

HDAC Inhibitors and Prostate Cancer

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Novel treatment regimens are required for castration-resistant prostate cancers (CRPCs) that become unresponsive to standard treatments, such as docetaxel and enzalutamide. Histone deacetylase (HDAC) inhibitors showed promising results in hematological malignancies, but they failed in solid tumors such as prostate cancer, despite the overexpression of HDACs in CRPC. Four HDAC inhibitors, vorinostat, pracinostat, panobinostat and romidepsin, underwent phase II clinical trials for prostate cancers; however, phase III trials were not recommended due to a majority of patients exhibiting either toxicity or disease progression. In this entry, the pharmacodynamic reasons for the failure of HDAC inhibitors were assessed and placed in the context of the advancements in the understanding of CRPCs, HDACs and resistance mechanisms.

Keywords: HDAC inhibitors ; histone deacetylases ; epigenetics ; prostate cancer ; androgen receptor-negative ; resistance mechanisms

1. Introduction

There were ~1.3 million new cases of prostate cancer worldwide in 2018 and it is the second most commonly diagnosed cancer in men ^[1]. Importantly, it ranks second among all cancer fatalities in males ^[2]. Even though the five-year survival for localized disease is 100%, it drops down to 29.3% if the prostate cancer metastasizes to other organs ^[3]. Furthermore, from 2005 to 2018 a 31% increase in the incidence of prostate cancer was observed from 974,000 to 1.3 million new cases ^{[1][4]}. The majority of prostate cancers are initially androgen-dependent ^[5]. In advanced prostate cancer, androgen deprivation therapy, either by chemical castration in the form of luteinizing hormone-releasing hormone agonists/antagonists or surgical castration has typically been used. After a median of 18–24 months of treatment, most patients progress to a metastatic castration-resistant prostate cancer (CRPC) ^{[6][7]}, which has a median survival time of 23–37 months ^[2]. Therefore, there is a need for novel therapeutic strategies that exploit the molecular signature of prostate cancers.

One of these strategies involves modulating histone acetylation and deacetylation mediated by histone acetyl transferases (HATs) and histone deacetylases (HDACs), respectively. HATs and HDACs affect both, histone and non-histone proteins ^[8]. Histone proteins, H2A, H2B, H3 and H4, form the core of the nucleosomes with DNA wrapped around them to form chromatin ^[9].

The histone core proteins have lysine residues on their N-terminal tails ^[10]. If these lysines are not acetylated, histones remain positively charged and ionically interact with the negatively-charged phosphate backbone of DNA (Figure 1). In this case, chromatin will remain condensed and the transcription machinery will not be able to access the underlying transcription site. Acetylation of lysine residues leads to chromatin decondensation and transcription initiation ^[11]. Importantly, the balance in the transcription of oncogenes and tumor suppressor genes is lost in cancer ^[12]. In addition to histones, non-histone proteins can be acetylated. Acetylation of transcription factors, such as p-53, NF- κ B, p50 and PC4, increases their sequence-specific binding to the DNA, whereas acetylation of other factors, such as FoxO1, HMG1 (Y) and p65, reduces their ability to bind DNA ^[8]. Regulation of acetylation via histones and non-histone proteins can have a wide repertoire of effects, including altered gene transcription, DNA damage repair, cell division, signal transduction, protein folding, autophagy and metabolism ^{[13][14]}. With such a wide range of actions, understanding the mechanisms of protein acetylation and deacetylation is essential to devise new therapies.

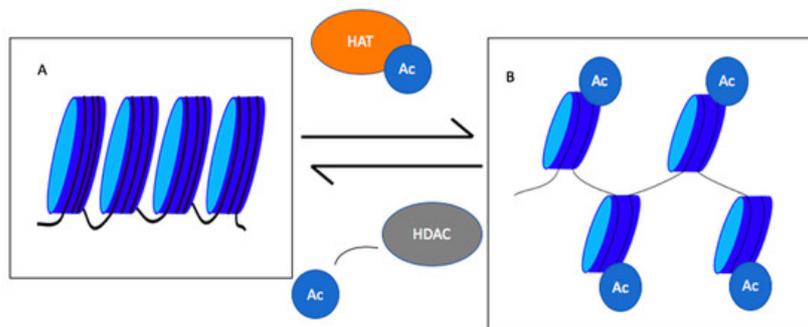


Figure 1. Roles of histone acetyl transferases (HATs) and histone deacetylase (HDACs) in chromatin condensation and decondensation. HATs add an acetyl group to histones decondensing chromatin, whereas HDACs remove acetyl groups leading to chromatin condensation. (A) represents the condensed chromatin, and (B) shows the decondensed chromatin. Ac = acetyl group [11].

2. HDAC Inhibitors

As HDAC expression has been shown to be associated with poor clinical outcome, HDAC inhibitors have been explored as a potential therapeutic option. The five classes of HDAC inhibitors include hydroxamic acids, cyclic tetrapeptides, short chain carboxylic acids, benzamides and keto-derivatives [15]. These inhibitors have a well accepted pharmacophore, consisting of a zinc-binding group, coordinating with the zinc ion in the active site, a linker that transverses the active site and a cap for interactions with the external surface [16].

Due to the role of HDAC inhibitors in inducing cell cycle arrest, apoptosis, autophagy, heat shock protein-90 (HSP90) inhibition and reactive oxygen species generation, this class of drugs has been trialed in cancers [17]. In 2006, the FDA approved the use of a hydroxamic acid drug, suberanilohydroxamic acid (SAHA, vorinostat), for the treatment of cutaneous T-cell lymphoma [18]. Since then, HDAC inhibitors, including belinostat, panobinostat and romidepsin, have been approved for peripheral T-cell cutaneous lymphoma and multiple myeloma (Figure 2). Hematological malignancies have shown promising responses to HDAC inhibitors [19]. However, HDAC inhibitors have not successfully cleared clinical trials for solid tumors, despite promising collective results in biologic, preclinical and phase I and II studies [17][20]. For example, in phase III trials for advanced hormone receptor-positive breast cancer, chidamide, a HDAC inhibitor, in combination with exemestane, increased the median progression-free survival to 7.4 months in comparison to 3.8 months with placebo and has been recommended for further testing [21]. Other HDAC inhibitors are undergoing trials in solid tumors, including entinostat in phase III trials for locally advanced or metastatic recurrent hormone receptor-positive breast cancer (Table 1) [22].

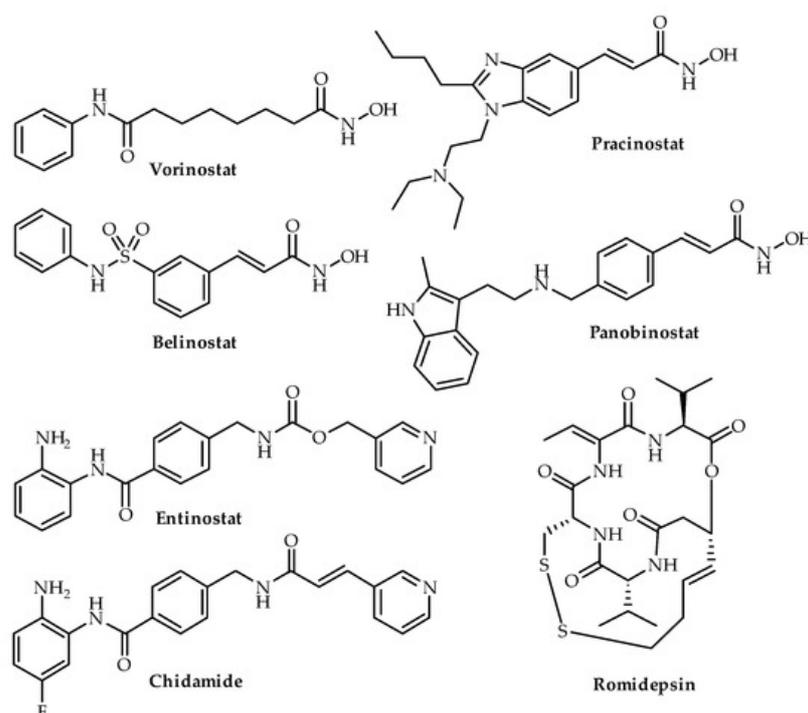


Figure 2. Chemical structures of four HDAC inhibitors (vorinostat, belinostat, romidepsin, pracinostat, entinostat, chidamide and panobinostat). Vorinostat, belinostat, romidepsin and panobinostat are FDA-approved.

Table 1. HDAC inhibitors undergoing clinical trials.

HDAC Inhibitor	Drug Combination	Tumor Type	Trial Phase Completed [Reference]
Entinostat	Exesmesane	Recurrent hormone receptor-positive breast cancer	Phase II trial [23]
	Permolizumab	Metastatic uveal melanoma	Phase II trial [24]
Chidamide	Exesmesane	Hormone receptor-positive, HER2-negative breast cancer	Phase III trial [21]
Vorinostat	Permolizumab	Advanced/metastatic Non-small cell lung cancer	Phase I trial [25]

SAHA, romidepsin, panobinostat and pracinostat (Figure 2) are the four HDAC inhibitors that have undergone clinical trials for CRPC and are discussed below. Apart from romidepsin, SAHA, panobinostat and pracinostat are classed as hydroxamic acid derivatives. There is no clear-cut description of structure activity relationship for testing mainly hydroxamic acid derivatives in the clinical trials conducted for CRPC. Qian and colleagues (2016) recommended entinostat, a benzamide, for clinical testing in prostate cancer based on in vitro and in vivo experiments [26]. Details around the effect of class and structure of HDAC inhibitors remain unexplored. No trends can be observed in the IC₅₀ values of different classes of HDAC inhibitors. Hydroxamic acid derivatives, including SAHA, panobinostat, belinostat and pracinostat, inhibit different classes with different efficacies; similarly, different compounds from the benzamide class, including chidamide, entinostat and mocetinostat exhibit different affinities for different HDACs (Table 2). Even while considering a pharmacokinetic parameter such as half-life. Chidamide, entinostat and mocetinostat had a half-life of 16–18, 51.58 and 7–11 h, whereas vorinostat, panobinostat, pracinostat and belinostat had a half-life of 0.8–3.9, 12, 5.6–8.9 and 0.9 h [27][28][29].

Table 2. IC₅₀ values of HDAC inhibition [16][30][31][32].

Compound	HDAC Inhibition IC ₅₀ (nM)										
	Class I				Class IIa				Class IIb		Class IV
	1	2	3	8	4	5	7	9	6	10	11
SAHA	38	144	6	38	>30000	>30000	>300000	>30000	10	21	28
Panobinostat	3	3	4	248	23	NA	18	6	3	ND	ND
Belinostat	41	125	30	216	115	NA	67	128	82	ND	ND
Pracinostat	49	96	43	140	56	47	137	70	1008	40	ND
Chidamide	95	160	67	733	>30000	>30000	>30000	>30000	>30000	78	432
Entinostat	262	306	499	2700	>30000	>30000	>30000	>30000	>30000	254	0.649
Mocetinostat	150	290	1660	>10000	>10000	>10000	>10000	ND	ND	ND	590
Romidepsin	36	47	ND	ND	510	ND	ND	ND	14000	ND	ND

ND = Not Determined.

3. Pharmacodynamic Rationale for Treatment Failure Using HDAC Inhibitors

As a single agent, HDAC inhibitors have shown poor activity in CRPC and other solid tumors [21][33]. Previously, this has been attributed to their pharmacokinetic profile [34]. This argument can be made in the case of SAHA, which is classified as a class IV drug in the Biopharmaceutical Classification System, due to its low water solubility (190 µg/mL) and low cell permeability (2×10^6 cm/s) [29]. Among other parameters, the drug exhibits poor oral bioavailability (around 11% and 2% in the dog and rat, respectively) and a short half-life of 12 min. Similarly, panobinostat exhibits poor oral bioavailability of 6% in rats and a moderate bioavailability of 33–50% in dogs, whereas romidepsin has not been administered orally in animals due to poor solubility [35]. However, pracinostat has been classified as a BioPharmaceutical Classification System class I compound, as it has a higher water solubility, higher oral bioavailability (34% and 65% in mice and dogs, respectively) and longer half-life of 4.1 h in dogs [29][36][37]. It is worth noting, however, that while some patients exhibited

disease progression, others have shown disease stabilization and PSA declines >50% [20][38][39][40]. Given the conflicting results, the pharmacodynamic profile of HDAC inhibitors is clearly favorable in some patients.

Recently, new insights have been shed on prostate cancer, bringing the centrality of AR, effects on different classes of HDACs by non-specific HDAC inhibitors and evolution of resistance mechanisms in response to HDAC inhibitors into question.

3.1. AR-Negative Prostate Cancers

AR is thought to play a central role in the progression of prostate cancers [41]. Most studies show HDAC-mediated inhibition of HSP-90 acetylation as one of the main mechanisms by which AR can be regulated [38][40]. However, studies report different percentages of AR expression in CRPC. In a study conducted by Leav and colleagues (2001), AR immunostaining was present in more than 95% of metastatic cells irrespective of tumor grade [42]. Pronounced AR staining in the nucleus was also seen in normal prostate secretory and stromal cells [42]. In contrast, a study conducted by Shah and colleagues (2004) indicated that AR expression was variable among patients who died from metastasized CRPC. In this study, AR staining was less than 10% in 41.6% of patients, which suggests that there are alternative AR bypass mechanisms [43]. However, no comparisons were made between different regions of the organs to show differences between adjacent tumor sites [43].

These findings are also supported by another study which showed that serum PSA declines of >50% were observed in 56% of 140 patients with metastatic CRPC following treatment with the AR antagonist enzalutamide [44]. The baseline CTC population decreased by 75% of chemotherapy-naïve and 37% of post-chemotherapy patients [43]. Interestingly, tumors classified from the pre-enzalutamide era (1997–2011) differed in molecular signatures from tumor tissues obtained after the approval of enzalutamide [41]. Data pooled from immunohistochemistry and RNA sequencing of 56 patients showed that 88.4% of patients expressed an AR+ prostate cancer, whereas 6.3% had a neuroendocrine (NE) variant and 6.3% had an AR-/NE- variant from the period of 1998- Compared with data from 2012 to 2016, the figure dropped to 63.3% of patients expressing AR, whereas the AR-/NE+ variant rose to 13.3% and the AR-/NE- variant rose to 23.3% [41]. Although enzalutamide-resistant cell lines have not been studied, knock-down models, including the knockdown of the AR in LnCAP cells, were investigated with the aim of understanding the AR bypass mechanisms. The FGF8/MAPK pathway was implicated as a major AR bypass mechanism in cell lines and a patient-derived xenograft model, however, studies in patients pertaining to this pathway have not been conducted [41].

Interestingly, there has been no correlation between AR and PSA, which may just be a trend seen in late stage prostate cancer [41]. However, it has been widely reported that AR is responsible for regulating PSA expression via indirect and direct means [41][45][46][47]. Given the shifting paradigm in the understanding of prostate cancer, the percentage expression of AR requires further investigation. To date, clinical trials have not studied AR expression. It will also be interesting to understand the correlation between AR expression and HDAC inhibition, given that HDAC plays a role in HSP90 acetylation.

3.2. Other Cellular Targets of HDACs

Although HDAC inhibitors induce apoptosis and cell cycle arrest in various tumors, they also lead to upregulation of epithelial to mesenchymal (EMT) transitioning proteins and confer protective effects in cancer cells via p21 inductions [48][49][50]. These findings can be attributed to the different functions of HDAC classes, as presented in the studies below. Noteworthy, the HDAC inhibitors, SAHA, romidepsin, panobinostat and pracinostat that underwent trials are not specific and inhibit a range of HDACs (Table 3) [16]. Therefore, rather than inhibiting tumor growth, HDAC inhibitors may facilitate its growth.

Table 3. HDAC inhibitors examined in clinical trials to treat prostate cancer [10].

Name	Structural Class	HDACs Inhibited	Prostate Cancer Clinical Trial Status
SAHA	Hydroxamic acid	I, IIb and IV	II failed
Depsipeptide (Romidepsin)	Cyclic peptide	HDAC 1, 2 and 4	II failed
Pracinostat (SB939)	Hydroxamic acid	I, IIa and HDAC10	II failed
Panobinostat	Hydroxamic acid	HDAC I, 4, 6, 7 and 9	II failed

Various studies support these theories. Kong and colleagues (2012) showed that HDAC inhibitors caused acetylation of histone 3 residues in the promoter regions of EMT-related factors such as vimentin, zinc finger E-box-binding homeobox 1

(ZEB1), Slug and matrix metalloproteinase (MMP2). The Western blotting analysis also revealed that the levels of HDAC1 and HDAC2 were unchanged and interacted with the promoters of vimentin, Slug and ZEB1 following treatment with trichostatin A (TSA) and SAHA. These findings suggested that the activity of HDACs was repressed, but the amount of HDACs were unchanged [48]. Using immunofluorescence and reverse transcription polymerase chain reaction, an accumulation of vimentin, ZEB1 and F-actin was observed in response to treatments with SAHA and TSA at concentrations of 5 μ M and 400 nM, respectively, in PC-3 cells [48]. Following treatment with SAHA or TSA, PC3, Du145 and ARCaPE cells showed an irregular fibroblastoid morphology, reminiscent of EMT. As PC-3 cells are AR- cells, AR+ LnCAP cells were also treated with SAHA (2.5 and 5 μ M) and TSA (200 and 400 nM). Increases in the mRNA expressions of ZEB1, Slug, vimentin and N-cadherin were seen, along with increasing protein expression of vimentin and fibronectin 24 h after treatment [48]. Nasopharyngeal, colon, liver and lung cancers have shown similar responses to sodium butyrate, valproic acid (VPA) and SAHA at similar concentrations via upregulations in Snail and Slug [51]. Therefore, active HDAC1 and 2 are capable of inhibiting EMT, and HDAC inhibitors can induce EMT.

Of the few studies examining changes in angiogenesis elicited by HDAC inhibitors, most of the studies have focused on modulating the expression levels of hypoxia inducible factor-1 α (HIF-1 α), vascular endothelial growth factor receptor (VEGFR)-2 and vascular endothelial growth factor (VEGF)-A in cancer cell lines [52][53]. Mixed results have been reported in response to different drugs. Specifically, SAHA (2.5–10 μ M) reduced VEGFA protein expression in lung cancer cell lines (PC9, HCC827, H1975 and NCI-H460) [52][53]. Interestingly, SAHA (5 mmol/L) and VPA (1 mmol/L) caused sprouting of endothelial cell spheroids from HUVECs via activation of β -catenin [54]. VPA was used at this concentration as it equates to patient plasma levels [54]. A further rationale for these studies was the premise that HDAC5 and HDAC7 induce endothelial cell migration [54]. In another study, SAHA (5 μ M) and TSA (400 nM) were able to stop the invasion of HUVECs into type I collagen gel [55]. TSA also inhibited the sprouting of capillaries from ex vivo rat aortic rings and VEGF-induced expression of VEGFR1, VEGFR2 and neuropilin-1 [55]. Furthermore, the VEGF competitive inhibitor, semaphorin, was upregulated by SAHA and TSA in HUVECs [55]. Interestingly, the differential effects of HDAC inhibitors have been linked to their concentrations. Aurora and colleagues (2010) showed that HDAC inhibitors at low concentrations synergized with the competitive inhibitor of angiogenesis, pigment epithelium-derived factor (PEDF), whereas at higher concentrations HDAC inhibitors antagonized PEDF [56].

Various class I and II HDACs exert anti-angiogenic effects [57]. Kruppel-like factor 4 (KLF4) expression in breast cancer modulates VEGF activity via HDAC2 and HDAC3. Ray and colleagues (2013) showed that an ectopic expression of individual KLF4, HDAC2 and HDAC3 decreased VEGF-CAT reporter activity in MCF-10A cells [58]. Chromatin immunoprecipitation (ChIP) analysis also revealed that KLF4, HDAC2 and HDAC3 interacted with the VEGF promoter [58]. In another setting, HDAC5 interacted with the promoter of FGF2 and SLIT2, factors that are critical for endothelial cell growth, as determined by microarray analysis and ChIP assays [59]. Accordingly, knockdown of HDAC5 by siRNA led to HUVEC sprouting [56]. Furthermore, silencing of HDAC7, a class II HDAC, led to an up-regulation of the pro-angiogenic factor, platelet derived growth factor-B (PDGF-B) in HUVEC cells [60].

The studies discussed above demonstrate that HDACs can in fact exert anti-tumorigenic effects, as seen in studies discussed above. Table 1 sums up the different functions of HDAC subtypes to further reinforce that HDACs can exert both anti-tumorigenic and pro-tumorigenic effects.

References

1. Rawla, P. Epidemiology of Prostate Cancer. *World J. Oncol.* 2019, 10, 63–89.
2. Kaushik, D.; Vashistha, V.; Isharwal, S.; Sedique, S.A.; Lin, M.-F. Histone deacetylase inhibitors in castration-resistant prostate cancer: Molecular mechanism of action and recent clinical trials. *Ther. Adv. Urol.* 2015, 7, 388–395.
3. Damodaran, S.; Kyriakopoulos, C.E.; Jarrard, D.F. Newly Diagnosed Metastatic Prostate Cancer: Has the Paradigm Changed? *Urol. Clin. North Am.* 2017, 44, 611–621.
4. Global Burden of Disease Cancer Collaboration; Fitzmaurice, C.; Allen, C.; Barber, R.M.; Barregard, L.; Bhutta, Z.A.; Brenner, H.; Dicker, D.J.; Chimed-Orchir, O.; Dandona, R.; et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol.* 2017, 3, 524.
5. Saraon, P.; Drabovich, A.P.; Jarvi, K.A.; Diamandis, E.P. Mechanisms of Androgen-Independent Prostate Cancer. *EJIFCC* 2014, 25, 42–54.

6. Sartor, A.O. Progression of metastatic castrate-resistant prostate cancer: Impact of therapeutic intervention in the post-docetaxel space. *J. Hematol. Oncol.* 2011, 4, 18.
7. Huang, Y.; Jiang, X.; Liang, X.; Jiang, G. Molecular and cellular mechanisms of castration resistant prostate cancer (Review). *Oncol. Lett.* 2018.
8. Singh, B.N.; Zhang, G.; Hwa, Y.L.; Li, J.; Dowdy, S.C.; Jiang, S.-W. Nonhistone protein acetylation as cancer therapy targets. *Expert Rev. Anticancer Ther.* 2010, 10, 935–954.
9. Drogaris, P.; Villeneuve, V.; Pomiès, C.; Lee, E.-H.; Bourdeau, V.; Bonneil, É.; Ferbeyre, G.; Verreault, A.; Thibault, P. Histone Deacetylase Inhibitors Globally Enhance H3/H4 Tail Acetylation Without Affecting H3 Lysine 56 Acetylation. *Sci. Rep.* 2012, 2, 220.
10. Li, Y.; Seto, E. HDACs and HDAC Inhibitors in Cancer Development and Therapy. *Cold Spring Harb. Perspect. Med.* 2016, 6, a026831.
11. Ropero, S.; Esteller, M. The role of histone deacetylases (HDACs) in human cancer. *Mol. Oncol.* 2007, 1, 19–25.
12. Lee, E.Y.H.P.; Muller, W.J. Oncogenes and Tumor Suppressor Genes. *Cold Spring Harb. Perspect. Biol.* 2010, 2, a003236.
13. Audia, J.E.; Campbell, R.M. Histone Modifications and Cancer. *Cold Spring Harb. Perspect. Biol.* 2016, 8, a019521.
14. Narita, T.; Weinert, B.T.; Choudhary, C. Functions and mechanisms of non-histone protein acetylation. *Nat. Rev. Mol. Cell Biol.* 2019, 20, 156–174.
15. Eckschlager, T.; Pich, J.; Stiborova, M.; Hrabeta, J. Histone Deacetylase Inhibitors as Anticancer Drugs. *Int. J. Mol. Sci.* 2017, 18, 1414.
16. Bertrand, P. Inside HDAC with HDAC inhibitors. *Eur. J. Med. Chem.* 2010, 45, 2095–2116.
17. Robey, R.W.; Chakraborty, A.R.; Basseville, A.; Luchenko, V.; Bahr, J.; Zhan, Z.; Bates, S.E. Histone Deacetylase Inhibitors: Emerging Mechanisms of Resistance. *Mol. Pharm.* 2011, 8, 2021–2031.
18. Mann, B.S.; Johnson, J.R.; Cohen, M.H.; Justice, R.; Pazdur, R. FDA Approval Summary: Vorinostat for Treatment of Advanced Primary Cutaneous T-Cell Lymphoma. *The Oncologist* 2007, 12, 1247–1252.
19. Navakauskienė, R. Combination Epigenetic Therapy. In *Handbook of Epigenetics*; Elsevier, 2017; pp. 623–632. ISBN 978-0-12-805388-1.
20. Bradley, D.; Rathkopf, D.; Dunn, R.; Stadler, W.M.; Liu, G.; Smith, D.C.; Pili, R.; Zwiebel, J.; Scher, H.; Hussain, M. Vorinostat in advanced prostate cancer patients progressing on prior chemotherapy (National Cancer Institute Trial 6862): Trial results and interleukin-6 analysis: A study by the Department of Defense Prostate Cancer Clinical Trial Consortium and University of Chicago Phase 2 Consortium. *Cancer* 2009, 115, 5541–5549.
21. Jiang, Z.; Li, W.; Hu, X.; Zhang, Q.; Sun, T.; Cui, S.; Wang, S.; Ouyang, Q.; Yin, Y.; Geng, C.; et al. Tucidinostat plus exemestane for postmenopausal patients with advanced, hormone receptor-positive breast cancer (ACE): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* 2019, 20, 806–815.
22. Yeruva, S.L.H.; Zhao, F.; Miller, K.D.; Tevaarwerk, A.J.; Wagner, L.I.; Gray, R.J.; Sparano, J.A.; Connolly, R.M. E2112: Randomized phase III trial of endocrine therapy plus entinostat/placebo in patients with hormone receptor-positive advanced breast cancer. *Npj Breast Cancer* 2018, 4, 1.
23. Yardley, D.A.; Ismail-Khan, R.R.; Melichar, B.; Lichinitser, M.; Munster, P.N.; Klein, P.M.; Cruickshank, S.; Miller, K.D.; Lee, M.J.; Trepel, J.B. Randomized Phase II, Double-Blind, Placebo-Controlled Study of Exemestane With or Without Entinostat in Postmenopausal Women With Locally Recurrent or Metastatic Estrogen Receptor-Positive Breast Cancer Progressing on Treatment With a Nonsteroidal Aromatase Inhibitor. *J. Clin. Oncol.* 2013, 31, 2128–2135.
24. Jespersen, H.; Olofsson Bagge, R.; Ullenhag, G.; Carneiro, A.; Helgadottir, H.; Ljuslinder, I.; Levin, M.; All-Eriksson, C.; Andersson, B.; Stierner, U.; et al. Concomitant use of pembrolizumab and entinostat in adult patients with metastatic uveal melanoma (PEMDAC study): Protocol for a multicenter phase II open label study. *BMC Cancer* 2019, 19, 415.
25. Gray, J.E.; Saltos, A.; Tanvetyanon, T.; Haura, E.B.; Creelan, B.; Antonia, S.J.; Shafique, M.; Zheng, H.; Dai, W.; Saller, J.J.; et al. Phase I/II Study of Pembrolizumab Plus Vorinostat in Advanced/Metastatic Non-Small Cell Lung Cancer. *Clin. Cancer Res.* 2019, 25, 6623–6632.
26. Qian, D.Z.; Wei, Y.-F.; Wang, X.; Kato, Y.; Cheng, L.; Pili, R. Antitumor activity of the histone deacetylase inhibitor MS-275 in prostate cancer models. *The Prostate* 2007, 67, 1182–1193.
27. Bumber, Y.; Younes, A.; Garcia-Manero, G. Mocetinostat (MGCD0103): A review of an isotype-specific histone deacetylase inhibitor. *Expert Opin. Investig. Drugs* 2011, 20, 823–829.
28. Chan, T.; Tse, E.; Kwong, Y.-L. Chidamide in the treatment of peripheral T-cell lymphoma. *OncoTargets Ther.* 2017, 10, 347–352.

29. McClure, J.J.; Li, X.; Chou, C.J. Advances and Challenges of HDAC Inhibitors in Cancer Therapeutics. In *Advances in Cancer Research*; Elsevier, 2018; Volume 138, pp. 183–211. ISBN 978-0-12-815127-3.
30. Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K.-H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; et al. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. *Cancer Res.* 2002, 62, 4916–4921.
31. Novotny-Diermayr, V.; Sangthongpitag, K.; Hu, C.Y.; Wu, X.; Sausgruber, N.; Yeo, P.; Greicius, G.; Pettersson, S.; Liang, A.L.; Loh, Y.K.; et al. SB939, a Novel Potent and Orally Active Histone Deacetylase Inhibitor with High Tumor Exposure and Efficacy in Mouse Models of Colorectal Cancer. *Mol. Cancer Ther.* 2010, 9, 642–652.
32. Fournel, M.; Bonfils, C.; Hou, Y.; Yan, P.T.; Trachy-Bourget, M.-C.; Kalita, A.; Liu, J.; Lu, A.-H.; Zhou, N.Z.; Robert, M.-F.; et al. MGCD0103, a novel isotype-selective histone deacetylase inhibitor, has broad spectrum antitumor activity in vitro and in vivo. *Mol. Cancer Ther.* 2008, 7, 759–768.
33. Halsall, J.A.; Turner, B.M. Histone deacetylase inhibitors for cancer therapy: An evolutionarily ancient resistance response may explain their limited success. *BioEssays* 2016, 38, 1102–1110.
34. Kantharaj, E.; Jayaram, R. Histone Deacetylase Inhibitors as Therapeutic Agents for Cancer Therapy: Drug Metabolism and Pharmacokinetic Properties. In *Drug Development—A Case Study Based Insight into Modern Strategies*; Rundfeldt, C., Ed.; InTech, 2011; ISBN 978-953-307-257-9.
35. Konsoula, Z.; Cao, H.; Velená, A.; Jung, M. Pharmacokinetics-pharmacodynamics and antitumor activity of mercaptoacetamide-based histone deacetylase inhibitors. *Mol. Cancer Ther.* 2009, 8, 2844–2851.
36. Rundfeldt, C. *Drug Development: A Case Study Based Insight into Modern Strategies*; InTech: Rijeka, Croatia, 2011; ISBN 978-953-307-257-9.
37. Wishart, D.S.; Feunang, Y.D.; Guo, A.C.; Lo, E.J.; Marcu, A.; Grant, J.R.; Sajed, T.; Johnson, D.; Li, C.; Sayeeda, Z.; et al. DrugBank 5.0: A major update to the DrugBank database for 2018. *Nucleic Acids Res.* 2018, 46, D1074–D1082.
38. Molife, L.R.; Attard, G.; Fong, P.C.; Karavasilis, V.; Reid, A.H.M.; Patterson, S.; Riggs, C.E.; Higano, C.; Stadler, W.M.; McCulloch, W.; et al. Phase II, two-stage, single-arm trial of the histone deacetylase inhibitor (HDACi) romidepsin in metastatic castration-resistant prostate cancer (CRPC). *Ann. Oncol.* 2010, 21, 109–113.
39. Eigel, B.J.; North, S.; Winqvist, E.; Finch, D.; Wood, L.; Sridhar, S.S.; Powers, J.; Good, J.; Sharma, M.; Squire, J.A.; et al. A phase II study of the HDAC inhibitor SB939 in patients with castration resistant prostate cancer: NCIC clinical trials group study IND195. *Invest. New Drugs* 2015, 33, 969–976.
40. Rathkopf, D.E.; Picus, J.; Hussain, A.; Ellard, S.; Chi, K.N.; Nydam, T.; Allen-Freda, E.; Mishra, K.K.; Porro, M.G.; Scher, H.I.; et al. A phase 2 study of intravenous panobinostat in patients with castration-resistant prostate cancer. *Cancer Chemother. Pharmacol.* 2013, 72, 537–544.
41. Bluemner, E.G.; Coleman, I.M.; Lucas, J.M.; Coleman, R.T.; Hernandez-Lopez, S.; Tharakan, R.; Bianchi-Frias, D.; Dumit, R.F.; Kaipainen, A.; Corella, A.N.; et al. Androgen Receptor Pathway-Independent Prostate Cancer Is Sustained through FGF Signaling. *Cancer Cell* 2017, 32, 474–489.e6.
42. Leav, I.; Lau, K.M.; Adams, J.Y.; McNeal, J.E.; Taplin, M.E.; Wang, J.; Singh, H.; Ho, S.M. Comparative studies of the estrogen receptors beta and alpha and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. *Am. J. Pathol.* 2001, 159, 79–92.
43. Shah, R.B.; Mehra, R.; Chinnaiyan, A.M.; Shen, R.; Ghosh, D.; Zhou, M.; MacVicar, G.R.; Varambally, S.; Harwood, J.; Bismar, T.A.; et al. Androgen-Independent Prostate Cancer Is a Heterogeneous Group of Diseases: Lessons from a Rapid Autopsy Program. *Cancer Res.* 2004, 64, 9209–9216.
44. Scher, H.I.; Beer, T.M.; Higano, C.S.; Anand, A.; Taplin, M.-E.; Efsthathiou, E.; Rathkopf, D.; Shelkey, J.; Yu, E.Y.; Alumkal, J.; et al. Antitumor activity of MDV3100 in castration-resistant prostate cancer: A phase 1–2 study. *The Lancet* 2010, 375, 1437–1446.
45. Nickols, N.G.; Dervan, P.B. Suppression of androgen receptor-mediated gene expression by a sequence-specific DNA-binding polyamide. *Proc. Natl. Acad. Sci. USA* 2007, 104, 10418–10423.
46. Kim, J.; Coetzee, G.A. Prostate specific antigen gene regulation by androgen receptor. *J. Cell. Biochem.* 2004, 93, 233–241.
47. Saxena, P.; Trerotola, M.; Wang, T.; Li, J.; Sayeed, A.; VanOudenhove, J.; Adams, D.S.; FitzGerald, T.J.; Altieri, D.C.; Languino, L.R. PSA regulates androgen receptor expression in prostate cancer cells. *The Prostate* 2012, 72, 769–776.
48. Kong, D.; Ahmad, A.; Bao, B.; Li, Y.; Banerjee, S.; Sarkar, F.H. Histone Deacetylase Inhibitors Induce Epithelial-to-Mesenchymal Transition in Prostate Cancer Cells. *PLoS ONE* 2012, 7, e45045.

49. Bernhart, E.; Stundl, N.; Kaltenecker, H.; Windpassinger, C.; Donohue, N.; Leithner, A.; Lohberger, B. Histone deacetylase inhibitors vorinostat and panobinostat induce G1 cell cycle arrest and apoptosis in multidrug resistant sarcoma cell lines. *Oncotarget* 2017, 8.
50. Kim, Y.B.; Ki, S.W.; Yosnida, M.; Horinouchi, S. Mechanism of Cell Cycle Arrest Caused by Histone Deacetylase Inhibitors in Human Carcinoma Cells. *J. Antibiot. (Tokyo)* 2000, 53, 1191–1200.
51. Jiang, G.-M.; Wang, H.-S.; Zhang, F.; Zhang, K.-S.; Liu, Z.-C.; Fang, R.; Wang, H.; Cai, S.-H.; Du, J. Histone deacetylase inhibitor induction of epithelial–mesenchymal transitions via up-regulation of Snail facilitates cancer progression. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* 2013, 1833, 663–671.
52. Zhao, Y.; Yu, D.; Wu, H.; Liu, H.; Zhou, H.; Gu, R.; Zhang, R.; Zhang, S.; Wu, G. Anticancer activity of SAHA, a potent histone deacetylase inhibitor, in NCI-H460 human large-cell lung carcinoma cells in vitro and in vivo. *Int. J. Oncol.* 2014, 44, 451–458.
53. Wei, Y.; Zhou, F.; Lin, Z.; Shi, L.; Huang, A.; Liu, T.; Yu, D.; Wu, G. Antitumor effects of histone deacetylase inhibitor suberoylanilide hydroxamic acid in epidermal growth factor receptor-mutant non-small-cell lung cancer lines in vitro and in vivo. *Anticancer Drugs* 2018, 29, 262–270.
54. Jin, G.; Bausch, D.; Knightly, T.; Liu, Z.; Li, Y.; Liu, B.; Lu, J.; Chong, W.; Velmahos, G.C.; Alam, H.B. Histone deacetylase inhibitors enhance endothelial cell sprouting angiogenesis in vitro. *Surgery* 2011, 150, 429–435.
55. Deroanne, C.F.; Bonjean, K.; Servotte, S.; Devy, L.; Colige, A.; Clausse, N.; Blacher, S.; Verdin, E.; Foidart, J.-M.; Nusgens, B.V.; et al. Histone deacetylase inhibitors as anti-angiogenic agents altering vascular endothelial growth factor signaling. *Oncogene* 2002, 21, 427–436.
56. Aurora, A.B.; Biyashev, D.; Mirochnik, Y.; Zaichuk, T.A.; Sanchez-Martinez, C.; Renault, M.-A.; Losordo, D.; Volpert, O. V. NF- κ B balances vascular regression and angiogenesis via chromatin remodeling and NFAT displacement. *Blood* 2010, 116, 475–484.
57. Kim, H.-J.; Bae, S.-C. Histone deacetylase inhibitors: Molecular mechanisms of action and clinical trials as anti-cancer drugs. *Am. J. Transl. Res.* 2011, 3, 166–179.
58. Ray, A.; Alalem, M.; Ray, B.K. Loss of Epigenetic Kruppel-like Factor 4 Histone Deacetylase (KLF-4-HDAC)-mediated Transcriptional Suppression Is Crucial in Increasing Vascular Endothelial Growth Factor (VEGF) Expression in Breast Cancer. *J. Biol. Chem.* 2013, 288, 27232–27242.
59. Dunaway, C.M.; Hwang, Y.; Lindsley, C.W.; Cook, R.S.; Wu, J.Y.; Boothby, M.; Chen, J.; Brantley-Sieders, D.M. Cooperative Signaling between Slit2 and Ephrin-A1 Regulates a Balance between Angiogenesis and Angiostasis. *Mol. Cell. Biol.* 2011, 31, 404–416.
60. Turtoi, A.; Mottet, D.; Matheus, N.; Dumont, B.; Peixoto, P.; Hennequière, V.; Deroanne, C.; Colige, A.; De Pauw, E.; Belahcène, A.; et al. The angiogenesis suppressor gene AKAP12 is under the epigenetic control of HDAC7 in endothelial cells. *Angiogenesis* 2012, 15, 543–554.