cancer [85]. However, the drug combination was poorly tolerated and the trial had to be stopped due to excessive toxicity. Another trial used vorinostat in 27 advanced prostate cancer patients progressing on prior chemotherapy but it was associated with significant toxicity as well as limiting efficacy assessment [86]. The median overall survival was 11.7 months and best response was stable disease in two patients (7%). Vorinostat continues to be a promising agent and warrants further investigation for possible therapeutic application in prostate cancer.

Conclusion

With a host of next-generation HDAC inhibitors on the horizon, it is high time that the optimal dosing of VPA with or without other chemotherapy drugs is effectively evaluated. Despite the initial optimism generated by successful preclinical studies, it has not translated into success on clinical trials. The enrollment on these trials is heterogeneous with few prostate cancer patients. Most of these trials have not shown any significantly impressive results. Alhough there have been a few successes along the way, they are few and far between. Only a very small percentage of patients on these trials have derived oncologic benefit. Moreover, clinical benefit with VPA in patients with chemoresistant prostate cancer has been even lower. Although not proven convincingly, it is likely that VPA produces its antitumor effect by inducing histone acetylation in tumor cells. These studies are limited by small patient populations and, therefore, more studies at multiple centers with larger number of patients are warranted to conclusively establish the utility of VPA in these patients. It does seem that the efficacy of VPA as a single agent is limited in solid tumors but can be exploited in combination therapy with other agents to produce significant antitumor effect with possible application in CRPC patients. Further investigation of other agents that might act synergistically with this agent to induce robust gene expression is needed. Another area that needs further evaluation is the development of

biomarkers. With the availability of multiple agents targeting various pathways, the ability to predict which therapy or combination of therapies is going to work is paramount. However, the precise combination of drugs, timing, dosage and sequence of administration still remains to be elucidated through well-designed trials.

Future perspective

VPA still remains an exciting prospect in the armamentarium against solid tumors, especially for prostate cancer. The clinical efficacy of VPA as a single agent from data generated thus far seems to be limited. However, results from some of these clinical trials are promising, especially those that employed VPA in combination with certain other chemotherapy agents. It is the authors' opinion that, although there has been some loss of interest in the field, it shall continue to remain an area of active research over the next several years. Multiple clinical trials employing HDAC inhibitors, including VPA for efficacy in prostate cancer, are underway and some success is expected over the forthcoming years. It goes without saying that a large proportion of these trials would involve combination with traditional and novel agents, especially those that have shown survival benefit in patients with metastatic CRPC. A lot of ground needs to be gained in development of novel biomarkers for assessing response and/or recurrence and considerable research efforts are expected to be focused in this area. The next decade of research will be critical in judging whether HDAC inhibitors have any clinical applicability in solid tumors, including prostate cancer, and will help us in determining the exact combination of drugs, doses and their timing to derive maximum clinical benefit in these patients.

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Executive summary

Evidence from preclinical studies

- Prostate cancer cells seem to be vulnerable to valproic acid, an antiepileptic agent that possesses HDAC-inhibiting properties.
- Valproic acid targets tumor growth by multiple mechanisms including cell-cycle arrest, cellular differentiation and tumor vasculature growth inhibition. It might also enhance sensitivity of prostate cancer cells to other antitumor agents.

Evidence from clinical studies

- Phase I and II trials employing valproic acid as a single agent have had some degree of success in achieving clinical response, although it is limited. Only a few studies involving small patient populations have been conducted.

Combination with other agents

- Multiple studies involving combination therapy with other agents have been successful in effectively determining maximum-tolerated dose and dose-limiting toxicities of this agent.
- Studies have shown variable degrees of clinical efficacy in achieving partial responses in patients with prostate cancer treated with various combination agents. The therapeutic benefit derived is, however, limited and more clinical trials are underway to develop an efficacious chemotherapy regimen.
- Development of biomarkers to assess response and for purposes of surveillance remains an area of active research.

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