Clinical Cancer Research

Epigenetic Therapy with Panobinostat Combined with Bicalutamide Rechallenge in Castration-Resistant Prostate Cancer

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Abstract

Purpose: This study assesses the action of panobinostat, a histone deacetylase inhibitor (HDACI), in restoring sensitivity to bicalutamide in a castration-resistant prostate cancer (CRPC) model and the efficacy and safety of the panobinostat/bicalutamide combination in CRPC patients resistant to second-line antiandrogen therapy (2^{nd}LAARx).

Patients and Methods: The CWR22PC xenograft and isogenic cell line were tested for drug interactions on tumor cell growth and on the androgen receptor (AR), AR-splice variant7, and AR targets. A phase I trial had a 3×3 panobinostat dose-escalation design. The phase II study randomized 55 patients to panobinostat 40 mg (A arm) or 20 mg (B arm) triweekly $\times 2$ weeks with bicalutamide 50 mg/day in 3-week cycles. The primary endpoint was to determine the percentage of radiographic progression-free (rPF) patients at 36 weeks versus historic high-dose bicalutamide.

Results: In the model, panobinostat/bicalutamide demonstrated synergistic antitumor effect while reducing AR activity. The dose-limiting toxicity was not reached. The probabilities of remaining rPF were 47.5% in the A arm and 38.5% in the B arm, exceeding the protocol-specified threshold of 35%. The A arm but not the B arm exceeded expectations for times (medians) to rP (33.9 and 10 weeks), and from PSA progression to rP (24 and 5.9 weeks). A arm/B arm events included: adverse events (AE), 62%/19%; treatment stopped for AEs, 27.5%/11.5%; dose reduction required, 41%/4%. The principal A-arm grade ≥ 3 AEs were thrombocytopenia (31%) and fatigue (14%).

Conclusions: The 40 mg panobinostat/bicalutamide regimen increased rPF survival in CRPC patients resistant to 2^{nd}LAARx. Panobinostat toxicity was tolerable with dose reductions. Epigenetic HDACI therapy reduces AR-mediated resistance to bicalutamide in CRPC models with clinical benefit in patients. The combination merits validation using a second-generation antiandrogen.

Introduction

The lineage dependence of prostate cancer on the androgen receptor (AR) pathway (1, 2) accounts for the therapeutic efficacy of castration (3) and for the response to antiandrogen therapy after the development of castration resistance. However, only a fraction of castration-resistant prostate cancer (CRPC) patients responded to first-generation antiandrogens (bicalutamide, flutamide, and nilutamide) or the adrenal androgen synthesis blocker ketoconazole, and the median time to secondary progression was 6 to 8 months (4, 5). "Re-challenge" treatment with the same or an alternative antiandrogen after progression on the first second-line antiandrogen treatment (2^{nd}LAARx) yielded fewer responses and progressively shorter times to succeeding progression (5, 6). Although, second-generation antiandrogens (enzalutamide and apalutamide) or the adrenal androgen synthesis blocker abiraterone acetate (7–10) significantly delays progression and increases overall survival, resistance inevitably follows, and the response to taxanes is short (11). Therefore, preventing or reversing resistance to AR-directed agents may potentially block lethal pathways of progression and prolong survival.

The development of resistance to AR-targeted agents involves a host of genomic alterations that directly affect AR gene structure and function (12–16) as well as cross-talk with growth factor signaling pathways (17–19). The most prevalent *AR* alteration is overexpression (12–14), which is necessary and sufficient to lead to CRPC progression and resistance to antiandrogens (20). AR mutation frequency is low, but treatment with antiandrogens can select for distinct mutations in the ligand-binding domain (LBD) that confer resistance and drive AR activity but not cross-resistance to other antiandrogens (21–25). Ligand-independent AR splice variants (ARSv)

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Translational Relevance

The development of resistance to androgen pathway–targeted agents is central to prostate cancer lethality. Preclinical studies indicate that this drug-resistant state can be attenuated or reversed by epigenetic modulation. We propose that to achieve this requires the real-time copresence of the epigenetic effector and resistance agent. The relevance of this hypothesis in castrate-resistant prostate cancer (CRPC) was tested in a phase I/II trial of the histone deacetylase inhibitor panobinostat in combination with continuous bicalutamide in patients who had secondarily progressed on bicalutamide and/or other first-generation antiandrogen(s). The results provide evidence that compared with historic controls treated with bicalutamide alone, the combination of bicalutamide with high-dose panobinostat administered intermittently delayed radiographic progression of the disease; toxicity was not limiting, although dose reductions were required to extend treatment. Our results provide initial proof-of-concept support for the potential effectiveness of epigenetic therapy to reverse/delay antiandrogen-resistant CRPC and warrant confirmation with currently available, more potent antiandrogens to further increase their survival benefits.

are also enhanced by antiandrogen treatment including bicalutamide, and they frequently sprout in response to second-generation antiandrogens (26–28). More importantly, the overexpression of ARv7 has been shown to accelerate CRPC progression via a distinct mitosis-dependent transcriptome program that imparts cross-resistance to antiandrogens (29–32). These observations of the importance of AR overexpression in antiandrogen resistance suggest that aberrant regulatory mechanisms could play an important role. Thus, it is of interest that prostate cancer cells have the potential to rapidly adapt to an adverse environment by modulating the dynamics of gene expression without necessarily acquiring new DNA mutations (33). Such adaptability allowed a subpopulation of drug-tolerant prostate cancer cells to develop and persist despite lethal drug exposure, which, remarkably, could be reverted to drug sensitive by continuous exposure to an epigenetic modulator, specifically, a histone deacetylase inhibitor (HDACI; ref. 34).

Epigenetic mechanisms, such as chromatin acetylation and histone methylation, are critically involved in AR mRNA transcription and splicing, as well as AR protein functions, (30, 35). The balance between histone acetylase and HDAC enzymes is crucial for fine-tuning transcriptional regulation of the AR and its downstream targets (36, 37). More broadly, HDACIs target the acetylation status of both histone and nonhistone proteins. For example, in AR-negative DU145 and PC3 cells, chronic treatment with valproic acid decreased proliferation and increased caspase-2 and caspase-3 activation (38). Alternatively, in AR-positive LNCaP cells, increased acetylation of heat shock protein 90, as a consequence of exposure to the HDACI LAQ824, led to proteasome-mediated AR degradation, reduction of PSA, inhibition of proliferation, and induction of apoptosis (39). Thus, HDACIs can affect the expression and function of multiple tumor-relevant pathways involved in disease progression and drug resistance, including those not controlled by AR (30, 35, 40, 41).

In prostate cancer cells, both HDAC1 and HDAC3 are required for optimal transcriptional modulation of nearly all AR target genes (42), although HDAC1 alone can directly interact with AR protein, to produce transcriptional repression (39). By disrupting transcriptional activation, the HDACIs LBH589 (panobinostat) and suberoylanilide hydroxamic acid (SAHA, Vorinostat) decreased AR mRNA synthesis and repressed transcriptional activation of the AR target genes *PSA* and *TMPRSS2* (42). In CRPC cell lines with AR overexpression, PSA expression was inhibited in a dose-dependent manner: 30%, 50%, and 85%, respectively, by 10, 20, and 40 nmol/L panobinostat (42). These findings suggested that patients with AR-driven CRPC may require higher doses of panobinostat monotherapy for an antitumor response than the dose approved for use in myeloma (43). In a clinical setting, this could be achieved using an intermittent oral schedule to decrease toxicity (42). In addition, given the effect of panobinostat on AR transcriptional activity and, also, PSA expression in model systems, it was proposed to use PSA changes as a correlative biomarker to monitor the effect of HDACI treatment on the AR pathway and to guide dose selection in clinical trials (42).

Using cell line prostate cancer models, we previously explored the hypothesis that resistance to bicalutamide can be overcome by concomitant administration of panobinostat (20). We demonstrated that the HDACIs trichostatin-A and panobinostat effectively reduced overexpressed AR mRNA and protein levels in an androgen-independent, bicalutamide-resistant LNCaP derivative (44, 45). Further, although bicalutamide alone was ineffective, when combined with panobinostat, the combination induced both synergistic growth arrest and apoptosis greater than panobinostat alone (46). These results suggested that panobinostat reverses resistance to bicalutamide and that both are necessary to achieve an antiproliferative effect. We now show that this drug combination is also effective in an additional prostate cancer model, namely a paired isogenic *in vitro/in vivo* system: the 22Rv1 prostate cancer cell line and the CWR22PC xenograft which express high levels of ARv7 relative to full-length (fl) AR and are resistant to both bicalutamide and enzalutamide (47–49). Together, our preclinical studies prompted us to test this concept in a phase I/II trial in CRPC patients after progression on 2ndLAARx.

Patients and Methods

Cell line culture, establishment of tumor xenografts, and methods to measure growth, targets expression, and tumor response are described in detail in Supplementary Materials and in Fig. 1. In brief, 22Rv1 cell cultures treated 72 hours with serial dilutions of panobinostat, bicalutamide, or both were used to evaluate viability, to identify the IC_{50} and determine the combination index effect. After 24-hour treatment at preselected doses of the agents, expression of flAR, ARv7 PSA, and TMPRSS2 was measured in extracted mRNA by qT-PCR. CWR22PC tumor xenografts growing in castrate nude mice were used to measure growth and toxicity during 19 days of treatment with panobinostat, bicalutamide, or both (IACUC #090705-01).

Patients

Eligible patients had one or more CRPC progression events while receiving 2ndLAARx agents. Prior docetaxel was permissible.

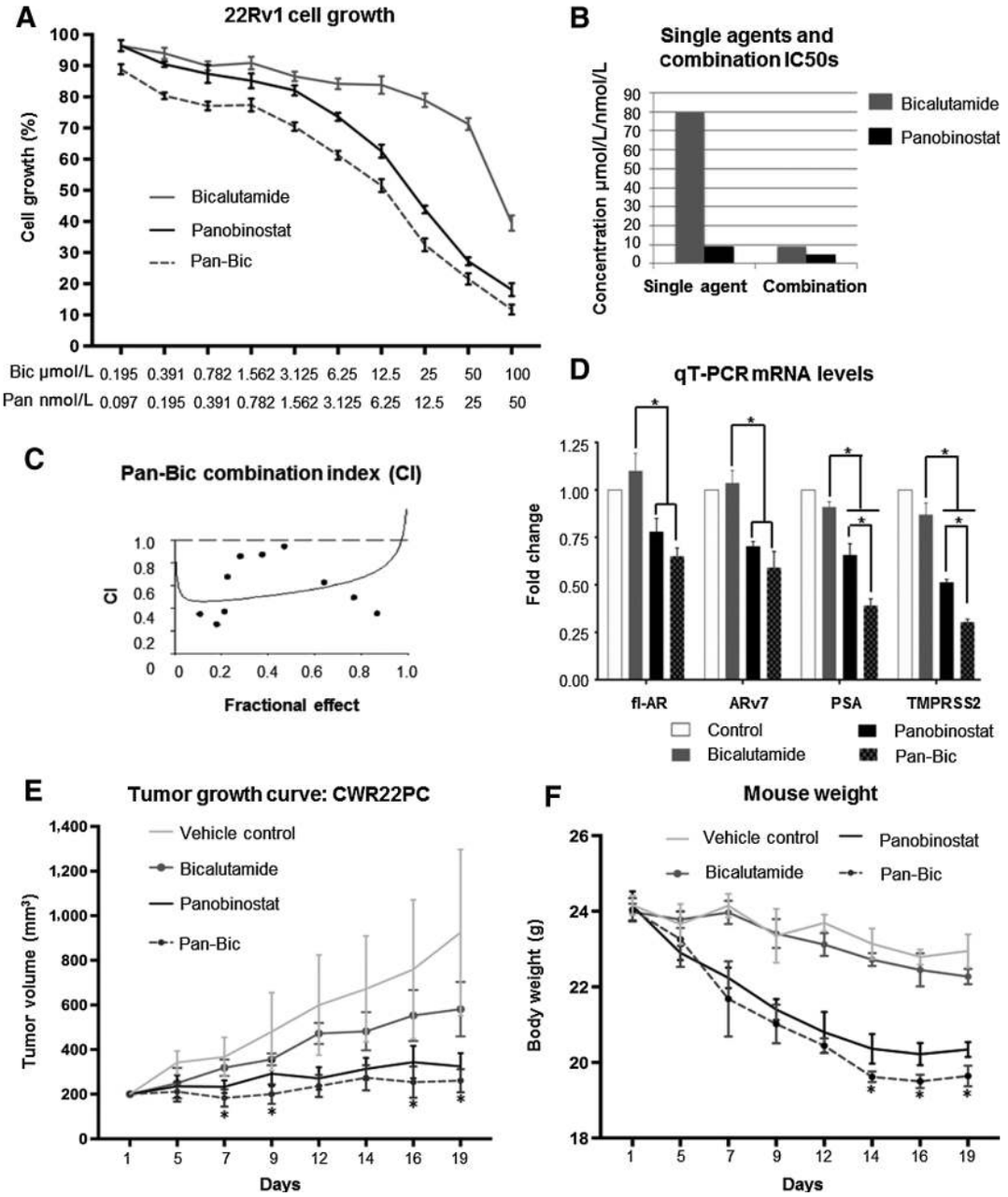

Figure 1.

Panobinostat reverses resistance of androgen deprivation-resistant prostate cancer cells to the antiandrogen bicalutamide by reducing the mRNA expression levels of spliced (ARv7) and flAR and downstream targets. **A,** Dose-response curves of serial dilutions of panobinostat (50 to 0.097 nmol/L), bicalutamide (100 to 0.195 µmol/L), and the combination of panobinostat with bicalutamide (Pan-Bic) on the viability of 22Rv1 cell after 72-hour treatment measured by MTT assay. **B,** Representation of the IC_{50} of either panobinostat, bicalutamide, or the combination in 22Rv1 cells. **C,** Isobologram of serial dilutions of panobinostat combined with bicalutamide shows a synergistic effect (combination index below 1 by Chou–Talalay method) at all concentrations. **D,** mRNA levels of flAR, ARv7, PSA, and TMPRSS2 in 22Rv1 after 24-hour treatment with panobinostat 10 nmol/L, bicalutamide 12.5 µmol/L, or the combination measured by qT-PCR. Asterisks denote statistically significant differences (P value < 0.05). **E,** 2×10^6 CWR22PC cells in 50 µL of Matrigel were s.c. injected into the flank of castrated athymic NCr nude mice ($n = 40$) in the presence of dihydrotestosterone (DHT) pellets. When tumors reached 200 mm^3, DHT pellets were extracted, and after documented tumor growth in castrate conditions, mice were randomized to treatment groups, 10 mice each. Mice were treated with bicalutamide 20 mg/kg p.o. daily (oral gavage), panobinostat 10 mg/kg i.p. on days 1 to 5 every 7 days, or the combination for 19 days. Tumor volume was measured at the indicated time points throughout the duration of the study. Asterisks denote statistically significance of the differences in tumor volume between panobinostat and the combination at the specified time points. **F,** Tumor-bearing mice were weighed at indicated time points throughout the duration of the study. Asterisks denote statistically significant difference just between panobinostat and the combination.

Required Eastern Cooperative Oncology Group performance status (ECOG PS) was 0–2. Complete eligibility criteria are outlined in the study protocol. The study was conducted according to the provisions of the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Conference on Harmonization. All patients provided written-informed consent before participating

Study design and endpoints

The phase I had a 3 × 3 dose-escalation design aimed to determine the MTD or a high-tolerable dose of oral panobinostat combined with continuous bicalutamide. The schedule of panobinostat was intermittent in cohorts 1 and 2 (2 weeks-on/1 week-off schedule) and without interruption in cohort 3. The phase II study randomized patients to test the combination with panobinostat at the MTD or highest-tolerable dose (A arm) and at a 50% lower dose (B arm) on an intermittent schedule to minimize toxicity and prolong administration, considered necessary for epigenetic "reprogramming" and antitumor activity (Fig. 2; ref. 34).

The primary endpoint was to determine the proportion of radiographic progression-free (rPF) patients after 36 weeks (9 months) of rechallenge with bicalutamide in combination with the two dose levels of panobinostat in order to select the most tolerable and active regimen based on Prostate Cancer Working Group 2 (PCWG2) recommendations (50). We chose the rPF at a fixed time point to gather pilot data as to whether the new combinations had the potential to achieve a clinically meaningful benefit to consider further testing (51). We also measured PSA levels frequently to evaluate the dynamics of response and to assess PSA as a correlative biomarker for monitoring the effect of panobinostat/bicalutamide and for guiding dose selection (42). The effectiveness of panobinostat in restoring tumor sensitivity to bicalutamide would be assessed by comparison with the activity of rechallenge 2ndLAARx in a historic study using bicalutamide (200 mg daily), where the PSA response rate was 26% and the time to PSA progression (≥50% PSA increase) was 3 to 4 months in patients who had progressed after first antiandrogen therapy (52). No data were available in earlier studies based on radiographic progression.

Secondary endpoints included safety and PSA changes. At the time of this pilot study design, PSA was the recommended correlative biomarker to monitor panobinostat activity on AR and to guide dose selection in clinical trials (34). The other available biomarker to monitor HDAC activity was accumulation of acetylated histones in peripheral blood mononuclear cells, but changes had no correlation with antitumor activity in metastatic CRPC (mCRPC; ref. 42). This study is registered in www.clinicaltrials.gov as NCT00878436.

Treatment

Phase I patients received bicalutamide 50 mg/day. The panobinostat triweekly p.o. doses were 20 mg (60 mg/week)

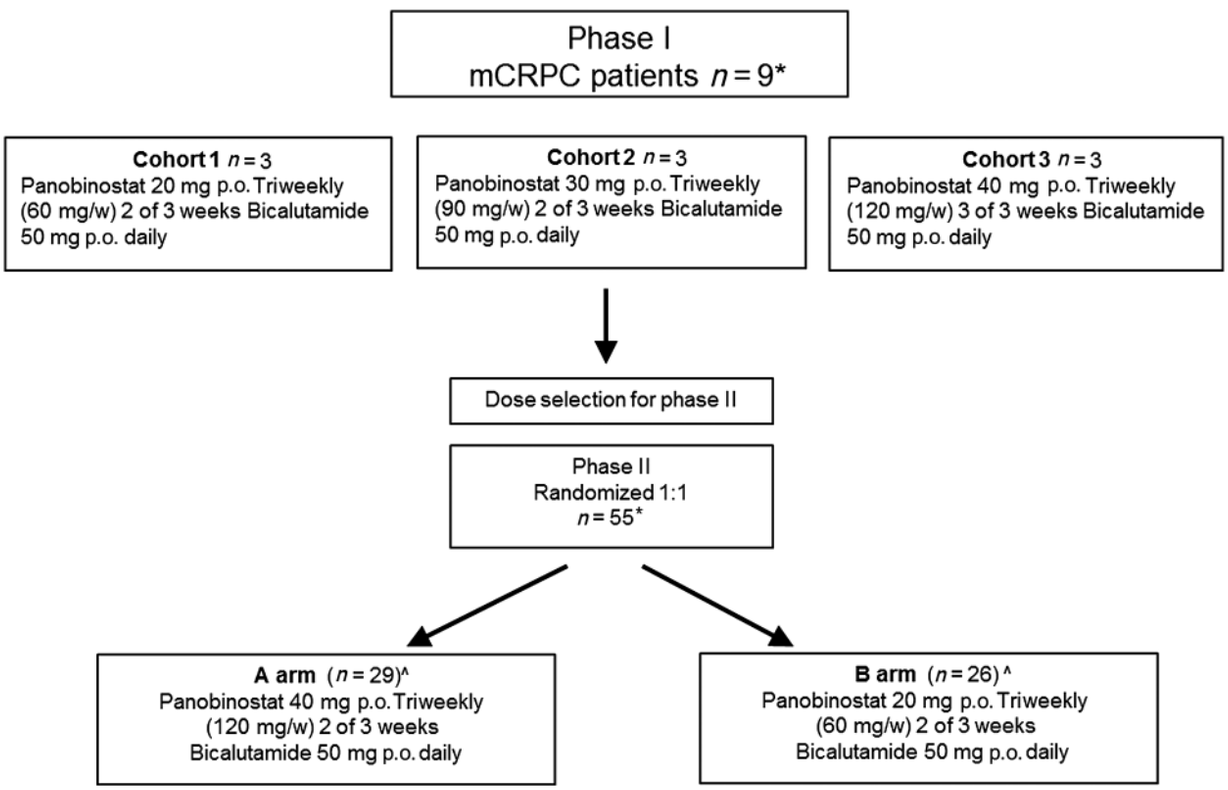

Figure 2.

CONSORT diagram. Dose A arm 40 mg p.o. triweekly × 2 weeks q 3 weeks, B arm 20 mg p.o. triweekly × 2 weeks q 3 weeks; *, safety evaluable all cases; ^, having received <2 cycles of therapy: A arm, 7; B arm, 5.

and 30 mg (90 mg/week) for 2 of 3 weeks in cohorts 1 and 2, respectively, and 40 mg (120 mg/week) for 3 of 3 weeks in cohort 3. Each cycle was repeated every 21 days. Novartis Pharmaceuticals provided oral panobinostat as hard gelatin capsules. The panobinostat MTD was defined as the highest dose at which no more than 1 of 6 patients experienced dose-limiting toxicity as defined by protocol.

Phase II patients were randomized 1:1 to panobinostat p.o. triweekly, 40 mg (A arm) or 20 mg (B arm), with bicalutamide 50 mg daily for 2-week of 3-week cycles. The choice of 40 mg dose was based on the preclinical evidence discussed above indicating that suppression of AR-driven CRPC by panobinostat required significantly higher concentrations than achieved by the 20 mg dosing (42) and on unpublished Novartis preclinical data in other solid tumors. To maximize long-term tolerance, we planned to decrease toxicity with an intermittent dosing schedule (triweekly, 2 weeks of 3 weeks cycles) and to allow two dose reductions for first and second adverse events (AE): A arm, 10 mg and 5 mg; B arm 5 mg each. Interruptions of $\leq$4 weeks were allowed.

Assessment

Treatment response was monitored by serum PSA every 3 weeks, bone scan and CT or magnetic resonance imaging of the abdomen and pelvis every 12 weeks, or as clinically indicated. Treatment tolerability required baseline electrocardiograms on days 1 and 2 of cycle 1 and monitoring every cycle, using criteria established by NIH-NCI Common Terminology Criteria for Adverse Events, version 3.0 CTCAEv3.0 (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). Response assessment followed PCWG2 criteria (50). PSA rises were not considered sufficient to remove patients from study in the absence of radiographic progression, clinical deterioration, or unacceptable toxicity.

Statistical analysis

The primary statistical objective was to assess, independently for each arm, the percentage (P) of patients that remained free of both the appearance of new or worsening radiographic metastasis and PSA progression for 9 months (36 weeks). A regimen would not be considered worthy of further investigation if the P was $\leq$15% and would be of clinical interest if P was $\geq$35%. The maximum sample size for each arm of the study was estimated to be 33 patients in order to achieve 95% power at the 20% significance level (53). Taking into account patient attrition on protocol, a Kaplan–Meier survival analysis was performed to estimate the probability of radiographic and PSA $\geq$25% progression-free survival (rPFS, PSA, 25%; ref. 54). Secondary objectives included safety and tolerability and best PSA response. No formal comparison between the two arms was planned.

Results

Panobinostat/bicalutamide-induced inhibition of growth, AR/ARv7 expression, and AR targets in 22Rv1 cells and CWR22PC xenograft

The effect of bicalutamide and panobinostat alone compared with the combined effect of the two agents on the growth of the AR+/ARv7+ 22Rv1 prostate cancer cell line was tested by serial dilutions (bicalutamide, 100–0.195 μmol/L; panobinostat, 50–0.097 nmol/L; Fig. 1A). Greater than 75% control cell growth was observed with exposure up to 50 μmol/L bicalutamide alone (IC_{50}, 80 μmol/L; Fig. 1B), indicating resistance. Panobinostat alone was inhibitory (IC_{50}, 9 nmol/L; Fig. 1B). The combination of panobinostat and bicalutamide was synergistic at all dose levels (Fig. 1C), with the IC_{50} dropping 2-fold for panobinostat (9 to 4.4 nmol/L) and 10-fold for bicalutamide (80 to 8.8 μmol/L). Tested at an approximate 50% growth-inhibitory concentration (10 nmol/L), panobinostat significantly reduced AR, ARv7, and 2 AR target gene (PSA, TMPRSS2) mRNA levels (Fig. 1D). When combined with 12.5 μmol/L bicalutamide, which alone was minimally growth inhibitory and had no effect on all 4 mRNA levels, 10 nmol/L panobinostat further reduced these mRNA levels compared with panobinostat alone, including nearly 2-fold reduction of the 2 AR target genes (P = 0.082 and <0.001, respectively).

To confirm the activity of the combination *in vivo*, we treated CWR22PC xenografts in castrate mice for a short period (19 days) with panobinostat, 10 mg/kg i.p. on days 1 to 5 weekly, and bicalutamide, 20 mg/kg by oral gavage daily, doses that are estimated to be active and achievable in humans (Novartis database). A similar pattern of significant tumor growth inhibition to that observed in the 22Rv1 cell line was observed with panobinostat alone or in combination with bicalutamide compared with bicalutamide alone (Fig. 1E); albeit less pronounced, inhibition was also significantly increased by the combination compared with panobinostat alone. Prolonged follow-up of a small cohort of xenografts treated with panobinostat alone showed that tumor growth was delayed by an additional 3 weeks over bicalutamide alone before the mice were sacrificed (data not shown). The toxicity of panobinostat alone or in combination, as defined by animal weight, was also significantly increased compared with bicalutamide alone (Fig. 1F). Although toxicity was higher with panobinostat, this was neither lethal nor did it compromise treatment up to 6 weeks.

Phase I

Nine CRPC patients were enrolled: median age, 65 years; median PSA, 9.26 ng/mL; bone metastases, 6 patients. The median number of panobinostat/bicalutamide cycles was 6. There were no G4 AEs. G3 AEs occurred predominantly in cohort 3, including 2 patients with thrombocytopenia without bleeding in cycles 1 to 3 at the end of the second week, which resolved spontaneously and/or were controlled by dose reduction. G1–2 AEs included thrombocytopenia 5, fatigue 5, hypothyroidism 2, dyspepsia 3, and anorexia 2. Dose-limiting toxicity was not observed; the MTD was not reached. Four cases had PSA decline from baseline: 1 in cohort 1, 60%; 3 in cohort 3, 56%, 17%, and 13% (55).

A 40 mg p.o. intermittent panobinostat dosing schedule (triweekly for 2 weeks with 1-week rest) was chosen as the highest oral dose that could be tolerated for prolonged administration based on the Novartis team experience with dose escalation in other solid tumors, indicating that higher or uninterrupted 40 mg doses would not be safe and tolerable.

Phase II

Patient characteristics. From April 2010 to October 2014, 56 CRPC patients were enrolled and 55 patients were randomized to the A arm (n = 29) or B arm (n = 26; Table 1). Median prior 2ndLAARx therapies were A arm, 2; B arm, 3; median age, 69 years;

Table 1. Baseline patient demographics and characteristics by treatment arm

Characteristics	Arm A *n* = 29	Arm B *n* = 26	All *n* = 55
Age in years, median (range)	69 (48–84)	69 (50–83)	69 (48–84)
Races			
White	25 (45%)	25 (45%)	50 (90%)
Black	3 (5%)	1 (2%)	4 (7%)
Asian	1 (2%)	0 (0%)	1 (2%)
Gleason score, *n*			
6–7	9 (16%)	13 (24%)	22 (40%)
8–9	17 (31%)	13 (24%)	30 (55%)
ECOG PS, *n*			
0	25 (45%)	24 (44%)	49 (89%)
1	5 (9%)	1 (2%)	6 (11%)
PSA ng/mL, median (range)	15.5 (0.56–392)	49.9 (2.1–583)	20 (0.56–583)
Measurable disease LN, *n* (%)	16 (55%)	20 (78%)	36 (65%)
Bone disease, *n* (%)	16 (55%)	17 (65%)	33 (60%)
No metastasis	2 (4%)	1 (2%)	3 (5%)

white, 50; African American, 4; Asian, 2. The median baseline PSA was: A arm, 15.5 ng/mL (range, 0.6–392); B arm, 49.9 ng/mL (range, 2.1–583). The Gleason score was 8 to 9 in 19 A-arm and 14 B-arm cases. All but 3 cases (94.5%) had mCRPC, involving bone, 55% and 65%, and/or lymph nodes (no visceral metastasis), 57% and 78%, A and B arms, respectively.

Treatment course. In both study arms, there were early treatment discontinuations by the first protocol checkpoint at 12 weeks (Fig. 3). In the A arm, the most frequent causes for early discontinuation were AEs or withdrawal for reasons other than disease progression (12 cases). In contrast, in the B arm, early discontinuation was mostly due to disease progression events: PSA increase, 4 cases; radiographic progression, 10 cases. On the A arm, the incidence of withdrawals for adverse/other events decreased after 12 weeks related to panobinostat dose reductions. On the B arm, there were no further dose reductions and only 1 withdrawal for an unusual case of stage I Kaposi sarcoma in an HIV-negative patient, with otherwise radiographic stable disease (SD). Overall, protocol discontinuation for disease progression-attributed causes occurred in 12 A-arm cases (4 PSA rises, 8 radiographic progressions) and 22 B-arm

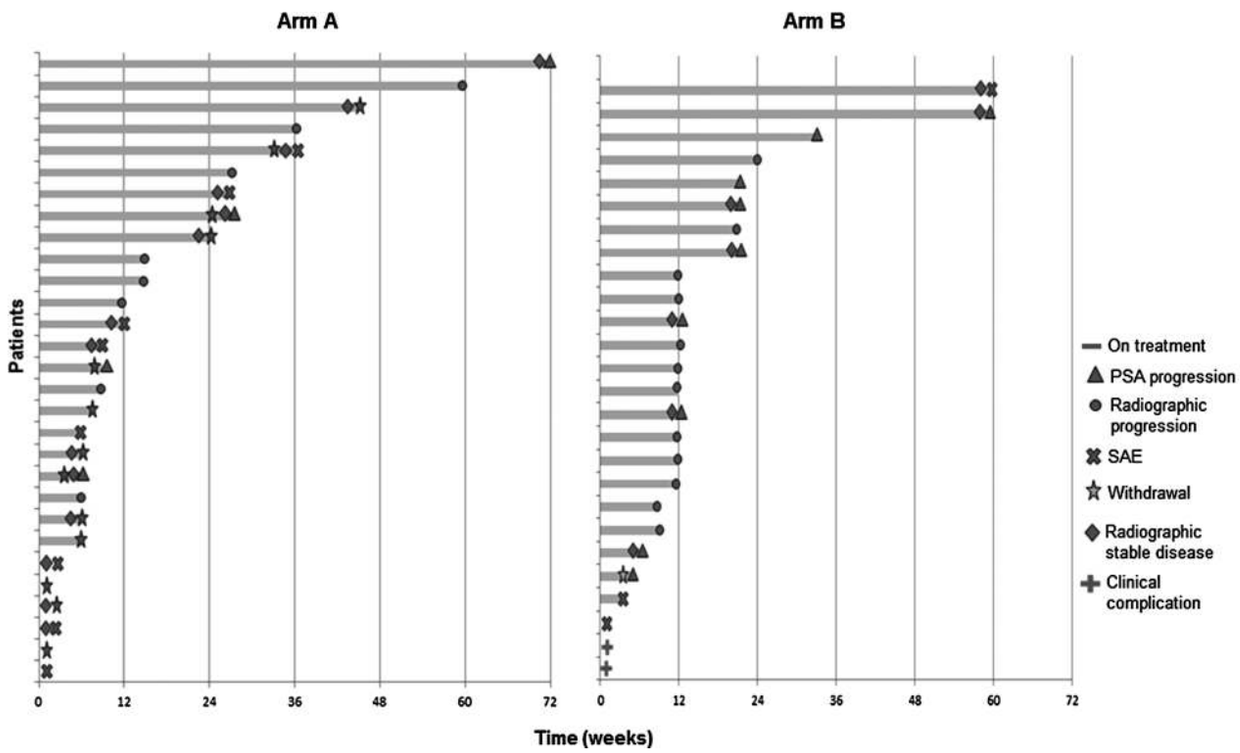

Figure 3.
Swim lane plots illustrating individual patient experience and treatment discontinuation reasons on arms A and B.

cases (10 PSA rises, 11 radiographic progressions). After relatively long protocol treatment (>24 weeks), 5 A-arm and 2 B-arm patients discontinued the protocol with radiographic SD without serious AEs or death.

Efficacy

PSA assessment. Assessed as best response, a PSA decline of >50% was observed in 2 patients in each arm, and a decline of >30% was observed in 5 A-arm and 4 B-arm patients (Fig. 4A). In total, a PSA decline from baseline was observed in 12 A-arm patients (44%) and 7 B-arm patients (28%). Over the entire treatment course, all of 47 evaluable patients (8 patients censored) experienced PSA progression (Fig. 4B). The median time to PSA progression was 9.4 weeks and 6.3 weeks, respectively, for the A and B arms. The time to PSA progression was unusually prolonged to 40 and 60 weeks in 2 B-arm patients. At the 12-weeks-on-treatment PSA checkpoint, 31 patients were evaluable (13 A-arm and 18 B-arm patients): 5 A-arm and 3 B-arm patient PSA values were below baseline; 4 A-arm (31%) and 12 B-arm values (67%) were increased $\geq$25%, the criterion for PSA progression; 2 A-arm (15%) and 9 B-arm (50%) values were increased $\geq$50% (Supplementary Fig. S1).

Radiographic assessment. No radiographic evidence of objective disease response was observed in either protocol arm. On an intent-to-treatment basis, 7 A-arm patients (24%) and 11 B-arm

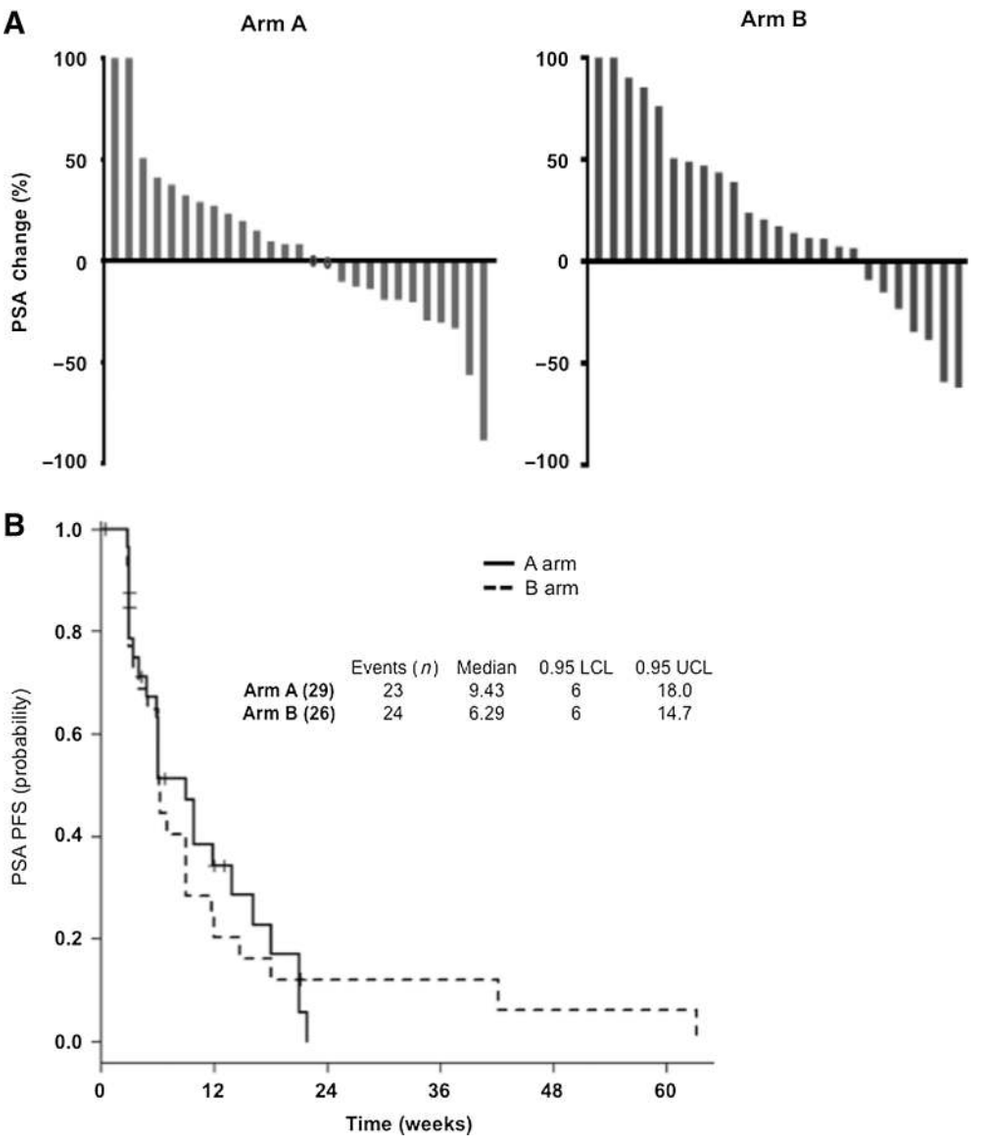

Figure 4.

A, Best PSA response depicted as the percent change from baseline to the lowest value on treatment below baseline or from baseline to the first recorded PSA rise. Two patients in each arm were excluded for insufficient data. **B,** Kaplan–Meier plots of PSA progression-free survival defined as $\geq$25% rise in PSA value above the lowest value at nadir or baseline from start of treatment and the time to reach the $\geq$25% PSA rise by treatment arm.

Table 2. rPFS probability at 12-week treatment intervals

Arms	12 weeks	24 weeks	36 weeks
A	0.713	0.634	0.475
$n = 29$	(0.528–0.963)	(0.434–0.926)	(0.241–0.939)
B	0.387	0.387	0.387
$n = 26$	(0.205–0.729)	(0.205–0.729)	(0.205–0.729)

patients (42%) had documented radiographic progression up to 36 weeks (9 months) of therapy (Fig. 3), the protocol endpoint for evaluation. In a survival analysis, the calculated probability of rPFS was 47.5% and 38.7% at week 36, respectively, for arms A and B (Table 2). These values compare favorably with the established pretrial statistical criterion for evidence of panobinostat/bicalutamide activity (rPFS ≥35% at 36 weeks) compared with the historic control using bicalutamide alone (5). For both arms, the lower limit of the 95% confidence interval (A arm, 24.1%; B arm, 20.5%) exceeds 15%, the rate specified in the protocol to reject a regimen as being of no further interest. From the survival analysis, the median time to radiographic progression was 33.9 weeks for A-arm and 10 weeks for B-arm patients (Fig. 5A). Radiographic progression was preceded by PSA progression in all cases by a median time of 24 weeks (6 months) and 5.8 weeks (1.2 months) on the A and B arms, respectively (Fig. 5B).

Safety

Grade ≤2 AEs affecting >20% of A-arm and B-arm cases were fatigue, diarrhea, thrombocytopenia, nausea, anorexia, and joint aches (Table 3). Dysgeusia, vomiting, and dizziness occurred only in the A arm. G≥3 AEs occurred in 42% of patients: 18 A arm (62%) and 5 (19%) B arm. G≥3 A-arm AEs included (patients/%) the following: thrombocytopenia without bleeding, 9/31 with only 1 withdrawal; fatigue and fainting, 4/14 each; neutropenia, 3/10; diarrhea, 2/7; prolonged QTc, 1/3. Most G≥3 AEs resolved after 1-week's break off panobinostat between cycles and/or by dose reductions. Twelve of 29 (41%) A-arm patients were dose-reduced, 11 of 12 patients before 4 cycles. Treatment was discontinued for AEs in 8 patients (27.5%). In the B arm, a G≥3 AE occurred in only 1 patient (4%) each, including thrombocytopenia without bleeding, constipation, dizziness, and a low-grade Kaposi sarcoma. The latter developed in an HIV-negative patient at 60 weeks of treatment and was managed with local surgical therapy. Only 1 patient was dose-reduced to 15 mg after the first cycle. Three patients (11.5%) discontinued for AEs. There were no skeletal-related events or toxicity-related deaths on either arm.

Discussion

In this report, we extend our previous preclinical studies (34, 41, 45, 46) to an isogenic cell line/xenograft model of CRPC, demonstrating that the synergistic antiproliferative activity of the HDACI panobinostat in combination with bicalutamide in castration- and antiandrogen-resistant prostate cancer cell lines translates into a significant combinational tumor reduction therapy of a human xenograft in castrate mice without lethal toxicity. In this model, the combinational antiproliferative activity was associated with significant reduction in the mRNA expression of flAR and its target genes PSA and TMPRSS2, as well as the androgen-independent splice variant ARSv7. These

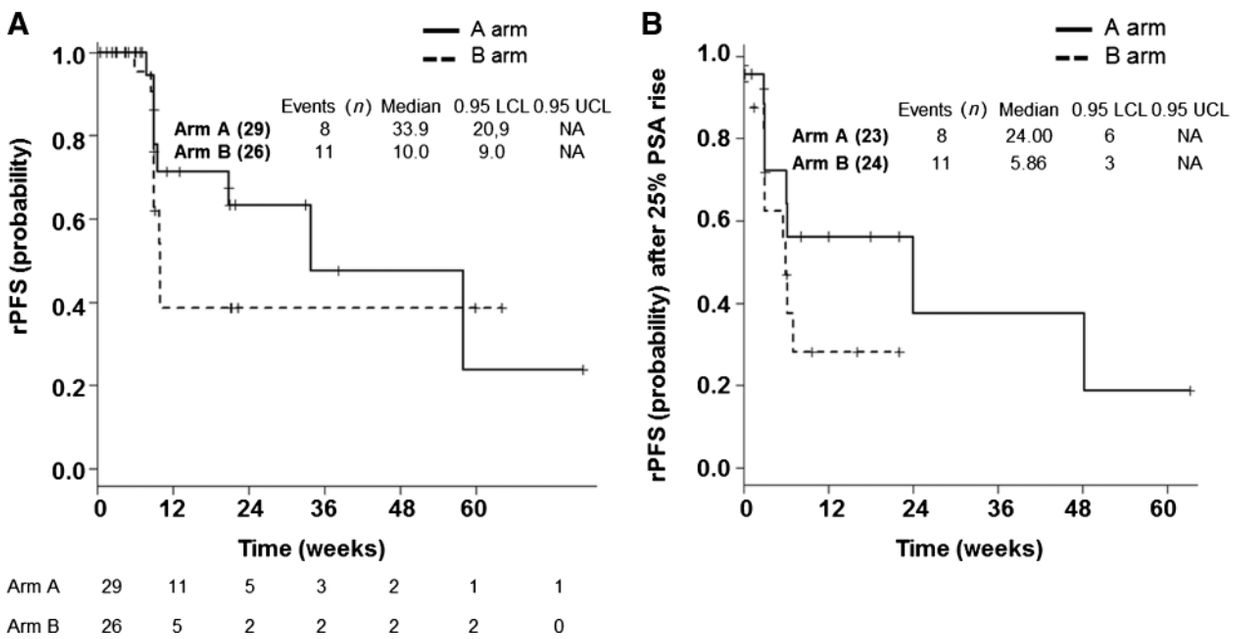

Figure 5.

A, Kaplan–Meier plots of rPFS probability and median time to radiographic progression per arm. For patients with no recorded scan, the days to end of treatment were used, and the event censored. **B,** Kaplan–Meier plots of rPFS probability after ≥25% PSA rise and time course to radiographic progression per treatment arm. Of the 47 patients with PSA ≥25% progression, 19 were followed by radiographic progression. PSA ≥ 25% progressions preceded radiographic progressions in all 19 cases.

Table 3. Adverse events

Arm	A (patients, $n = 29$)			B (patients, $n = 26$)		
AE	**Grade 1+2, *n* (%)**	**Grade 3 + 4 *n* (%)**	**Any grade, *n* (%)**	**Grade 1 + 2 *n* (%)**	**Grade 3 + 4 *n* (%)**	**Any grade, *n* (%)**
Fatigue	12 (41)	4 (14)	16 (55)	17 (65)	0 (0)	17 (65)
Diarrhea	13 (45)	2 (7)	15 (51)	7 (27)	0 (0)	7 (27)
Thrombocytopenia	5 (17)	9 (31)	14 (48)	9 (35)	1 (4)	10 (38)
Nausea	13 (45)	1 (3)	14 (48)	10 (38)	0 (0)	10 (38)
Anorexia	11 (38)	0 (0)	11 (37)	6 (23)	0 (0)	6 (23)
Joint aches	10 (34)	0 (0)	10 (34)	14 (54)	0 (0)	14 (54)
Dysgeusia	8 (28)	0 (0)	8 (27)	2 (8)	0 (0)	2 (8)
Vomiting	7 (24)	1 (3)	8 (24)	2 (8)	0 (0)	2 (8)
Constipation	6 (21)	0 (0)	6 (20)	4 (15)	1 (4)	5 (19)
Creatinine	6 (21)	0 (0)	6 (20)	4 (15)	0 (0)	4 (15)
Dizziness	6 (21)	0 (0)	6 (20)	3 (12)	1 (4)	4 (15)
Infection	5 (17)	0 (0)	5 (17)	5 (19)	0 (0)	5 (19)
Weight loss	5 (17)	0 (0)	5 (17)	2 (8)	0 (0)	2 (8)
Cardiopulmonary	3 (10)	1 (3)	4 (14)	0 (0)	1 (4)	1 (4)
Syncope (fainting)	0 (0)	4 (14)	4 (14)	1 (4)	1 (4)	2 (8)
Dyspepsia	4 (14)	0 (0)	4 (14)	3 (12)	0 (0)	3 (12)
Hemoglobin	1 (3)	2 (7)	3 (10)	3 (12)	0 (0)	3 (12)
Neutropenia	0 (0)	3 (10)	3 (10)	1 (4)	0 (0)	1 (4)
Prolonged QTc interval	1 (3)	1 (3)	2 (6)	0 (0)	0 (0)	0 (0)
Secondary malignancy	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	1 (4)

gene expression changes are consistent with our hypothesis that transcriptional regulatory changes in the AR pathway or interacting genes involved in antiandrogen resistance are favorably altered by combined HDACI-antiandrogen therapy. Mechanistically, it was shown that conformational changes in overexpressed flAR interfere with the binding and recruitment of the complement of coactivators and corepressors required for bicalutamide antagonist activity (20). Thus, reduction of flAR by a HDACI likely re-establishes the stoichiometric relationships needed for bicalutamide to regain its antagonist activity. Because the diminution of flAR is incomplete and the epigenetic effect may be reversible, the continued presence of the HDACI may be required to maintain bicalutamide effectiveness. An additional antiproliferative effect of the HDACI is likely due to reduction of ARSv7, which commands a transcriptional program enriched in cell-cycle genes and has independent proproliferative activity (31). Further studies of the mechanistic details are needed to fully understand how HDACIs reverse the adaptive response of prostate cancer cells associated with overexpression of AR and ARSv.

The current exploratory phase I/II study of combined panobinostat and continuous bicalutamide rechallenge therapy is to our knowledge the first of its kind in CRPC patients after progression on one or more 2ndLAARxs. Panobinostat and bicalutamide have previously been tested for clinical activity as monotherapies in small sample studies of CRPC patients with similarities in characteristics to those in our combined agent study. Panobinostat was administered intravenously to heavily-treated patients with mCRPC in order to achieve the high drug levels that were required for effective antitumor cell activity in preclinical prostate cancer models; no significant beneficial clinical effect was found, although toxicity was modest (56). In an older study of bicalutamide monotherapy, prostate cancer patients who had progressed on flutamide as a component of combined androgen blockade with leuprolide were treated with continuous 200 mg/day bicalutamide; the median time to PSA progression (50% increase from baseline) was 3 to 4 months (12–16 weeks; ref. 5). The latter study, which provided the only available data for a disease progression endpoint in patients with mCRPC previously treated with an antiandrogen, was adopted as the historic control for bicalutamide alone in the current trial. Neither this historic study nor any subsequent study in mCRPC patients who had progressed on $\geq$1 antiandrogens provides data regarding time to radiographic progression, the primary endpoint of the current trial, chosen because it has a stronger association with clinically meaningful benefit than PSA (51). Two recent publications provide useful information for making comparative projections from the historic control: (1) in a retrospective analysis of 436 patients treated with $\geq$1 courses of secondary hormonal therapies, there was a successive decline in the time to disease progression from 8 months on the first to 2 months on the fourth antiandrogen course (6); (2) in a 1:1 randomization study of bicalutamide 50 mg daily versus enzalutamide in 396 CRPC patients who had failed primary ADT, two-thirds of whom had mCRPC and one-half of whom had previously received an antiandrogen (bicalutamide excluded), the interval from PSA progression (5.7 months) to radiographic progression on the bicalutamide arm was 2.6 months (9 weeks; ref. 57). In the current study, the time to PSA progression at 50% over baseline (the historic control criterion), the A arm did not differ from the historic controls (15 vs. 16 weeks; not shown). However, it must be considered that only 1 antiandrogen course was given in the control versus 2 to 3 courses in the current trial, and the bicalutamide dose was 4 times higher in the control (200 mg vs. 50 mg). Most tellingly, the time to radiographic progression on the A arm (33.9 weeks; 8.5 months) considerably exceeds that for the projected time in the historic control (16 weeks to PSA progression + projected 9 weeks to radiographic progression = 25 weeks). These considerations strongly support our conclusion, based on the protocol statistical criterion to adjudge the trial outcome of clinical interest (proportion of patients remaining rPF at 36 weeks >35%), that this primary endpoint was clearly exceeded on the A arm (rPFS 45.7% at 36 weeks). Furthermore, the primary effect of the high-dose panobinostat/bicalutamide combination was to extend the interval between PSA progression (at 9.4 weeks) and radiographic progression

(24 weeks): ratio, 1:2.6. Notably, this ratio is the reverse of that observed in the large recent trial cited above in which single-agent bicalutamide was used as a control arm for the enzalutamide arm in mCRPC: ratio, 2.1:1 [5.7 weeks to PSA (25%) progression; 2.6 weeks to subsequent radiographic progression; ref. 57]. Overall, we conclude that the high-dose panobinostat regimen considerably increased time to objective disease progression compared with single-agent bicalutamide rechallenge alone.

In contrast to the A arm, the 20 mg panobinostat B arm showed no improvement from the historic control (6.3 weeks to PSA progression, 10 weeks to radiographic progression). Notably, 10 (38%) cases of radiographic progression occurred on the B arm versus 3 (10%) cases on the A arm by the first protocol checkpoint at 12 weeks of treatment. This suggests that full high-dose treatment is critical for achieving maximal early antitumor efficacy. Possibly, a lower dose of panobinostat has some effectiveness after 12 weeks, because only 1 additional radiographic progression occurred in 5 at-risk cases on the 20 mg B arm. On the other hand, 5 subsequent radiographic progressions occurred in 11 at-risk A-arm patients, 4 of who had been dose-reduced to 30 or 25 mg panobinostat for toxicity. Overall, the much higher incidence of G3–4 toxicities on the A arm, even though 40 mg was demonstrated in the phase I trial component to be below the MTD, and the lack of antitumor efficacy of the 20 mg B arm indicate a narrow therapeutic window.

In view of the clinical inefficacy of panobinostat alone (56) or of 20 mg panobinostat in combination with bicalutamide, we speculate, based on our preclinical data, that high-dose panobinostat is effective in overcoming acquired resistance to bicalutamide by its dual effect on AR-related transcripts: decreasing the overexpression of flAR, which facilitates the binding and antagonistic activity of bicalutamide, and decreasing ARSvs that independently drive proliferation. In this context, it is notable that all measures of serum PSA response are more favorable in the A arm than the B arm, suggesting that PSA may, indeed, serve as a biomarker for monitoring HDACI activity on AR transcriptional activity in mCRPC (42).

The spectrum of AEs was within that previously observed for single-agent panobinostat, and the severity and incidence of AEs was directly related to dose level. G1–2 AEs were similar in both arms, most commonly fatigue, joint aches, and diarrhea. G$\geq$3 AEs prevailed in the A arm (62%): most commonly, thrombocytopenia without bleeding (31%). The latter was reversible after 1-week off panobinostat and was avoidable by dose reduction. Panobinostat dose reduction was required before 4 cycles in 41% of cases. Treatment was discontinued for AEs in 27.5% of cases, most commonly for G2 fatigue and diarrhea. On the B arm, G$\geq$3 AEs were low (19%): 1 case of thrombocytopenia (only B-arm case dose-reduced) and 1 case of low-grade Kaposi sarcoma in an HIV-negative patient after 14 months of treatment with otherwise stable mCRPC. A literature search of HDACI side effects revealed no other mention of Kaposi sarcoma. We conclude that the 40 mg dose of panobinostat is the highest dose that can be given in the intermittent regimen with continuous bicalutamide without excessive G3–4 events over a time period sufficient to increase rPFS provided that dose reductions are implemented early.

There are intrinsic limitations of this hypothesis-exploring, dose-finding study. Only serum PSA was monitored during the trial, which provides an incomplete assessment of the changes in AR-related molecules on treatment, but more advanced assays were not available before 2010, when the trial was designed. The lack of a bicalutamide-alone arm in the phase II trial created complexity in assessing the efficacy of the panobinostat/bicalutamide combination. However, randomization at 2 different panobinostat dose levels was considered more important given pretrial information that 40 mg panobinostat was liable to have considerable toxicity-limiting treatment time, whereas other information suggested that prolonged treatment at lower doses might be necessary for antitumor activity. The power of the trial to assess efficacy was also diminished by accrual shortfall (56 of 66 patients needed for 95% power), primarily due to competing trials with highly promising second-generation antiandrogens, and by a few withdrawals without serious AEs, deterioration, or death that did not have radiographic evaluation. Despite these limitations, we believe that the trial results demonstrate a narrow therapeutic window for the efficacy of the high-dose panobinostat/bicalutamide regimen.

Based on our pilot evidence that panobinostat/bicalutamide has a clinically beneficial effect by retarding the progression of objective disease, a successor clinical trial in an equivalent group of mCRPC patients seems warranted to substantiate and extend our findings using a second-generation antiandrogen, such as enzalutamide (9). Although more potent than bicalutamide, acquired resistance to enzalutamide or alternative antiandrogen therapy also inevitably develops in patients with mCRPC. Like bicalutamide, enzalutamide works by binding to the LBD of the AR (58), and the development of resistance is linked to flAR overexpression and the emergence of ARSv7 (49), which are targets of panobinostat and other HDACIs (27, 42, 59). Although a phase I will be necessary to find an acceptable dose schedule of the combination, the phase II can have a single-agent control arm, and from what we learned, implementation of early dose reductions will avoid dropouts. Correlative laboratory studies by quantitative molecular monitoring of circulating tumor cells and/or cell-free DNA in peripheral blood together with serum PSA will provide insights into the relationship of the activity of the agents on AR-related transcripts and the retardation of radiographic progression.

Disclosure of Potential Conflicts of Interest

M.N. Stein is a consultant/advisory board member for Merck Sharp & Dohme, and reports receiving commercial research support from Advaxis, Bristol-Myers Squibb, Janssen Oncology, Lilly, and Oncoceutics. E.S. Barnett is an employee of Memorial Sloan Kettering Cancer Center. T.M. Beer is a consultant/advisory board member for Novartis. No potential conflicts of interest were disclosed by the other authors.

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A.C. Ferrari reports having received funds for preclinical studies and conduct of this study from 2011 to 2015. T.M. Beer reports having received consulting fees from Novartis.

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