Histone deacetylase inhibitors in castration-resistant prostate cancer: molecular mechanism of action and recent clinical trials

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Abstract: Historically, androgen-deprivation therapy has been the cornerstone for treatment of metastatic prostate cancer. Unfortunately, nearly majority patients with prostate cancer transition to the refractory state of castration-resistant prostate cancer (CRPC). Newer therapeutic agents are needed for treating these CRPC patients that are unresponsive to androgen deprivation and/or chemotherapy. The histone deacetylase (HDAC) family of enzymes limits the expression of genomic regions by improving binding between histones and the DNA backbone. Modulating the role of HDAC enzymes can alter the cell's regulation of proto-oncogenes and tumor suppressor genes, thereby regulating potential neoplastic proliferation. As a result, histone deacetylase inhibitors (HDAC*i*) are now being evaluated for CRPC or chemotherapy-resistant prostate cancer due to their effects on the expression of the androgen receptor gene. In this paper, we review the molecular mechanism and functional target molecules of different HDAC*i* as applicable to CRPC as well as describe recent and current clinical trials involving HDAC*i* in prostate cancer. To date, four HDAC classes comprising 18 isoenzymes have been identified. Recent clinical trials of vorinostat, romidepsin, and panobinostat have provided cautious optimism towards improved outcomes using these novel therapeutic agents for CPRC patients. Nevertheless, no phase III trial has been conducted to cement one of these drugs as an adjunct to androgen-deprivation therapy. Consequently, further investigation is necessary to delineate the benefits and drawbacks of these medications.

Keywords: castration-resistant prostate cancer, clinical trials, histone deacetylase inhibitors

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Introduction

Prostate cancer is estimated to comprise 26% of newly diagnosed male cancers in the USA in 2015, making it the most commonly diagnosed cancer in men and the second leading cause of cancer-related death in men for this year [Siegel *et al.* 2015]. Androgen-deprivation therapy has been the mainstay treatment for advanced prostate cancer and induces remission in 80–90% of men with advanced disease, resulting in a median disease progression-free survival of 12–33 months. Unfortunately, in a majority of patients, neoplastic cells will subsequently continue to proliferate despite previous response to androgen deprivation. This progressive state is termed castration-resistant prostate cancer (CRPC), which carries a median overall survival of 23–37 months starting from the initial onset of androgen deprivation [Hellerstedt and Pienta, 2008]. Therefore, studies evaluating a newer generation of agents are necessary to prolong life expectancy and quality of life for patients suffering from CRPC.

The molecular mechanisms underlying the proliferation of prostate cancer cells under an androgen-deprivation environment are currently under investigation. One of these mechanisms is the covalent acetylation and deacetylation of histone proteins. These covalent modifications are important in regulating the transcription of proto-oncogenes and tumor suppressor genes. The binding and retraction of acetyl groups to histones are

reversible and heritable from one generation to the next. These modifications are mediated by two sets of enzymes, histone deacetylase (HDAC) and histone acetyltransferase (HAT).

In particular, the HDAC family of enzymes is of current interest in urology because these proteins offer a novel therapeutic target to limit prostate cancer proliferation. HDAC regulates the expression of several functional genes, including the androgen receptor (AR) in prostate cells. Consequently, histone deacetylase inhibitors (HDAC*i*) are now being evaluated for their effects on CRPC patients. The following is a discussion of the molecular mechanisms and functional target molecules of different HDAC*i* as applicable to CRPC as well as a description of the current clinical trials involving HDAC*i* in prostate cancer.

Epigenetics and prostate cancer

Epigenetics and the HDAC family of enzymes
Epigenetics is the study of heritable changes in gene expression that are not concomitantly accompanied by changes in DNA sequences. The key modifications of DNA involving epigenetics are the DNA methylation of CpG islands in the promoter region of genes and the covalent modifications involving the acetylation and deacetylation of histones [Bode and Dong, 2004]. Histones are proteins that form a scaffold allowing genomic DNA to wrap in a systematic fashion. The expression of genes in a particular genomic region is thereby regulated by its winding around histones. Modification of these histone proteins by acetylation and deacetylation controls the tightness of DNA winding around histones, and therefore, controls the expression of the genes at that histone's location. HAT enzymes transfer acetyl moieties to lysines in the N-terminal histone tails through use of a cofactor, acetyl-coenzyme A. This results in the neutralization of the negative charge of the nitrogen in the ε-amino group of the lysine residue, which in turn, leads to a more open form of chromatin that is associated with activation of gene expression. Contrarily, the acetyl groups are in turn cleaved off by HDAC enzymes leading to a more condensed form of chromatin and gene silencing [Wagner *et al.* 2010]. In summation, HDAC represents a family of enzymes that cooperate with the HAT family of enzymes to modulate chromatin structure and transcriptional activity *via* changes in the acetylation status of nucleosomal histones.

To date, four HDAC classes comprising 18 isoenzymes have been identified (Table 1). Class I HDAC is primarily localized in the nucleus and ubiquitously expressed in all tissues. Class I consists of HDACs 1, 2, 3, and 8. Class I HDACs have the deacetylase domain located at their N-terminal and carry a variable Carbon-terminal (C-terminal) depending on the specific HDAC of the class. Class II HDACs are localized both in the nucleus as well as the cytoplasm. Class II consists of HDACs 4, 5, 6, 7, 9, and 10. Class II HDACs have the deacetylase domain at the C-terminal with the exception of HDAC 6, which contains two acetylase domains at both the N- and C-terminals. Class III HDACs are homologues of yeast silent information regulator 2 proteins and consist of sirtuins 1–7. Class III HDACs are distinct from Class I and II HDACs due to their enzymatic dependence on coenzyme nicotinamide adenine dinucleotide for deacetylase activity. Contrarily, Class I and II HDACs have a zinc coordinated active site. Class IV HDAC has the property of both class I and class II, and consists of HDAC 11 [Perry *et al.* 2010; Shankar and Srivastava, 2008].

In cancer, uncontrolled HDAC expression results in the deacetylation of histone proteins. Deacetylation causes DNA to be wrapped tightly by histones, thereby inhibiting gene expression. If tightly wound DNA incorporates tumor suppressor genes, a neoplastic proliferation of cells may indefinitely result [Abbas and Gupta, 2008].

HDAC in prostate cancer
Recently, it has been shown that a majority of recurrent prostate cancers are neither hormone refractory nor androgen independent, but are rather dependent on the AR signaling axis [Montgomery *et al.* 2008]. The AR is a cytoplasmic protein that binds to testosterone or dihydrotestosterone before entering the nucleus leading to the alteration of gene transcription. Several mechanisms exist for the increased signaling of the AR. These include the amplification and overexpression of the AR, enhanced AR signal transduction through alterations in coactivators/corepressors, and activation of the AR or downstream regulatory molecules by cross-talk with other signaling pathways [Feldman and Feldman, 2001].

Intracrine androgen production may play a critical role in maintaining tumoral androgen levels and development of CRPC [Stanbrough *et al.*

Table 1. Current histone deacetylase classification.

HDAC class	Members	Localization	Activity domain
Class I	HDAC 1	N	Zinc-coordinated active site, N-terminal HDAC activity
	HDAC 2	N	
	HDAC 3	N	
	HDAC 8	N/C	
Class II	HDAC 4	N/C	Zinc-coordinated active site, C-terminal HDAC activity
	HDAC 5	N/C	
	HDAC 6	C	
	HDAC 7	N/C	
	HDAC 9	N/C	
	HDAC 10	C	
Class III	SIRT 1	N	Coenzyme nicotinamide adenine dinucleotide-dependent active site
	SIRT 2	C	
	SIRT 3	N/C	
	SIRT 4	C	
	SIRT 5	C	
	SIRT 6	N	
	SIRT 7	N	
Class IV	HDAC 11	N/C	N- and C-terminal HDAC activity

C, cytoplasmic; HDAC, histone deacetylase; N, nuclear; SIRT, sirtuin.

2006]. We have previously shown that prostate-derived factor promotes AR-positive prostate-tumor progression through autocrine and paracrine stimulation by upregulating cell proliferation *via* the extracellular signal-regulated kinase 1/2 signal pathway [Chen *et al.* 2007]. Dillard and colleagues have shown that advanced prostate cancer cells are able to synthesize their own testosterone from cholesterol indicating supporting intracrine function seen in advanced CRPC. One of the key players for this autonomous testosterone synthesis is the AR, which may undergo changes leading to its subsequent deregulation [Dillard *et al.* 2008]. A portion of this deregulation of the AR is often governed at the epigenetic level. Epigenetic changes are potentially reversible, or in other words, they may be amenable to pharmacologic interventions. Therefore, a lot of work in recent years has focused on the development of inhibitors of histone-modifying enzymes.

Several lines of evidence have shown that HDACs are abundantly expressed and upregulated in prostate cancer [Waltregny *et al.* 2004; Weichert *et al.* 2008]. Weichert and colleagues studied the expression patterns of HDACs 1, 2, and 3 in prostate cancer using a patient cohort of 192 patients who underwent radical prostatectomy. Their data showed that HDACs 1 and 2 correlated positively with Gleason scores, with high-grade tumors expressing both isoforms at higher rates. In addition, expression of HDACs 1, 2, and 3 correlated significantly with the Ki-67-positive proliferative fraction of prostate cancer cells, indicating increased cellular proliferation. They also demonstrated a significantly lower probability of disease-free survival based on the Kattan nomogram for patients with high-HDAC 2 tumors compared with patients with low-HDAC 2 tumors [Weichert *et al.* 2008].

HDAC plays a critical role in the regulation of the AR, therefore, HDAC*i* are an important therapeutic consideration in CRPC. In the following sections, we will discuss some important molecular pathways where HDAC*i* impact cellular signaling and cancer cell growth. The effects of HDAC*i* on various enzymes are depicted in Figure 1.

Molecular mechanism of action of HDAC*i*

Effect of HDAC*i* on the AR and heat shock protein-90
The AR is bound to the heat shock protein-90 (Hsp90) molecular chaperone complex, which
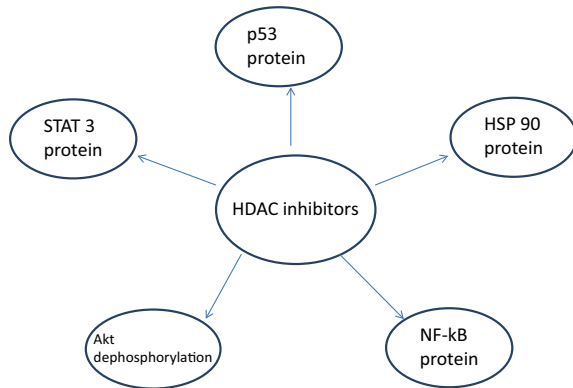
Figure 1. The unique proteins modifying cell survival and proliferation that HDAC inhibitors have been to shown to affect: Akt, protein kinase B; Hsp90, heat shock protein-90; NF-kB, nuclear factor-kappa B; STAT 3, signal transducers and activators of transcription.

is essential for AR stability, activation, and maturation [Fang *et al.* 1996; Neckers and Ivy, 2003].

Hsp90 clients include several proteins of potential importance in mediating prostate cancer progression, including the wild-type and mutated AR, human epidermal growth factor receptor 2, and protein kinase B (Akt). Therefore, the inhibition of Hsp90 function causes the proteasomal degradation of proteins that require this chaperone for maturation and stability. Hence, Hsp90 has been targeted in prostate-cancer therapy [Solit *et al.* 2003].

Modification of Hsp90 by acetylation has been reported by Yu and colleagues [Yu *et al.* 2002]. It was observed that a depsipeptide HDAC inhibitor, romidepsin (FK228), could deplete Hsp90 client proteins Raf-1, ErbB1, and ErbB2. They further showed that romidepsin induced Hsp90 acetylation in nonsmall-cell lung cancer cell lines, which correlated with the loss of its binding activities to adenosine triphosphate (ATP) and client proteins.

Chen and colleagues described the HDAC inhibitor LAQ824 and its role [Chen *et al.* 2005]. LAQ824 affects all Hsp90 client proteins, including the depletion of AR in LNCaP cells by inducing Hsp90 acetylation. This results in the inhibition of its ATP-binding activity and induces the dissociation of the Hsp90–AR complex leading to AR degradation.

Effect of HDACi on p53

The p53 tumor suppressor exerts antiproliferative effects, including growth arrest, apoptosis, and cell senescence, in response to various types of stress. It is well known that phosphorylation and acetylation are involved in the posttranslational modifications of p53 [Bode and Dong, 2004; Appella and Anderson, 2001; Roy and Tenniswood, 2007].

It has been shown that the HAT p300/CREB binding protein (CBP) strongly potentiates p53-dependent transcriptional activation, and that acetylation of p53 by p300 markedly stimulates its sequence-specific DNA-binding activity and transcriptional activity [Gu and Roeder 1997; Gu *et al.* 1997]. Therefore, HDAC*i* may be used to potentiate the biological effects of p53 on growth suppression.

Effect of HDACi on the signal transducers and activators of transcription family of proteins

The signal transducers and activators of transcription (STAT) family of proteins are self-signaling transcription factors in cytoplasm. They are utilized by most cytokine receptors to rapidly turn on genetic expression in nuclei. Function-related posttranslational modifications of STAT proteins in response to treatment with cytokine or growth factor include phosphorylation of a single tyrosine residue. Yuan and colleagues reported that STAT proteins undergo acetylation of a single amino-acid residue, lysine 685, in response to cytokine stimulation. They concluded that this modification is essential for STAT proteins to form stable dimers and to activate transcription [Yuan *et al.* 2005].

Effect of HDACi on Akt dephosphorylation

Akt is a serine/threonine kinase that functions as a regulator of cell survival and metabolism through effects on several downstream proteins [Favard *et al.* 2010]. Chen and colleagues demonstrated that HDAC*i* facilitate dephosphorylation of Akt by altering the dynamics of HDAC–protein phosphatase 1 (PP1) complexes. The group used U87MG human glioblastoma cells and PC3 prostate cancer cell lines to reveal the novel histone acetylation-independent mechanism by which HDAC*i* mediate the dephosphorylation of Akt through the disruption of HDAC–PP1 complexes. HDAC*i* selectively target HDACs 1 and 6 to disrupt the respective HDAC–PP1complexes

resulting in an increased association of PP1 with Akt [Chen *et al.* 2005].

Effect of HDAC*i* on nuclear factor-kappa B

Nuclear factor-kappa B (NF-kB) corresponds to an inducible transcription factor complex that plays an important role in anti-apoptotic responses in mammals. It has a subunit named ReIA and phosphorylation of an N-terminal site on this subunit by protein kinase A facilitates NF-kB assembly with CBP/p300. Both CBP and p300 are coactivators and contain HAT activity that has been implicated in the regulation of gene expression. This subunit can be deacetylated through interaction with HDAC 3 [Chen *et al.* 2001].

Recent clinical trials of HDAC*i* in CRPC

Vorinostat

Vorinostat is a small molecular inhibitor of class I and II HDACs. It has been shown to inhibit the growth of PC-3, DU-145, LNCaP human prostate cancer cell lines, and suppression of PC-3 xenograft tumors. It mediates Akt dephosphorylation through the disruption of the complexes of HDAC bound to PP1, leading to more PP1–Akt association complexes, resulting in increased PP1–Akt association [Kulp *et al.* 2006]. The drug recently underwent a phase II clinical trial [ClinicalTrials.gov identifier: NCT00330161] as described in Table 2 [Bradley *et al.* 2009]. Patients received open-label oral vorinostat 400 mg daily continuously. The primary endpoint was the proportion of patients who did not demonstrate disease progression at 6 months. A total of 27 patients were enrolled, and all of them had received previous chemotherapy. A total of 11 patients (41%) discontinued therapy because of toxicity and 13 patients (48%) were removed because of disease progression. The best objective response obtained was stable disease in two patients (7%). No declines in prostate-specific antigen (PSA) of more than 50% were observed [Bradley *et al.* 2009].

Two other clinical trials are concurrently evaluating oral vorinostat. One is a phase I trial [ClinicalTrials.gov identifier: NCT01174199] with the Roswell Park Cancer Institute evaluating vorinostat with intravenous temsirolimus for patients with metastatic disease. In addition to evaluating clinical outcomes and PSA changes,

the study will also interestingly use positron emission tomography/computed tomography to monitor decrease in interval tumor size. The other current ongoing trial is a National Cancer Institute phase II study [ClinicalTrials.gov identifier: NCT00589472] known as the 'Total Androgen-Receptor Gene Expression Targeted Therapy (TARGET) trial', which evaluates neoadjuvant vorinostat with oral bicalutamide and intramuscular leuprolide acetate or subcutaneous goserelin acetate 4–8 weeks prior to radical prostatectomy. This is an exciting phase II study combining androgen-deprivation therapy with an HDAC inhibitor to weigh outcomes in the neoadjuvant setting. Such a trial may start to shift our view of the clinical roles for vorinostat and other HDAC*i*.

Romidepsin

Romidepsin (depsipeptide, FK228, FR901228, NSC630176) is a member of the cyclic depsipeptide class of HDAC*i*. Romidepsin can induce the hyperacetylation of Hsp90, disrupting the complex between Hsp90 and its client proteins, inhibiting their synthesis and function [Yu *et al.* 2002].

A phase II study [ClinicalTrials.gov identifier: NCT00106418] of 35 patients with metastatic CRPC revealed minimal clinical activity of romidepsin is also described in Table 2. Two patients had a partial response that lasted longer than 6 months with a PSA decline of over 50%, but 11 patients (31%) had to discontinue the medication due to toxicity [Molife *et al.* 2010]. In total, two patients fulfilled response evaluation criteria in solid tumors. We could not identify any other ongoing clinical trials that are studying romidepsin.

Pracinostat

Pracinostat (SB939) is an orally active hydroxamic acid-based HDAC inhibitor. The pharmacokinetic profile has been excellent in both mouse models and calculated extrapolation for humans [Javaraman *et al.* 2011; Novotny-Diermayr *et al.* 2010]. In a recent phase II trial [ClinicalTrials.gov identifier: NCT01075308] of 32 patients, two patients achieved greater than 50% PSA reduction, and seven patients had objectively stable disease at 1.6–8 months. Five patients suffered from fatigue and four patients had neutropenia as the most common side effect

Table 2. Current and recent trials of histone deacetylase inhibitors in prostate cancer.

Histone deacetylase inhibitor	Trial phase	Study design/results	ClinicalTrials.gov identifier
Vorinostat	Phase II	Trial of 400 mg daily PO vorinostat. Disease progression measured at 6 months. A total of 27 patients: 41% removed due to toxicity; 48% had disease progression; 7% did not progress.	NCT00330161
Vorinostat	Phase I	Trial of PO vorinostat and IV temsirolimus. Currently ongoing.	NCT01174199
Vorinostat	Phase II	Trial of PO vorinostat and PO bicalutamide with either IM leuprolide or SC goserelin acetate followed by radical prostatectomy. Currently ongoing.	NCT00589472
Romidepsin	Phase II	Trial of IV romidepsin. Disease progression measured at 6 months. A total of 35 patients: 31% removed due to toxicity; 6% filled RECIST criteria; 6% had more than 50% PSA decline.	NCT00106418
Pracinostat SB939	Phase II	Trial of PO pracinostat SB939. A total of 32 patients: 25% with PSA response; 6.3% with more than 50%; 22% with objectively stable disease at 1.6–8 months.	NCT01075308
Panobinostat	Phase I	Trial of IV panobinostat *versus* IV panobinostat, docetaxel, and prednisone. Disease progression measured at 6 months. A total of 16 patients, 8 in each arm. No apparent synergistic effect of combination.	NCT00663832
Panobinostat	Phase II	Trial of IV panobinostat. Disease progression measured at 24 weeks. A total of 35 patients: 14% had PSA decrease (none more than 50%).	NCT00667862
Panobinostat	Phase I/II	Trial of PO panobinostat with bicalutimide measured at 9 months. A total of 9 patients: 22% with more than 50% PSA reduction; 33% with stable PSA. Limited toxicity.	NCT00878436

IM, intramuscular; IV, intravenous; PO, by mouth; PSA, prostate-specific antigen; RECIST, response evaluation criteria in solid tumors; SC, subcutaneous.

of treatment [Eigl *et al.* 2012]. No current trials of pracinostat are ongoing.

Panobinostat

Similar to pracinostat, panobinostat belongs to the structurally novel cinnamic hydroxamic acid class of compounds. Treatment of LNCaP, an AR-positive prostate cancer cell line, with less than 100 nM panobinostat resulted in significant degradation of the AR as well as inhibition of cell growth, through acetylation and subsequent inhibition of the Hsp90 chaperone function [Chen *et al.* 2005].

In a combination study [ClinicalTrials.gov identifier: NCT00663832] of oral panobinostat *versus* oral panobinostat plus docetaxel in patients with advanced prostate cancer, all patients in the panobinostat monotherapy arm progressed under therapy, despite evidence of detectable levels of histone hyperacetylation in peripheral blood mononuclear cells as described in Table 2. Contrarily, 63% (5/8) of patients in the combination arm showed a PSA decline of greater than 50% [Rathkopf *et al.* 2008]. Furthermore, intravenous panobinostat was studied alone in a phase II trial [ClinicalTrials.gov identifier: NCT00667862] involving 35 patients. In this trial 14% of patients had a PSA decrease, but none of these decreases were greater than 50%. Fatigue and thrombocytopenia were the major adverse effects [Rathkopf *et al.* 2013]. Lastly, a phase I/II trial of oral panobinostat of nine patients was conducted with bicalutamide with outcomes measured at 9 months. Three patients had stable PSA and two patients had a greater than 50% reduction in PSA. The most concerning side effect was only

thrombocytopenia without bleeding noted in three patients [Ferrari *et al.* 2011].

Conclusions and prospective

Together, these findings indicate the complexity in the mechanism of HDAC*i* that underlies their high potency in suppressing tumor growth *in vitro* and *in vivo*. These agents offer a possible means towards treating patients suffering from CRPC. Further phase II trials are needed to identify individual CRPC subsets that may particularly benefit from these medications based on either epidemiologic or genetic criteria. Such investigations will offer insight towards not only the benefits and adverse effects of HDAC*i* but also our overall understanding of these novel therapeutic agents.

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Conflict of interest statement

The authors declare that there is no conflict of interest to disclose.

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